Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Identification of Shared Biomarkers in Chronic Kidney Disease and Diabetic Nephropathy Using Single-Cell Sequencing

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Background: Chronic kidney disease (CKD) and diabetic nephropathy (DN) represent significant renal health challenges, with overlapping pathogenic mechanisms. This study evaluated shared biomarkers in CKD and DN through single-cell sequencing, aiming to identify potential diagnostic and therapeutic targets and provide new insights into their common pathogenesis.

Methods: In this study, single-cell RNA sequencing was performed on nine columns of human blood samples, including three control cases, three CKD cases, and three DN cases. Following sequencing, single-cell analysis was conducted to identify different cell types. Differential expression analysis was then performed to compare the disease samples (CKD and DN) with control samples, resulting in the identification of differentially expressed genes (DEGs). The intersection of DEGs between the disease samples and the control samples was extracted, and a Protein-Protein Interaction (PPI) network was constructed using these intersecting genes, with biomarkers identified through the STRING database. Additionally, Gene Set Enrichment Analysis and GeneMANIA were applied to explore the potential mechanisms underlying these biomarkers.

Results: Findings revealed elevated IRF7 expression within dendritic cells (DC), while MX1 showed specifically elevated expression in both DN and CKD samples. MX1 and IRF7 exhibited notable high expression in DC. Four biomarkers were all enriched in the Oxidative Phosphorylation pathway in CKD, and in DN, they were all enriched in the FcγR Mediated Phagocytosis pathway. STAT1 and ISG15 were widely expressed across macrophages, monocytes, NK cells, and NK T cells. In conclusion, the four biomarkers were expressed differently in the disease and control groups of different immune cells.

Conclusion: Our study successfully identified MX1, IRF7, STAT1, and ISG15 as shared biomarkers in CKD and DN, revealing their distinct expression patterns and potential roles in disease mechanisms.

Keywords: chronic kidney disease, diabetic nephropathy, gene set enrichment analysis, pseudotime, single-cell RNA-sequencing

Introduction

The global prevalence of chronic kidney disease (CKD) has been rising steadily, currently affecting approximately 10.8% of the total population.[1] CKD is characterized by abnormalities in kidney structure or function persisting for over 3 months, affecting overall health. A key indicator is a glomerular filtration rate (GFR) of less than 60 mL/ (min·1.73 m²), accompanied by at least one of the following markers of kidney injury: albuminuria, abnormal urinary sediment (eg, hematuria), electrolyte disturbances due to renal tubule dysfunction, histological abnormalities, structural changes in imaging, or a history of kidney transplantation.[2] CKD often presents gradually, with subtle or atypical symptoms in the early stages, making timely detection challenging. At onset, typical indicators include hypertension, hyperglycemia, and microalbuminuria, which are not highly sensitive to standard diagnostic tests, contributing to a poor clinical prognosis.

As the condition advances, it can evolve into nephrotic syndrome, chronic nephritis, or acute nephritis, with some patients progressing to end-stage renal disease (ESRD). The long waiting period for kidney transplantation, due to limited donor availability, results in many patients with ESRD relying on dialysis for survival. Over 60% of these

Received: 30 December 2024
Accepted: 19 June 2025
Published: 5 July 2025

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patients undergo dialysis for more than a year, with approximately 23% needing long-term dialysis for over 3 years.[3] This not only imposes a significant physiological, psychological, and financial burden but also affects the quality of life.

Treatment for CKD primarily focuses on slowing nephron damage, managing hyperfiltration, addressing complications, and providing renal replacement therapy. However, hemodialysis often leads to poor functional outcomes, including uremia-related malnutrition and muscle wasting, and carries risks of infection and vascular complications, further compromising patient quality of life. While kidney transplantation offers improved quality of life, recipients face persistent CKD-related symptoms and complications from immunosuppressive therapies.[4] Consequently, more effective and scientifically-based methods are needed for the diagnosis and treatment of CKD.

Diabetic nephropathy (DN) is a common and serious microvascular complication of diabetes, particularly prevalent in type 2 diabetes mellitus (T2DM), primarily induced by hyperglycemia. Its clinical manifestations include proteinuria, progressive renal dysfunction, hypertension, and edema. In China, approximately 20% to 40% of patients with diabetes are affected by DN, with most cases in the early, asymptomatic stages.[5] Current research suggests that the pathogenesis of DN is closely associated with hyperglycemia, the accumulation of advanced glycosylation end products, as well as inflammatory and immune responses.[6] Currently, DN has surpassed glomerulonephritis as the leading cause of new cases of CKD in China.[7] As DN advances, patients face an increased risk of developing CKD due to factors such as RAAS activation and microvascular damage induced by sustained hyperglycemia and hypertension.[8] Early screening and prompt diagnosis of DN are essential to prevent progression to CKD and end-stage nephropathy. However, the precise mechanisms by which DN contributes to CKD remain unclear. Thus, studying DN is vital not only for understanding the underlying mechanisms of CKD but also for identifying new therapeutic targets for both DN and CKD prevention and treatment.

Single-cell RNA sequencing (scRNA-seq) is a cutting-edge high-throughput sequencing technique that enables the analysis of gene expression profiles at the single-cell level. By analyzing cellular composition, gene enrichment pathways, and intercellular communication, scRNA-seq offers insights into the underlying pathological processes of diseases. Recently, scRNA-seq has gained widespread use due to its sensitivity, accuracy, and efficiency. Unlike traditional sequencing methods, scRNA-seq enables detailed analysis of the cellular spectrum, identification of specific cell types, and mapping of gene expression patterns in heterogeneous cell samples. This allows for the study of gene expression at the single-cell level, providing a microscopic view of disease progression.[9] The application of scRNA-seq in kidney research is promising, as it enhances understanding of cellular heterogeneity in CKD and DN, as well as identifying the potential mechanisms between these conditions. Additionally, scRNA-seq can help elucidate the correlation between CKD and DN, offering valuable insights for identifying biomarkers that can predict disease progression and inform patient-specific treatment strategies.[10]

In this study, biomarkers associated with DN and CKD were identified through single-cell analysis, differential expression analysis, and protein-protein interaction (PPI) network construction. A comprehensive bioinformatics approach was utilized, including cell communication analysis, pseudotime series analysis, and gene set enrichment analysis (GSEA). The mechanisms of these biomarkers were examined, with an emphasis on key cell types and immune responses in patients with DN and CKD. This research provides a theoretical foundation and novel perspectives for studying disease associations, advancing diagnostic methods, and identifying therapeutic targets. Additionally, cellular-level validation of biomarker expression offers valuable insights for distinguishing between these two conditions.

Materials and Methods

Single-Cell RNA-Sequencing

Nine blood samples were collected for scRNA-seq, including three control samples, three CKD samples (All were stage 5 chronic kidney disease), and three DN samples (One sample was stage 4 chronic kidney disease, and the rest were stage 5 chronic kidney disease, and all were stage 5 diabetic nephropathy). Sample grouping information is shown in Supplementary Table 1.

Patients with DN were selected based on the 2020 Kidney Disease: Improving Global Outcomes (KDIGO) Guidelines, using the following criteria: (1) A urine albumin-creatinine ratio (UACR) of ≥ 30 mg/g, measured at least twice over a 3 to 6-month period, with other factors excluded; (2) An estimated glomerular filtration rate (eGFR) of <

60mL • min-1 • (1.73 m^2) −1 persisting for more than 3 months; (3) Renal biopsy results indicating pathological changes consistent with DN. Patients with CKD met the 2020 KDIGO Guidelines for CKD diagnosis in the absence of diabetes. The exclusion criteria were: (1) Severe infections; (2) Malignant tumors; (3) Active autoimmune diseases; (4) Concurrent cardiovascular or cerebrovascular events; (5) Pregnancy. This study was approved by our hospital's Ethics Committee, and informed consent was obtained from all participants.

Chronic Kidney Disease is classified into stages based on GFR and albuminuria, as outlined by the KDIGO guidelines. The KDIGO 2012 Classification includes: Stage 1: GFR ≥ 90 mL/min/1.73 m² with evidence of kidney damage (eg, albuminuria, structural abnormalities, or genetic disorders); Stage 2: GFR 60–89 mL/min/1.73 m² with kidney damage; Stage 3a: GFR 45–59 mL/min/1.73 m²; Stage 3b: GFR 30–44 mL/min/1.73 m²; Stage 4: GFR 15–29 mL/min/1.73 m²; Stage 5: GFR < 15 mL/min/1.73 m² or kidney failure requiring dialysis/transplantation.[11]

DN progression is classified based on GFR and albuminuria, integrating criteria from both diabetes and CKD guidelines. The KDIGO framework is widely used, with modifications specific to Diabetic Kidney Disease. Stage 1: GFR > 90 mL/min/1.73 m² (elevated due to renal hyperfiltration), early glomerular hypertrophy and hyperfiltration; Stage 2: GFR normal or mildly elevated (≥ 90 mL/min/1.73 m²), persistently elevated albumin-to-creatinine ratio (ACR 30–300 mg/g, moderately increased), kidney structural damage (eg, glomerular basement membrane thickening); Stage 3: GFR 60–89 mL/min/1.73 m² (CKD Stage 2), ACR ≥ 300 mg/g (severely increased), with clinical signs of hypertension and progressive proteinuria; Stage 4: GFR 15–59 mL/min/1.73 m² (CKD Stages 3–4), persistent ACR ≥ 300 mg/g, complications include declining kidney function, edema, and cardiovascular risks; Stage 5: kidney failure, GFR < 15 mL/min/1.73 m² (CKD Stage 5), requiring dialysis or transplantation.[12]

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood samples using Ficoll density gradient centrifugation. Cell viability, assessed using AO/PI double fluorescent staining on a Countstar Rigel (S2) instrument, was required to exceed 85%. Following quality inspection, the single-cell suspension met the quality control criteria and proceeded with library construction, adhering to the SOP "ChromiumNextGEMSingleCell3_3.1_rev_d" from 10x Genomics. The Illumina Nova-seq 6000 PE150 platform was employed for sequencing the single-cell library.

Data Filtering

Sequencing data were initially examined for data volume, sequencing base quality, and sequencing saturation, followed by sequence statistics analysis using CellRanger (v 7.0).[13] Single-cell analysis was then conducted on the RNA-sequencing dataset using the Seurat package (v 3.1.5).[14] A Seurat object was created with parameters min.cells = 100 and min.features = 100 to filter out low-quality cells. Next, the scDblFinder package (v 1.17.2) was applied to identify and eliminate doublet cells.[15] Cell screening criteria were as follows: library size exceeding 500 but below the 95th percentile (10,000 cells), gene counts below the 95th percentile (10,000 cells), and mitochondrial content restricted to less than 10%. Gene expression in each cell was normalized using the LogNormalize method.

Principal Component Analysis (PCA) and Cell Annotation

The FindVariableFeatures function with the variance-stabilizing transformation (vst) method was employed to identify genes exhibiting significant variation across cells. From this analysis, the top 2000 genes with the highest variability were selected. To minimize the effects of differing sequencing batches, data from the nine samples were integrated. The FindIntegrationAnchors function was used to identify anchors from a set of Seurat objects, and the IntegrateData function was then applied to merge the samples based on these anchors. PCA was applied to scale and reduce the data dimensionality. Principal components (PCs) with higher rankings in PCA encapsulate more diverse and valuable differential features. An elbow plot was constructed to identify the appropriate number of PCs for clustering analysis. Cells were clustered in an unsupervised manner using the FindNeighbors and FindClusters functions (resolution = 1). t-distributed stochastic neighbor embedding (t-SNE) was used to visualize cell clusters. Marker genes for each cluster were identified and annotated by comparing them with known cell type marker genes from the CellMarker database (http://biocc.hrbmu.edu.cn/CellMarker/) for cell annotation. A bar chart illustrating cell proportions across samples was generated to represent the distribution of cells within each sample.

Cell Correlation Analysis

The relationship between different cells in the PCA space was examined by constructing cluster dendrograms based on PCA dimensions, with the aim of analyzing the Euclidean distances between the cells. Additionally, the correlation among various cell types was evaluated using the average gene expression data.

GSEA and Gene Set Variation Analysis (GSVA)

Differential expression analysis was conducted for each cell type, comparing control samples with DN and CKD samples. The log2FoldChange (FC) values for each gene were sorted in descending order for each cell type. GSEA was then performed using the clusterProfiler package (v 3.16.0), with the Kyoto Encyclopedia of Genes and Genomes (KEGG) gene set as the background (|Normalized Enrichment Scores (NES)| > 1, NOM $p < 0.05$).[16] The GSVA package (v 1.46.0) was used to compute GSVA scores across all samples for different cell types based on the h.all.v2022.3.Hs.symbols.gmt gene set.[17] The limma package (v 3.54.0) was then applied to assess the statistical significance in pathway differences between control samples and CKD or DN samples ($p < 0.05$).[18]

Cell Communication and Pseudotime Analysis

CellPhoneDB analysis was performed separately on the control, CKD, and DN samples. The receptor-ligand pairs were filtered with a threshold of $p < 0.05$ and a minimum mean expression value > 1. Key cells were selected based on annotated cell types according to literature reports.[19,20] To examine the differentiation status of key cells at different periods, pseudotime analysis was conducted using Monocle (v 2.14.0), providing insights into the progression of cellular differentiation over time.[21]

Identification of Candidate Genes

To identify candidate genes, differentially expressed genes (DEGs) in key cell types were compared between control and DN samples, and between control and CKD samples. DEGs were selected based on the following criteria: |average log2FC| > 0.25, pct > 0.1, and adj.$p < 0.05$. The DEGs identified between control and DN samples were designated as DEGs1, while those between control and CKD samples were designated as DEGs2. A Venn diagram tool (http://bioinformatics.psb.ugent.be/webtools/Venn/), was used to find the intersection of DEGs1 and DEGs2, resulting in a list of candidate genes shared by both DN and CKD groups. Gene Ontology (GO) and KEGG pathway analyses were subsequently performed using the clusterProfiler package (v 3.16.0) to investigate the shared functions and pathways of these candidate genes.[16]

PPI Network Analysis

To explore interactions among candidate genes, a PPI network was constructed using the STRING database (https://string-db.org) with a confidence threshold of 0.4. Proteins not connected from the main network were excluded, allowing the focus to remain on the hub genes. The MCODE function in Cytoscape (v 3.10.1) was then used to analyze sub-networks of these hub genes, specifically highlighting the TOP1 sub-network based on the following parameters: degree cutoff = 2, node score cutoff = 0.2, K-core = 2, and max depth = 100).[22] The CytoHubba plugin was used to rank hub genes according to four scoring methods (MCC, MNC, Closeness, and Degree). The top 10 genes from each scoring method were selected and genes that consistently appeared across all four methods were designated as biomarkers.

Enrichment Analysis and Gene Co-Expression Network of Biomarkers

The correlation coefficients between gene expression in the control versus DN samples and control versus CKD samples were calculated and ranked. This ranking allowed for further GSEA analysis with thresholds set at (|NES| > 1 and adj.$p <$ 0.05). GO and KEGG gene sets were used as background for this analysis. The GeneMANIA database (http://genemania.org) was employed to predict genes that interact with the biomarkers and explore their associated biological functions, facilitating the construction of a gene co-expression network.

To investigate the activity of upstream pathways associated with the biomarkers, their corresponding pathways were retrieved using the SPEED2 database (https://speed2.sys-bio.net/). The activities of these enriched upstream pathways were quantified using the Bates test and subsequently ranked.

Biomarker-Drug-Disease Network

The Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/) was used to predict drugs targeting the biomarkers and to screen relationship pairs in human species. Additionally, the CTD database provided the diseases associated with the identified drugs, which were then visualized in a biomarker-drug-disease network.

Expression Analysis of Biomarkers

The "polygenic query" function in GTEx (v 8, https://www.gtexportal.org/home/) was utilized to analyze the expression of biomarkers across different cells and tissues. The expression levels of biomarkers in the annotated cells were assessed, followed by a comparison of expression differences between the control, CKD, and DN groups. Additionally, the expression patterns of biomarkers were analyzed throughout pseudotime based on previous pseudotime analysis results.

Statistical Analysis

Data processing and analysis were performed using R software (version 4.2.1). In the bioinformatics analysis, the Wilcoxon rank-sum test was used to examine the differences between the 2 groups. A P-value less than 0.05 was regarded as statistically significant. In addition, the bioinformatics tools and databases used in this study are shown in Supplementary Table 2.

Results

Annotation of 10 Cell Types

The quality of sequencing was assessed, with a Q30 value for all samples exceeding 74%, and the Q20 value surpassing 82% (Supplementary Table 3). Moreover, the sequencing saturation for all samples was greater than 81%, with a mapping rate of over 85% (Supplementary Tables 4 and 5). These results confirm that the sequencing quality of all samples was high, making them suitable for further analysis. After filtering out low-quality cells, the initial cell count of 61,935 was reduced to 28,938 (Supplementary Figure 1). To minimize computational load, the top 2000 most variable genes were selected for PCA (Supplementary Figure 2). The genes from the top nine PCs are shown in Figure 1A, and the top 20 PCs, chosen based on the elbow plot, were used for unsupervised clustering (Figure 1B). A total of 27 clusters were identified, and 10 cell types were annotated: natural killer (NK) T cells, T cells, NK cells, monocytes, B cells, macrophages, mast cells, dendritic cells, and plasma cells (Figure 1C and D, Supplementary Figure 3). T cells, NK T cells, and monocytes were most prevalent across the samples (Figure 1E).

Pathway Similarities Between CKD and DN Across Cell Types

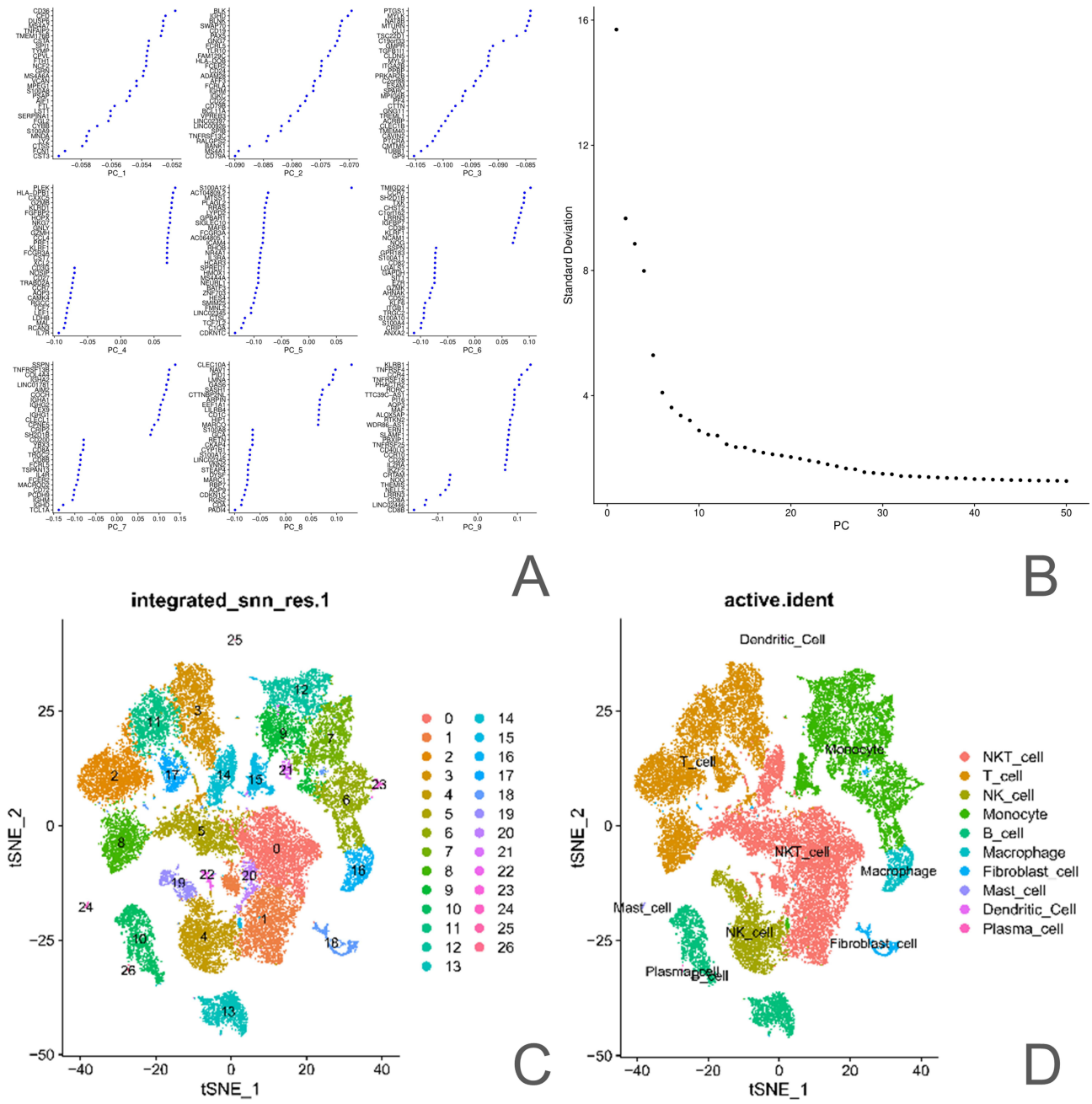
The dendrogram showed that cells in close proximity demonstrate higher similarity. Notably, NK cells and NK T cells showed a greater degree of similarity to each other, followed by a closer resemblance to T cells (Figure 2A). A strong positive correlation was also observed between NK cells and NK T cells (Figure 2B). The DEGs in dendritic cells between control and CKD as well as DN samples were enriched in pathways such as the proteasome, endometrial cancer, and apoptosis. In macrophages, the NOD-like receptor signaling pathway was the enriched pathway, while oxidative phosphorylation, non-alcoholic fatty liver disease, and valine, leucine, and isoleucine degradation were the key pathways in mast cells. The enriched pathways for the nine cell types are shown in Supplementary Figure 4 Plasma cells from DN samples were not analyzed due to an insufficient sample size). Significant differences were observed in most pathways between DN and normal samples in T cells, NK T cells, monocytes, B cells, and NK cells. Similarly, notable pathway differences were found between CKD and control samples in T cells, NK T cells, monocytes, and NK cells. The pathway activities in T cells, NK T cells, monocytes, and NK cells were found to be elevated in both CKD and DN, with similar activation patterns suggesting a resemblance between the two conditions (Figure 2C and D).

Disruption and Imbalance of Cell Communication in DN and CKD

Cell communication patterns varied markedly between conditions, with a significant increase in cell communication frequency observed in CKD compared to the control group, while the frequency of cell communication was significantly reduced in DN (Figure 3A–F). This indicates that the occurrence of DN and CKD may be associated with disruptions and imbalances in cell communication.

Identification of 119 Candidate Genes Associated with Both CKD and DN

Analysis of myeloid cell subtypes, including monocytes, macrophages, mast cells, and dendritic cells, identified these as key cell types involved in both CKD and DN. Differential expression analysis revealed 297 DEGs (DEGs1) between the control



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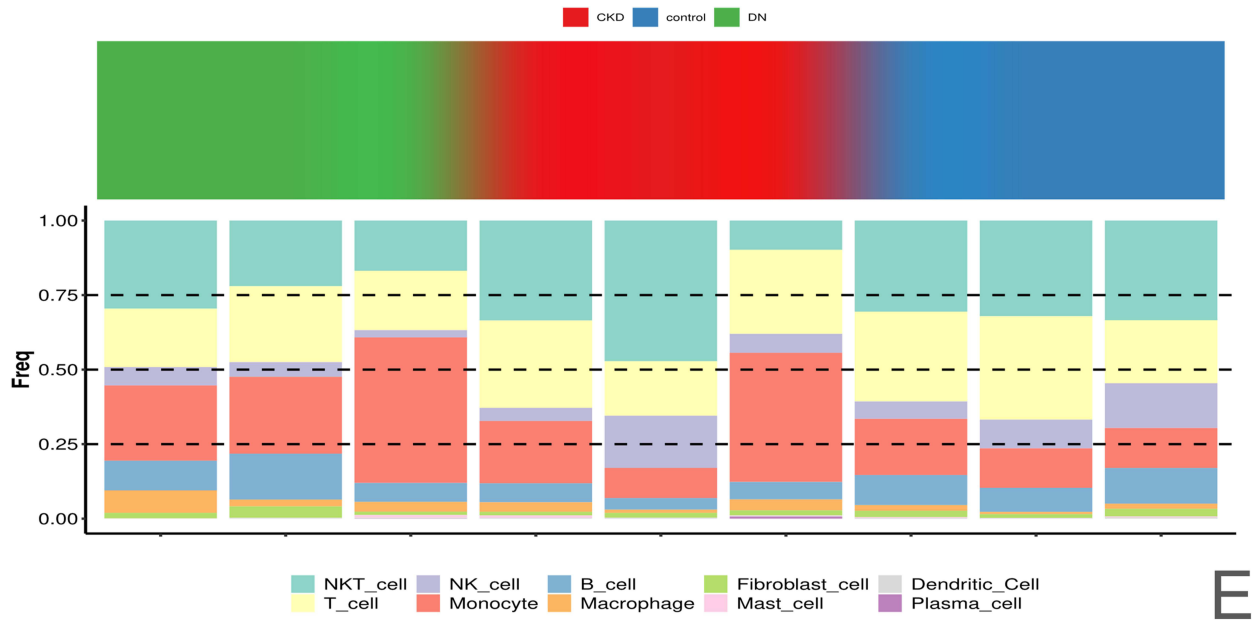

Figure 1 Annotation of cluster subtypes and unsupervised clustering analysis of single-cell samples. (**A** and **B**) PCA analysis and elbow plot for determining the optimal PCs. (**C** and **D**) Heatmap of cell clustering based on genes involved in t-SNE dimensionality reduction across the samples. (**E**) Proportion of cell clusters in control, DN, and CKD samples. **Abbreviations**: PCA, Principal component analysis; PCs, Principal components; t-SNE, t-distributed stochastic neighbor embedding; DN, Diabetic nephropathy; CKD, Chronic kidney disease.

and CKD samples and 277 DEGs (DEGs2) between the control and DN samples in these cell types (Figure 4A and B). Upon intersecting these datasets, 119 candidate genes associated with both CKD and DN were obtained (Figure 4C). The involvement of these candidate genes in disease was linked to several KEGG pathways, such as viral life cycle, measles, malaria, and B cell receptor signaling. Additionally, GO functions related to these genes included negative regulation of MAP kinase activity, toll-like receptor 4 signaling pathway, immunological synapse, platelet alpha granule lumen, amyloid-beta binding, and chemokine receptor binding (Figure 4D and E).

MX1, IRF7, STAT1, and ISG15 Were Identified as Biomarkers

From the initial 119 candidate genes, 21 genes corresponding to discrete proteins were excluded, resulting in a PPI network consisting of 98 proteins (Figure 5A). The top subnetwork identified by the MCODE function included 25 nodes and 267 edges, revealing strong interactions among the proteins (Figure 5B). By intersecting the top 10 genes obtained from the MCC, MNC, Closeness, and Degree scores in the cytoHubba plugin, four biomarkers were identified: MX1, IRF7, STAT1, and ISG15 (Figure 5C). A gene co-expression network was constructed, identifying 20 genes interacting with these biomarkers, primarily involved in functions such as response to type I interferon, cellular response to type I interferon, and viral response (Figure 5D). Additionally, pathway analysis showed that the JAK-STAT, TLR, and TNFa signaling pathways displayed elevated biological activity, while the Hippo and Wnt pathways showed down-regulation in their activities (Figure 5E).

Lysosome Pathway Enrichment of MX1, IRF7, STAT1, and ISG15

To further explore the functions and pathways associated with the identified biomarkers, GSEA analysis was conducted. The results indicated that MX1 was enriched in pathways such as lysosome, degradation of other glycans, and glutathione metabolism in both CKD and DN (Supplementary Figure 5A and B). IRF7 showed involvement in lysosome-related processes, and was additionally enriched in pathways associated with Vibrio cholerae and Leishmania infections in both CKD and DN (Supplementary Figure 5C and D). STAT1 was linked to lysosome, insulin signaling pathway, Fc gamma R (FcγR)-mediated phagocytosis, and other pathways across CKD and DN (Supplementary Figure 5E and F). Lysosome, systemic lupus erythematosus, glutathione metabolism, and other pathways

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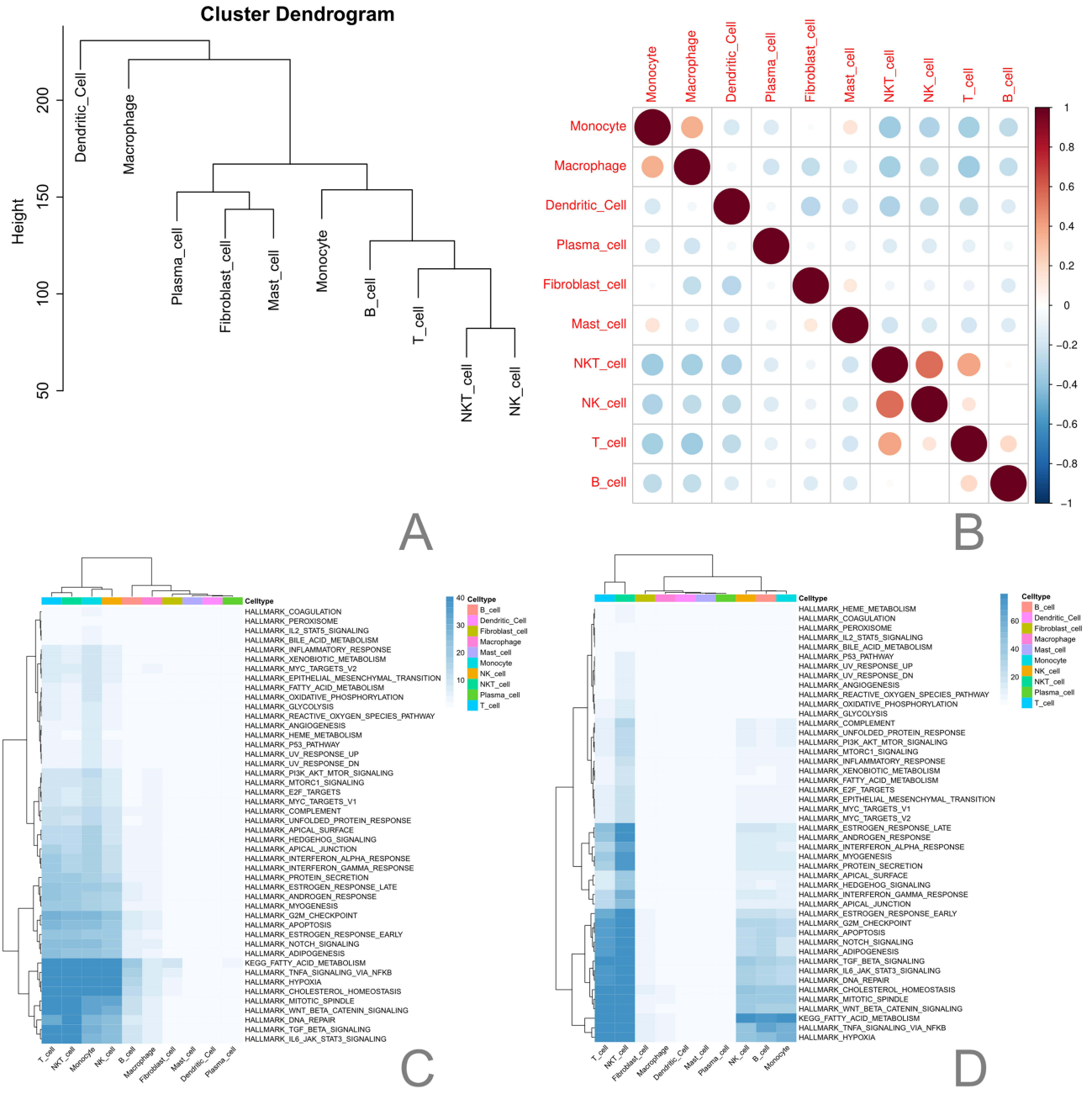

Figure 2 CKD and DN samples enriched in pathways of nine cell types. (**A**) Clustering tree diagram. (**B**) Heatmap showing cell correlation. (**C** and **D**) GSVA analysis of cell subgroups between the control and CKD groups, and the control and DN group groups.
Abbreviations: CKD, Chronic kidney disease; DN, Diabetic nephropathy; GSVA, Gene set variation analysis.

were enriched by ISG15 in both CKD and DN (Supplementary Figure 5G and H). All biomarkers showed enrichment in the lysosome pathway. Moreover, in CKD, MX1, IRF7, STAT1, and ISG15 were all enriched in the oxidative phosphorylation pathway. In DN, they were all enriched in the FcγR mediated phagocytosis pathway. In addition, a biomarker-drug-disease network was constructed, comprising 165 nodes, including the four biomarkers, 151 drugs (eg, acetylcysteine, acrolein, and alpha-pinene), and 10 diseases (eg, diabetes mellitus, diabetes complications, and diabetic angiopathies), with a total of 635 interaction pairs (Supplementary Figure 6).

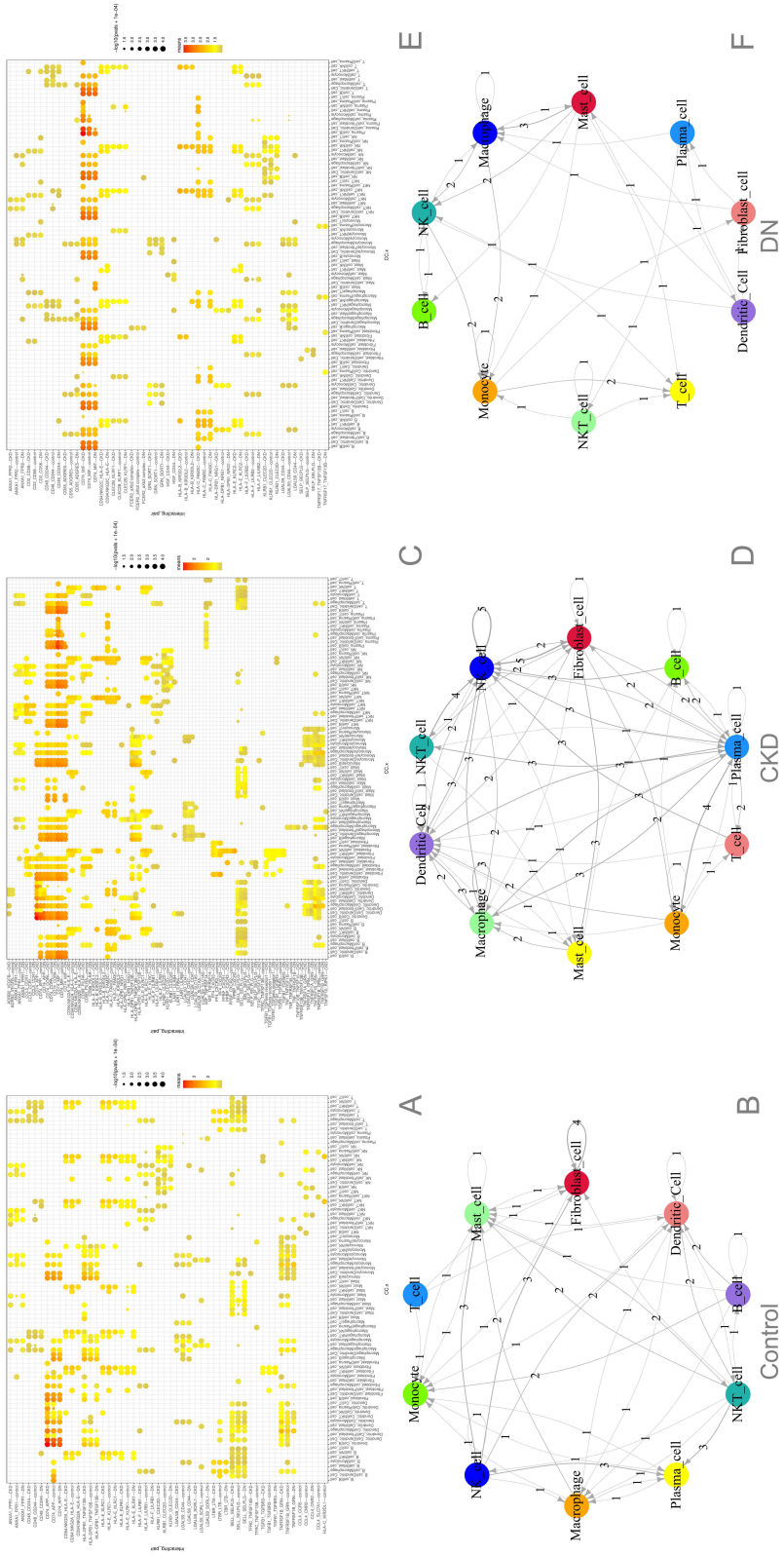

Figure 3 Disrupted and imbalanced cell communication in DN and CKD. (**A, C, E**) Heatmaps displaying the relationship between the selected CKD and DN genes and their corresponding expression pathways in the control group, along with changes in gene expression levels. (**B, D, F**) Cell communication trajectories for control, CKD, and DN samples.
Abbreviations: DN, Diabetic nephropathy; CKD, Chronic kidney disease.

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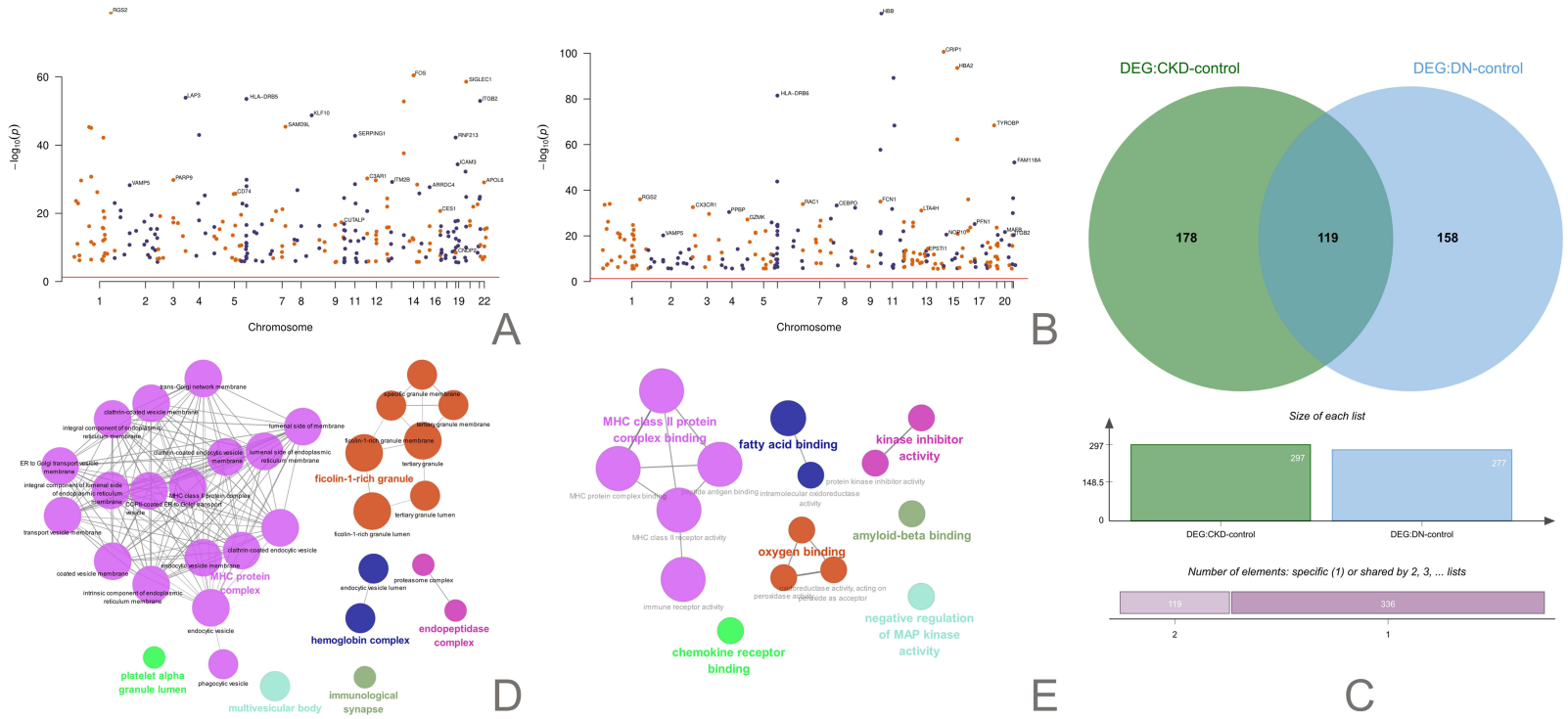

Figure 4 Screening of key genes. (**A** and **B**) Manhattan plots illustrating differentially expressed genes across each chromosome for CKD vs control and DN vs control, respectively (left to right). The y-axis represents -log10(p) values, and the x-axis represents chromosomes, visualizing gene expression across the genome. (**C**) Intersection of candidate genes relevant to both CKD and DN. (**D**) KEGG and GO analysis of candidate genes. (**E**) Bubble plot showing distinct enrichment items, with each node representing a specific biological function. KEGG pathways identified include viral life cycles, viral protein-cytokine receptor interactions, measles, B-cell receptor signaling, African trypanosomiasis, malaria, phagosome, cell adhesion molecules, antigen processing and presentation, and hematopoietic cell lineage.

Abbreviations: DN, Diabetic nephropathy; CKD, Chronic kidney disease; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

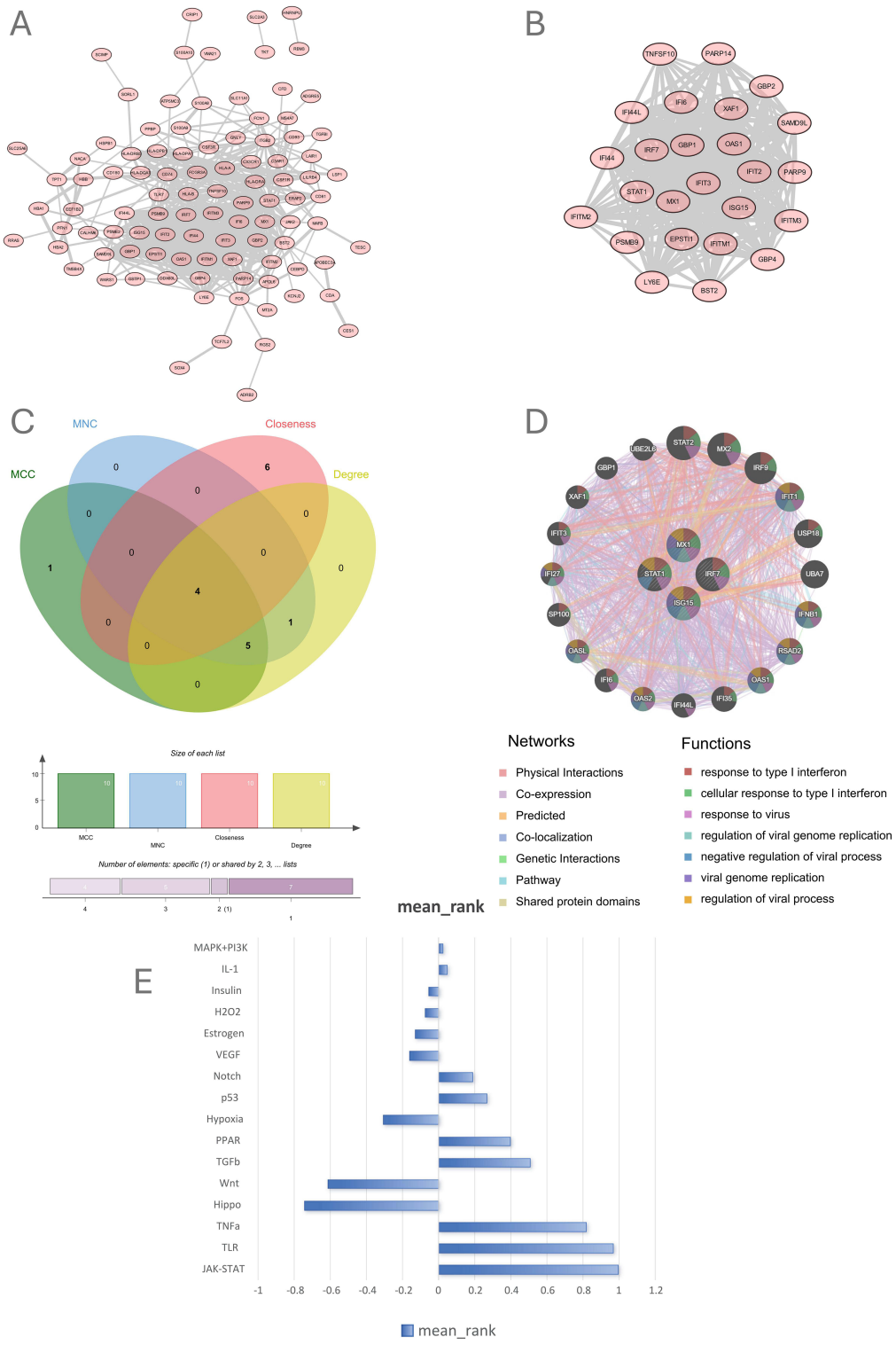

Figure 5 Associations between key genes and biomarkers in the sample. (**A** and **B**) Interaction analysis of 119 candidate genes using a PPI network constructed using STRING (https://string-db.org), with a confidence score of 0.4, identifying 21 discrete proteins and a network comprising 98 interacting proteins. The network contains 98 nodes and 569 edges, visualized using Cytoscape (version 3.10.1). (**C**) Biomarker identification through MCC, MNC, Closeness, and Degree scores using the cytoHubba plugin, examining the expression activity of four biomarkers across 16 major cell communication signaling pathways. (**D**) GeneMANIA network analysis, displaying the four biomarker genes in the inner circle, with the outer circle showing other genes related to them. Each gene color denotes its biological pathway, with a high correlation density indicating essential biological functions and significant interactions with other genes. (**E**) Upstream pathway analysis of the biomarkers.

Abbreviations: PPI, protein-protein interaction; MNC, Maximum Neighborhood Component; MCC, Maximal Clique Centrality; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins.

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Elevated Expression of MX1 and IRF7 in Dendritic Cells

The expression levels of the biomarkers MX1, STAT1, and ISG15 were highest in the neuronal cells of the esophagus muscularis, while expression data for IRF7 was unavailable (Figure 6A). The expression levels of the biomarkers in each cell type across different group samples (control, CKD, DN) are shown in Figure 6B. IRF7 showed high expression in dendritic cells in all samples, and MX1 exhibited elevated expression specifically in dendritic cells, particularly in DN and CKD samples (Figure 6C). In contrast, STAT1 and ISG15 were widely expressed in macrophages, monocytes, NK cells, and NK T cells. Notably, these four biomarkers showed significant differential expression in NK cells, T cells, B cells, and NK T cells (Figure 6D–G). The pseudotime analysis highlighted that the significantly elevated expression of MX1 and IRF7 in dendritic cells is associated with myeloid cells differentiation into dendritic cells (Figure 7A–G).

Discussion

T2DM and CKD are both widespread chronic diseases. The most common microvascular complication of T2DM is DN, which is the leading cause of CKD.[23] Currently, the diagnosis of these two conditions relies on traditional markers such as estimated glomerular filtration rate (eGFR), urinary albumin measurement, and creatinine levels, especially in the absence of renal biopsy.[24] Early diagnosis of CKD and DN is often subjective due to the lack of non-invasive biomarkers.[25] This limitation complicates the design of clinical trials, impeding efforts to identify effective treatments, facilitate early detection, and ensure timely diagnosis. Additionally, reducing cardiovascular mortality and slowing the progression to ESRD remain significant unmet medical needs for patients with CKD and DN.[26]

This study offers new insights into the molecular mechanisms underlying DN and CKD, by identifying common biomarkers and exploring the biological processes involved. Using scRNA-seq technology, we analyzed cell-specific gene expression changes across control, CKD, and DN groups, identifying differentially expressed genes within distinct cell subpopulations. Through further examination of the signaling pathways within these cell clusters, this study provides a theoretical foundation for understanding biomarkers related to the progression of CKD and DN, their roles in immune response, and their potential as therapeutic targets.

In this study, four biomarkers—MX1, IRF7, STAT1, and ISG15—were identified through differential expression analysis and the PPI network analysis, showing notable expression in both DN and CKD samples.

Interferon-stimulated gene 15 (ISG15) is a 15kD ubiquitin-like protein induced by the binding of interferon-α (IFN-α) to the promoters of interferon (IFN) regulatory factor (IRF) and the interferon-stimulated response element. ISG15 has been implicated in several intracellular processes, including autophagy, apoptosis, and signal transduction. As a cytokine, it activates Janus kinase (JAK) and the JAK/STAT signaling pathway, which mediates various physiological and pathological responses, such as cell proliferation, differentiation, apoptosis, and immune regulation.[27] Recent studies indicate that JAK/STAT pathway activation exacerbates renal fibrosis and glomerulosclerosis, while ISG15 overexpression contributes to systemic inflammation and CKD.[28,29] At the same time, ISG15 can modify viral proteins or host proteins, thereby inhibiting viral replication.[30] ISG15 modification can directly interfere with the life cycle of the virus.[31] And the resistance of ISG15-deficient cells to paramyxovirus is reduced, indicating their direct antiviral activity.[32] In oral squamous cell carcinoma, tumor cell-derived ISG15 promotes fibroblast recruitment, promoting tumor growth and metastasis through CD11a-dependent glycolytic reprogramming.[33] In pancreatic and renal clear cell carcinoma, ISG15 levels are elevated and high expression is associated with adverse clinical outcomes.[34,35] ISG15 can also promote tumor cell migration and immunosuppression by inducing the macrophage M2-like phenotype.[36] To sum up, ISG15 has shown a pleiotropic effect in antiviral immunity, tumor progression and metastasis, and tumor microenvironment regulation. Its complex functional mechanism in different pathophysiological processes provides rich and promising research directions for the development of prevention and treatment strategies for related diseases.

This study found that MX1 was specifically highly expressed in DN and CKD samples. The MX1 gene encodes an interferon-induced protein that is involved in the cell's antiviral immune response.[37] In the context of diabetes, persistent hyperglycemia and lipid metabolism disorders may activate MX1, leading to chronic inflammation, which may promote pathological changes in DN.[38] In CKD, MX1 may reduce the inflammatory response caused by viral infection by inhibiting viral replication, thereby producing a protective effect on CKD.[39,40] Additionally, the degree of methylation of

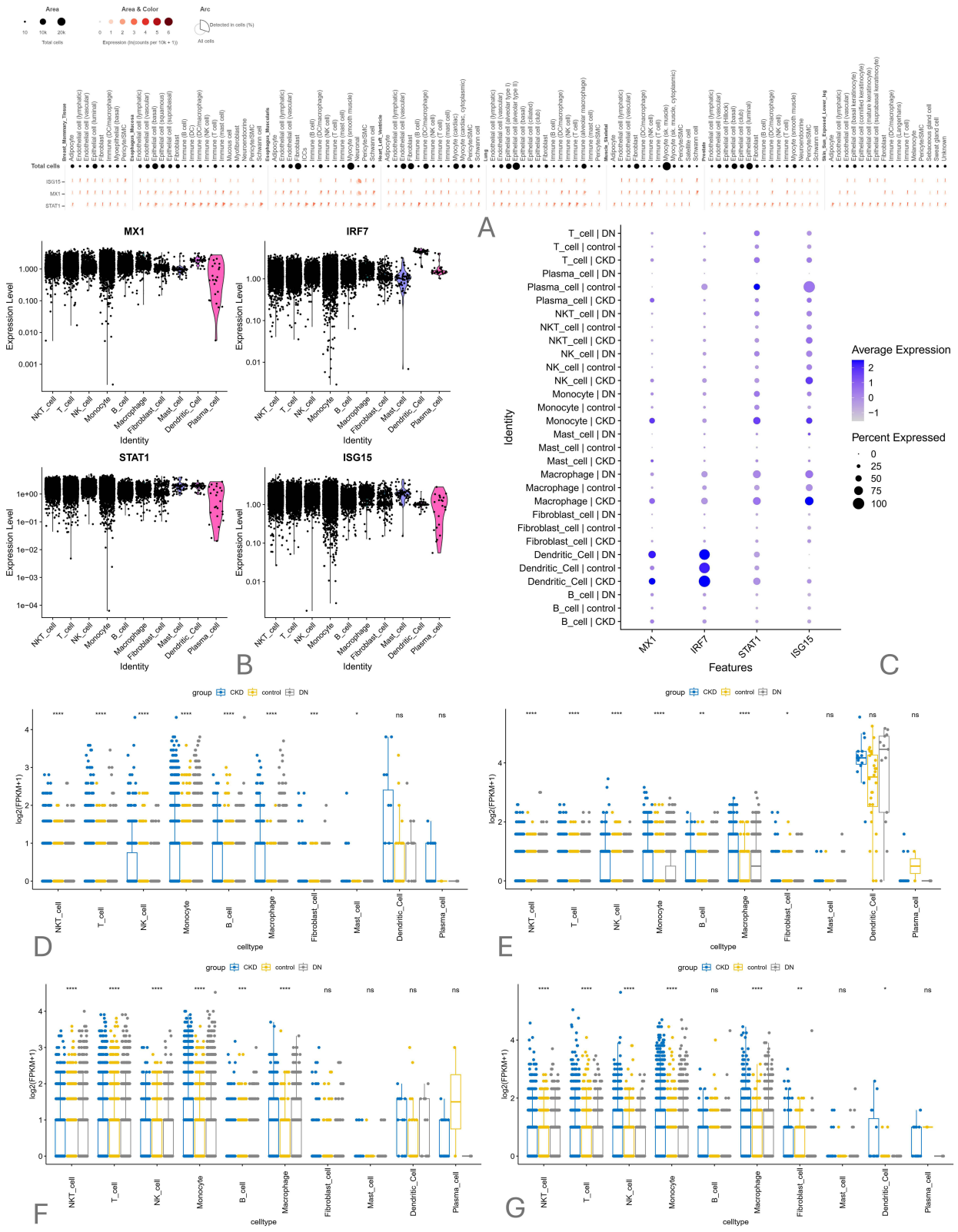

Figure 6 Connection between expression levels of biomarkers and each cell type. (**A**) Expression levels of biomarkers across different tissues and cells. (**B**) Expression levels of biomarkers in individual cell types. (**C**–**G**) Differential expression of the four biomarkers in each cell type, presented in boxplots. These results show significant differences in the expressions of MX1, STAT1, ISG15, and IRF7 in NK cells, T cells, B cells, and NKT cells. *p<0.05, there is evidence of significant difference; **p<0.01; ***p<0.005; ****p<0.001, with strong evidence of significant difference (P value was used as the standard for figure (**D**–**G**) screening of differentially expressed genes).
Abbreviations: ns, no significant difference.

https://doi.org/10.2147/DMSO.S514319

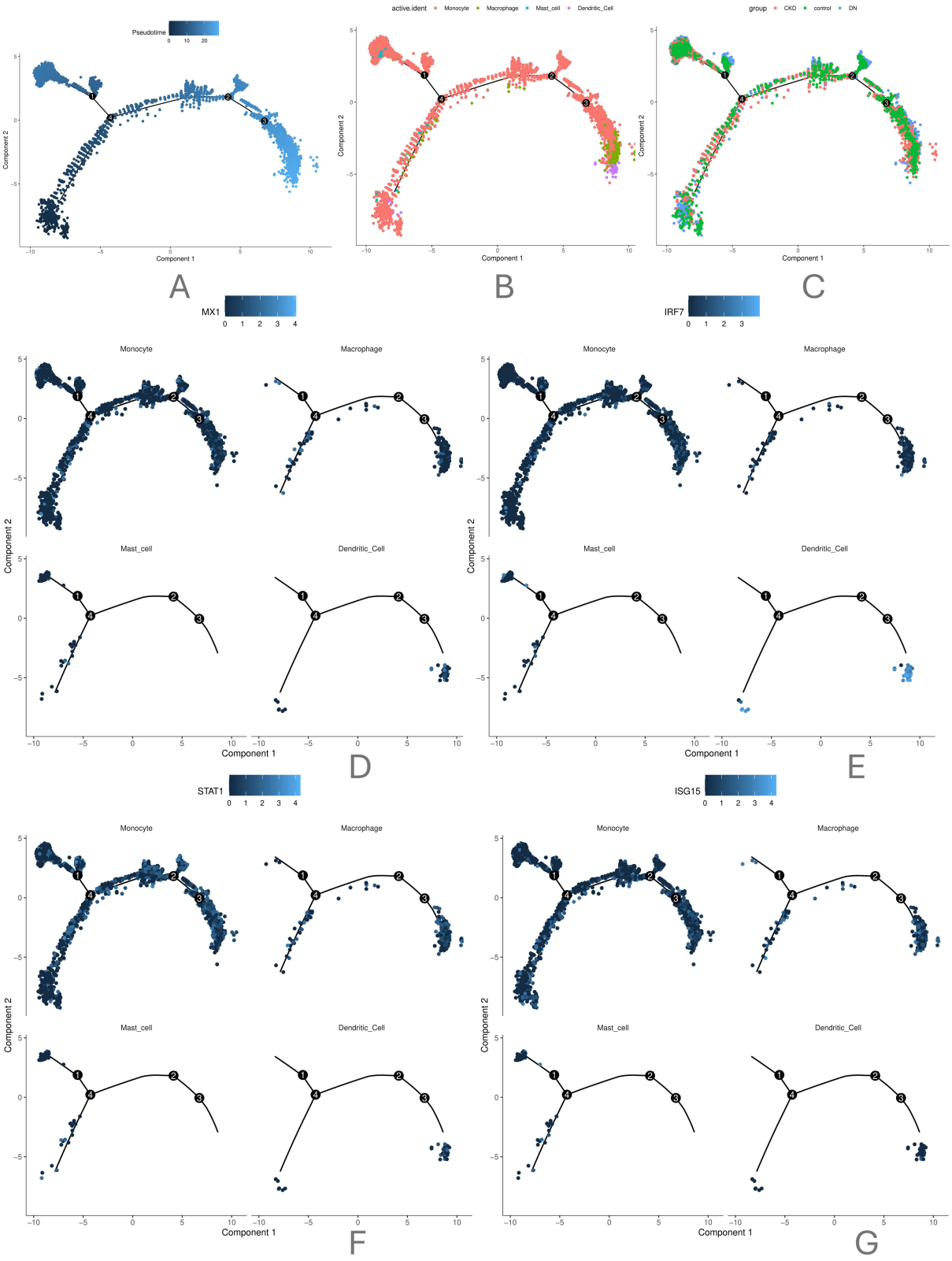

Figure 7 Pseudotime analysis showing the differentiation states of cells. Branch points indicate potential decision points in cellular processes. (**A**–**C**) Each point represents a cell, with cells exhibiting similar cellular states grouped together. Branch points in the pseudotime trajectory indicate potential decision points in the cell's biological process (eg, four branch points in this analysis). Cells are color-labeled according to pseudotime, state, and group. Integrating biomarker expression data, the differentiation states of genes related to disease progression can be identified. (**D**–**G**) Pseudotime analysis of biomarkers. The results reveal that MX1 and IRF7 genes show significantly higher expression levels in dendritic cells, suggesting that MX1 and IRF7 may play a role in the differentiation of myeloid cells into dendritic cells. In contrast, the other two biomarkers (STAT1 and ISG15) did not exhibit significant differences in expression, indicating they may have a lesser impact on this differentiation process.

the MX1 gene promoter is correlated with the severity of COVID-19 and there may be gender differences.[41] This suggests that the expression level of MX1 can be used as an early indicator of viral infection, and its gene polymorphism is also related to the risk of autoimmune disease, and is of great value in the judgment of infection type, individualized treatment and disease risk assessment.

Signal transducer and activator of transcription 1 (STAT1) is a cytoplasmic transcription factor activated by various stimuli, regulating the human immune system.[42] Moreover, STAT1 mediates interferon signaling pathways and plays a crucial role in antiviral (eg, HBV, HCV, HIV) and antibacterial (eg, *Mycobacterium tuberculosis*) immune responses. Its phosphorylation levels reflect the progression of infection.[43–45] Additionally, STAT1 gain-of-function mutations are associated with chronic mucosal skin candidiasis and systemic lupus erythematosus (SLE), while loss-of-function mutations lead to severe immunodeficiency.[46,47] STAT1 exhibits a dual role in cancer: high expression in prostate and breast cancers may indicate a better prognosis, but it may also promote immune escape in some solid tumors.[48] These findings suggest that STAT1 could serve as a monitoring indicator for infectious disease progression, a molecular diagnostic marker for autoimmune diseases, and a potential target for tumor prognosis evaluation. Regulating STAT1 may provide novel strategies for precise treatment of related diseases.

IRF7 (Interferon Regulatory Factor 7) is a key member of the IRF family, playing a pivotal role in innate immunity and antiviral responses.[49,50] Studies have shown that IRF7 expression correlates with disease activity in SLE patients, with elevated mRNA levels positively correlated with serum IFN levels, IFN scores, and SLEDAI scores.[51,52] In acute myeloid leukemia, inhibiting TOX exerts anti-tumor effects by upregulating IRF7 expression.[53] Furthermore, IRF7 mediates the transcription of MCP-1, an obesity-related molecule.[54] These findings indicate that changes in IRF7 expression are linked to disease development and may serve as a potential biomarker for diagnosis.

MX1, IRF7, STAT1, and ISG15 are core regulatory molecules in the type I interferon (IFN-α/β) response pathway, interacting with each other in complex ways. STAT1, as a central signal node, is phosphorylated by JAK kinase under IFN-γ or IFN-α/β stimulation. This results in the formation of a dimer that translocates to the nucleus and directly activates IRF7 transcription.[55] IRF7, in turn, amplifies IFN-α/β production, creating a positive feedback loop, and induces MX1 and ISG15 expression.[56] MX1, an antiviral effector protein, relies on the STAT1-IRF7 axis, while ISG15 stabilizes STAT1 and IRF7 proteins via ubiquitination (ISGylation) and enhances their transcriptional activity.[57] Additionally, ISG15 regulates MX1 oligomerization through non-covalent binding, impacting its antiviral function.[58] In CKD and DN, these complex interactions may lead to the oversecretion of pro-inflammatory factors (eg, TNF-α, IL-6) and dysregulated cytotoxic immune responses, accelerating tissue damage.

Furthermore, exploring the relationship between MX1, IRF7, STAT1, and ISG15 and traditional renal function markers (eg, serum creatinine, urea nitrogen, and urine protein) is valuable. Serum creatinine levels are influenced by muscle metabolism and glomerular filtration capacity,[59] urea nitrogen reflects protein metabolism and renal excretion function,[60] and urinary protein indicates impaired glomerular filtration barrier.[61] In contrast, MX1, IRF7, STAT1, and ISG15 are more involved in immunomodulation and inflammatory response pathways.[62,63] In kidney disease progression, local inflammation in the kidney and immune cell activation[64,65] can alter the expression of these biomarkers. In early CKD, the immune-inflammatory response begins subtly, and the expression of MX1, IRF7, etc., may change before traditional markers like serum creatinine or urea nitrogen significantly deviate. At this stage, urine protein may also remain at critical levels.[66–69] This suggests that biomarkers such as MX1 could serve as early warning indicators, complementing traditional markers. As the disease progresses and traditional markers become more abnormal, these novel biomarkers may increase, providing a more comprehensive basis for disease assessment.

GSEA results indicated that all four biomarkers were enriched in the Oxidative Phosphorylation pathway in CKD, while in DN, they were enriched in the FcγR-mediated Phagocytosis pathway. Kidney cells typically rely on Oxidative Phosphorylation to maintain physiological functions like reabsorption and secretion, processes requiring substantial energy.[70] However, in CKD, kidney cell metabolic pathways are altered, and their dependence on Oxidative Phosphorylation is enhanced.[71] Oxidative Phosphorylation is a major source of intracellular reactive oxygen species (ROS). In CKD, metabolic disturbances and inflammatory responses may overactivate this pathway, resulting in excessive ROS production.[71] Excessive ROS can damage cellular proteins, lipids, and DNA, triggering oxidative stress and accelerating renal fibrosis and functional decline.[72] FcγR-mediated phagocytosis is crucial for immune function,

facilitating the uptake and clearance of phagocytes (eg, macrophages, neutrophils) that recognize target cells or granules bound to antibodies.[73] In early DN, immune complexes may accumulate in renal tissues, activating the complement system and inflammatory responses, thereby exacerbating kidney damage.[74] If phagocytosis fails to clear target cells completely, residual antigens may continue to stimulate the immune response, leading to chronic inflammation and renal fibrosis.[75] These findings highlight the differences in enriched pathways between CKD and DN, shedding light on the distinct pathogenesis and progression of each disease. This insight is crucial for understanding disease mechanisms and developing targeted therapeutic strategies.

This study found that IRF7 expression was elevated in dendritic cells (DCs). In CKD and DN, immune cells like DCs are continuously activated during disease progression.[76] Plasmacytoid DCs (pDCs) are the primary producers of IFN-I,[77] and in pDCs, IRF7 expression is regulated by NFATC3, which enhances IFN production.[78] Upon stimulation by TLR7 or TLR9, IRF7 is activated, promoting IFN-α secretion.[79] High IRF7 expression in DCs strengthens their antiviral immune response,[50,80] enhances antigen presentation, and regulates the Th1 immune response and inflammatory factor release.[81–84] In CKD and DN, abnormal IRF7 expression may contribute to disease progression via two mechanisms: excessive activation can cause persistent inflammation, macrophage infiltration, and proinflammatory factor release (eg, TNF-α, IL-6), exacerbating fibrosis;[38,85,86] additionally, IRF7 may worsen podocyte damage and glomerular basement membrane thickening under metabolic stress (eg, high sugar or glycosylation end product stimulation).[87] Notably, the high-sugar environment in DN amplifies IRF7-mediated inflammation, creating a vicious "metabolic-inflammatory" cycle.[88] Thus, understanding IRF7's role in DCs provides key insights into the pathogenesis of CKD and DN, offering future directions for developing targeted therapies to block disease progression.[74–77]

Our study also highlights that STAT1 and ISG15 are widely expressed in macrophages, monocytes, NK cells, and NKT cells. In macrophages, IFN-γ stimulates the phosphorylation of STAT1 by JAK1/JAK2, promoting STAT1 dimerization, nuclear translocation, and the expression of pro-inflammatory genes such as IRF1 and CXCL10, enhancing M1 polarization and antibacterial function.[89,90] ISG15 regulates cytokine expression (eg, TNF-α, IL-6) by activating the JAK-STAT pathway, modulating immune response intensity.[91] In monocytes, STAT1 enhances the inflammatory response via the TLR/MyD88 pathway,[92] while ISG15 may regulate cell migration and phagocytosis through NF-κB signaling.[58,93] For NK and NKT cells, STAT1 mediates the IFN-γ feedback loop, promoting granzyme and perforin expression,[94,95] while ISG15 supports IFN-γ production and cytotoxic function by stabilizing STAT1/STAT4.[56,96] Free ISG15 also enhances NK cell cytotoxicity via LFA-1 receptors.[57] Importantly, STAT1 can compete with STAT3 for DNA binding to regulate immune balance, but over-activation may lead to chronic inflammation and is associated with autoimmune and metabolic diseases like diabetic nephropathy.[55,97] These findings underscore the core role of STAT1 and ISG15 in the innate immune system, offering new insights into immune cell activation and laying the foundation for targeted therapies in inflammatory and metabolic diseases.[90]

Furthermore, sodium-glucose cotransporter 2 inhibitors have been shown to interfere with the polarization of DCs by reducing receptor pairing between M2 macrophages and T cells. In this study, cell communication analysis of DN and CKD groups revealed that B cells, NK cells, T cells, and monocytes exhibited the closest interactions. The four biomarkers, MX1, IRF7, STAT1, and ISG15, were widely expressed in these cell populations. These findings suggest that these biomarkers play central roles in the immune response and the progression of CKD and DN. These biomarkers could serve as valuable targets for predicting disease progression.

However, this study has certain limitations. First, single-cell sequencing technology presents challenges such as high costs and time consumption, making it difficult to fully assess the accuracy of these markers in disease evaluation. More importantly, the specific functional mechanisms, clinical translational potential, and diagnostic value of these biomarkers require systematic validation in independent cohorts. Additionally, the dynamic changes in relevant signaling pathways during CKD and DN progression, their correlation with disease stage and severity, and the clinical application value of these markers still need further investigation. To address these limitations, future research will focus on enhancing the clinical applicability of single-cell sequencing, including cost reduction, time efficiency, and improved accuracy and reliability. Interdisciplinary collaboration will be promoted to facilitate its clinical use. We plan to explore the impact of biomarkers on disease-related cell behavior and physiological processes through cell culture and animal models (eg, PDO, PDX). This will involve verifying whether biomarker regulation can reverse disease phenotypes and elucidate

underlying mechanisms. Concurrently, large-scale, multicenter clinical samples will be collected to investigate the MX1/IRF7/STAT1/ISG15 pathway using gene-editing technologies. Furthermore, we will compare biomarker expression across CKD, DN, and other renal diseases to assess specificity. In terms of clinical translation, we will collaborate with the Clinical Center for Nephrology to examine the correlation between biomarkers, disease staging, and treatment response. Clinical measurement techniques such as ELISA, mass spectrometry, and immunohistochemistry will be used to correlate biomarker expression with clinical data. Samples from different geographic regions will be collected to assess the generalizability of our findings.

Conclusion

In this study, through single-cell RNA sequencing and the application of a series of bioinformatics methods, four biomarkers (MX1, IRF7, STAT1, ISG15) in CKD and DN were identified. During the clinical diagnosis process, detecting the expression levels of biomarkers in patients may serve as a means of auxiliary diagnosis for CKD and DN, and also as an important basis for predicting disease progression. Meanwhile, in the clinical treatment of CKD and DN, these biomarkers can be considered as therapeutic targets.

Data Sharing Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of The Second Hospital Affiliated to Kunming Medical University (PJ-2021-36). This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

Acknowledgments

We would like to acknowledge the hard and dedicated work of all the staff that implemented the intervention and evaluation components of the study.

Funding

Yunnan Revitalization Talent Support Program (Youth talent project: NO.YNWR-QNBJ-2020-269) and (Famous doctors project: NO.YNWR-MY-2019-075). Reserve Talents Project for Young and Middle-aged Academic and Technical leaders in Yunnan Province (202005AC160024). Yunnan Fundamental Research Kunming Medical University Joint Projects (grant NO. 202201AY070001-101). National Clinical Research Center of Chronic Kidney Disease, the Second Affiliated Hospital of Kunming Medical University.(Project Number: GF2020003). The Second Affiliated Hospital of Kunming Medical University talent echelon cultivation project-Academic leader (RCTDXS-202303).

Disclosure

The authors declare that they have no competing interests.

References

1. Ammirati AL. Chronic kidney disease. *Rev Assoc Med Bras*. 2020;66Suppl 1(Suppl 1):s03–s09. PMID: 31939529. doi:10.1590/1806-9282.66.S1.3
2. McGrath K, Edi R. Diabetic kidney disease: diagnosis, treatment, and prevention. *Am Fam Physician*. 2019;99(12):751–759.
3. Jun Z, Xiaoying L, Min L. Analysis of correlation between heart rate variability and vascular endothelial injury and microinflammation in patients with chronic kidney disease. *Chinese Medical Engineerin*. 2024;32(05):85–88. doi:10.19338/j.issn.1672-2019.2024.05.019
4. Stevens PE, Levin A; Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med*. 2013;158(11):825–830. doi:10.7326/0003-4819-158-11-201306040-00007
5. Yamanouchi M, Furuichi K, Hoshino J, Ubara Y, Wada T. Nonproteinuric diabetic kidney disease. *Clin Exp Nephrol*. 2020;24(7):573–581. doi:10.1007/s10157-020-01881-0
6. Chen TK, Hoenig MP, Nitsch D, et al. Advances in the management of chronic kidney disease. *BMJ*. 2023;383:e074216. doi:10.1136/bmj-2022-074216

7. Hou JH, Zhu HX, Zhou ML, et al. Changes in the spectrum of kidney diseases: an analysis of 40,759 biopsy-proven cases from 2003 to 2014 in China. *Kidney Dis*. 2018;4(1):10–19. doi:10.1159/000484717
8. Komici K, Femminella GD, de Lucia C, et al. Predisposing factors to heart failure in diabetic nephropathy: a look at the sympathetic nervous system hyperactivity. *Aging Clin Exp Res*. 2019;31(3):321–330. doi:10.1007/s40520-018-0973-2
9. Berest I, Tangherloni A. Integration of scATAC-Seq with scRNA-Seq data. *Methods Mol Biol*. 2023;2584:293–310. doi:10.1007/978-1-0716-2756-3_15
10. Macosko EZ, Basu A, Satija R, et al. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell*. 2015;161(5):1202–1214. doi:10.1016/j.cell.2015.05.002
11. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO 2022 clinical practice guideline for diabetes management in chronic kidney disease. *Kidney Int*. 2022;102(5S):S1–S127. doi:10.1016/j.kint.2022.06.008
12. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO 2020 clinical practice guideline for diabetes management in chronic kidney disease. *Kidney Int*. 2020;98(4S):S1–S115. doi:10.1016/j.kint.2020.06.019
13. Liao Y, Raghu D, Pal B, Mielke LA, Shi W. cellCounts: an R function for quantifying 10x Chromium single-cell RNA sequencing data. *Bioinformatics*. 2023;39(7):btad439. doi:10.1093/bioinformatics/btad439
14. Hao Y, Hao S, Andersen-Nissen E, et al. Integrated analysis of multimodal single-cell data. *Cell*. 2021;184(13):3573–3587.e29. doi:10.1016/j.cell.2021.04.048
15. Germain PL, Lun A, Garcia Meixide C, Macnair W, Robinson MD. Doublet identification in single-cell sequencing data using *scDblFinder*. *F1000Res*. 2021;10:979. doi:10.12688/f1000research.73600.1
16. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation*. 2021;2(3):100141. doi:10.1016/j.xinn.2021.100141
17. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinf*. 2013;14(1):7. doi:10.1186/1471-2105-14-7
18. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. doi:10.1093/nar/gkv007
19. Conway BR, O'Sullivan ED, Cairns C, et al. Kidney single-cell atlas reveals myeloid heterogeneity in progression and regression of kidney disease. *J Am Soc Nephrol*. 2020;31(12):2833–2854. doi:10.1681/ASN.2020060806
20. Bell RMB, Denby L. Myeloid heterogeneity in kidney disease as revealed through single-cell RNA sequencing. *Kidney360*. 2021;2(11):1844–1851. doi:10.34067/KID.0003682021
21. Qiu X, Mao Q, Tang Y, et al. Reversed graph embedding resolves complex single-cell trajectories. *Nat Methods*. 2017;14(10):979–982. doi:10.1038/nmeth.4402
22. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498–2504. doi:10.1101/gr.1239303
23. Rayego-Mateos S, Rodrigues-Diez RR, Fernandez-Fernandez B, et al. Targeting inflammation to treat diabetic kidney disease: the road to 2030. *Kidney Int*. 2023;103(2):282–296. doi:10.1016/j.kint.2022.10.030
24. Aldrich S, Ashjian E. Use of GLP-1 receptor agonists in patients with T2DM and chronic kidney disease. *Nurse Pract*. 2019;44(3):20–28. doi:10.1097/01.NPR.0000553396.65976.bb
25. Anders HJ, Huber TB, Isermann B, Schiffer M. CKD in diabetes: diabetic kidney disease versus nondiabetic kidney disease. *Nat Rev Nephrol*. 2018;14(6):361–377. doi:10.1038/s41581-018-0001-y
26. Doshi SM, Friedman AN. Diagnosis and management of type 2 diabetic kidney disease. *Clin J Am Soc Nephrol*. 2017;12(8):1366–1373. doi:10.2215/CJN.11111016
27. Mirzalieva O, Juncker M, Schwartzenburg J, Desai S. ISG15 and ISGylation in human diseases. *Cells*. 2022;11(3):538. doi:10.3390/cells11030538
28. Jia J, Xu LH, Deng C, et al. Hederagenin ameliorates renal fibrosis in chronic kidney disease through blocking ISG15 regulated JAK/STAT signaling. *Int Immunopharmacol*. 2023;118:110122. doi:10.1016/j.intimp.2023.110122
29. He T, Xia Y, Yang J. Systemic inflammation and chronic kidney disease in a patient due to the RNASEH2B defect. *Pediatr Rheumatol Online J*. 2021;19(1):9. doi:10.1186/s12969-021-00497-2
30. Kespohl M, Bredow C, Klingel K, et al. Protein modification with ISG15 blocks coxsackievirus pathology by antiviral and metabolic reprogramming. *Sci Adv*. 2020;6(11):eaay1109. doi:10.1126/sciadv.aay1109
31. Freitas BT, Scholte FEM, Bergeron É, Pegan SD. How ISG15 combats viral infection. *Virus Res*. 2020;286:198036. doi:10.1016/j.virusres.2020.198036
32. Holthaus D, Vasou A, Bamford CGG, et al. Direct antiviral activity of IFN-stimulated genes is responsible for resistance to paramyxoviruses in ISG15-deficient cells. *J Immunol*. 2020;205(1):261–271. doi:10.4049/jimmunol.1901472
33. Wang SH, Chen YL, Huang SH, et al. Tumor cell-derived ISG15 promotes fibroblast recruitment in oral squamous cell carcinoma via CD11a-dependent glycolytic reprogramming. *Oncogenesis*. 2025;14(1):6. doi:10.1038/s41389-025-00549-2
34. Meng Y, Bian L, Zhang M, et al. ISG15 promotes progression and gemcitabine resistance of pancreatic cancer cells through ATG7. *Int J Biol Sci*. 2024;20(4):1180–1193. doi:10.7150/ijbs.85424
35. Xie W, Zhang Y, Zhang Z, Li Q, Tao L, Zhang R. ISG15 promotes tumor progression via IL6/JAK2/STAT3 signaling pathway in ccRCC. *Clin Exp Med*. 2024;24(1):140. doi:10.1007/s10238-024-01414-z
36. Chen RH, Xiao ZW, Yan XQ, et al. Tumor cell-secreted ISG15 promotes tumor cell migration and immune suppression by inducing the macrophage M2-like phenotype. *Front Immunol*. 2020;11:594775. doi:10.3389/fimmu.2020.594775
37. Wang G, Hua R, Chen X, et al. MX1 and UBE2L6 are potential metaflammation gene targets in both diabetes and atherosclerosis. *PeerJ*. 2024;12:e16975. doi:10.7717/peerj.16975
38. Matoba K, Takeda Y, Nagai Y, Kawanami D, Utsunomiya K, Nishimura R. Unraveling the role of inflammation in the pathogenesis of diabetic kidney disease. *Int J Mol Sci*. 2019;20(14):3393. doi:10.3390/ijms20143393
39. Salomon R, Staeheli P, Kochs G, et al. Mx1 gene protects mice against the highly lethal human H5N1 influenza virus. *Cell Cycle*. 2007;6(19):2417–2421. doi:10.4161/cc.6.19.4779
40. Jakhotia S, Kavvuri R, Raviraj S, Baishya S, Pasupulati AK, Reddy GB. Obesity-related glomerulopathy is associated with elevated WT1 expression in podocytes. *Int J Obes*. 2024;48(8):1080–1091. doi:10.1038/s41366-024-01509-3

41. Ghoreshi ZA, Abbasi-Jorjandi M, Asadikaram G, Sharifzak M, Rezazadeh-Jabalbarzi M, Rashidinejad H. Evaluation of MX1 gene promoter methylation in different severities of COVID-19 considering patient gender. *Clin Lab*. 2022;68(10). doi:10.7754/Clin.Lab.2022.220104
42. Spitaels J, Van Hoecke L, Roose K, Kochs G, Saelens X. Mx1 in hematopoietic cells protects against thogoto virus infection. *J Virol*. 2019;93(15): e00193–19. doi:10.1128/JVI.00193-19
43. Jung SR, Ashhurst TM, West PK, et al. Contribution of STAT1 to innate and adaptive immunity during type I interferon-mediated lethal virus infection. *PLoS Pathog*. 2020;16(4):e1008525. doi:10.1371/journal.ppat.1008525
44. Lei WT, Lo YF, Tsumura M, et al. Immunophenotyping and therapeutic insights from chronic mucocutaneous candidiasis cases with STAT1 gain-of-function mutations. *J Clin Immunol*. 2024;44(8):184. doi:10.1007/s10875-024-01776-9
45. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. *Nat Rev Immunol*. 2014;14(1):36–49. PMID: 24362405. doi:10.1038/nri3581
46. Kristensen IA, Veirum JE, Møller BK, Christiansen M. Novel STAT1 alleles in a patient with impaired resistance to mycobacteria. *J Clin Immunol*. 2011;31(2):265–271. PMID: 21057861. doi:10.1007/s10875-010-9480-8
47. Uzel G, Sampaio EP, Lawrence MG, et al. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J Allergy Clin Immunol*. 2013;131(6):1611–1623. doi:10.1016/j.jaci.2012.11.054
48. Khodarev NN, Beckett M, Labay E, Darga T, Roizman B, Weichselbaum RR. STAT1 is overexpressed in tumors selected for radioresistance and confers protection from radiation in transduced sensitive cells. *Proc Natl Acad Sci U S A*. 2004;101(6):1714–1719. doi:10.1073/pnas.0308102100
49. Qing F, Liu Z. Interferon regulatory factor 7 in inflammation, cancer and infection. *Front Immunol*. 2023;14:1190841. doi:10.3389/fimmu.2023.1190841
50. Ma W, Huang G, Wang Z, Wang L, Gao Q. IRF7: role and regulation in immunity and autoimmunity. *Front Immunol*. 2023;14:1236923. doi:10.3389/fimmu.2023.1236923
51. Renaudineau Y, Charras A, Natoli V, et al. UK jSLE cohort study. Type I interferon associated epistasis may contribute to early disease-onset and high disease activity in juvenile-onset lupus. *Clin Immunol*. 2024;262:110194. doi:10.1016/j.clim.2024.110194
52. Xu WD, Zhang YJ, Xu K, et al. IRF7, a functional factor associates with systemic lupus erythematosus. *Cytokine*. 2012;58(3):317–320. PMID: 22455868. doi:10.1016/j.cyto.2012.03.003
53. Huang S, Chen Z, Zhong S, et al. Inhibition of TOX exerts anti-tumor effects in acute myeloid leukemia by upregulating IRF7 expression. *Eur J Pharmacol*. 2025;987:177163. doi:10.1016/j.ejphar.2024.177163
54. Kuroda M, Nishiguchi M, Ugawa N, et al. Interferon regulatory factor 7 mediates obesity-associated MCP-1 transcription. *PLoS One*. 2020;15(5): e0233390. doi:10.1371/journal.pone.0233390
55. Seffens A, Herrera A, Tegla C, et al. STAT3 dysregulation in mature T and NK cell lymphomas. *Cancers*. 2019;11(11):1711. doi:10.3390/cancers11111711
56. Swaim CD, Canadeo LA, Monte KJ, Khanna S, Lenschow DJ, Huibregtse JM. Modulation of extracellular ISG15 signaling by pathogens and viral effector proteins. *Cell Rep*. 2020;31(11):107772. doi:10.1016/j.celrep.2020.107772
57. Swaim CD, Scott AF, Canadeo LA, Huibregtse JM. Extracellular ISG15 signals cytokine secretion through the LFA-1 integrin receptor. *Mol Cell*. 2017;68(3):581–590.e5. doi:10.1016/j.molcel.2017.10.003
58. Nowak K, Jabłońska E, Ratajczak-Wrona W. NF-κB-an important player in xenoestrogen signaling in immune cells. *Cells*. 2021;10(7):1799. doi:10.3390/cells10071799
59. Butt B, Ghulam B, Bashir Z, et al. Enhanced creatinine level in diabetic patients maximizing the possibilities of nephropathy and its association with blood urea nitrogen and glomerular filtration rate. *Cureus*. 2024;16(9):e70482. doi:10.7759/cureus.70482
60. Ahmad S, Khan MA, Ali R. Blood urea nitrogen (BUN) levels in renal failure: unraveling the complex interplay of protein metabolism and kidney health. *Professional Med J*. 2024;31(3):7908. doi:10.29309/TPMJ/2024.31.03.7908
61. Lee SW, Baek SH, Paik JH, et al. Tubular B7-1 expression parallels proteinuria levels, but not clinical outcomes in adult minimal change disease patients. *Sci Rep*. 2017;7:41859. PMID: 28150736; PMCID: PMC5288792. doi:10.1038/srep41859
62. Cui N, Liu C, Tang X, et al. ISG15 accelerates acute kidney injury and the subsequent AKI-to-CKD transition by promoting TGFβR1 ISGylation. *Theranostics*. 2024;14(11):4536–4553. doi:10.7150/thno.95796
63. Da G, Wang J, Shang J, et al. Nuclear PCGF3 inhibits the antiviral immune response by suppressing the interferon-stimulated gene. *Cell Death Discov*. 2024;10(1):429. doi:10.1038/s41420-024-02194-x
64. Cantero-Navarro E, Rayego-Mateos S, Orejudo M, et al. Role of macrophages and related cytokines in kidney disease. *Front Med*. 2021;8:688060. doi:10.3389/fmed.2021.688060
65. Imig JD, Ryan MJ. Immune and inflammatory role in renal disease. *Compr Physiol*. 2013;3(2):957–976. doi:10.1002/cphy.c120028
66. Shimizu Y, Yasuda S, Kimura T, et al. Interferon-inducible Mx1 protein is highly expressed in renal tissues from treatment-naïve lupus nephritis, but not in those under immunosuppressive treatment. *Mod Rheumatol*. 2018;28(4):661–669. doi:10.1080/14397595.2017.1404711
67. Smith JD, Doe JM. The impact of dialysis on patient quality of life. *Open J Nephrol*. 2021;11(3):31. doi:10.4236/ojneph.2021.113031
68. Fu Y, Xiang Y, Wang Y, et al. The STAT1/HMGB1/NF-κB pathway in chronic inflammation and kidney injury after cisplatin exposure. *Theranostics*. 2023;13(9):2757–2773. PMID: 37284446; PMCID: PMC10240827. doi:10.7150/thno.81406
69. Zheng C, Shang F, Cheng R, Bai Y. STAT1 aggravates kidney injury by NOD-like receptor (NLRP3) signaling in MRL-lpr mice. *J Mol Histol*. 2024;55(4):555–566. PMID: 38856930. doi:10.1007/s10735-024-10208-2
70. Johnson AR, Smith LK. Advances in mitochondrial dynamics and cellular energy regulation. *Mitochondrial Commun*. 2024;3(3):Article100234. doi:10.1016/j.mitoco.2024.03.002
71. Shi J, Yang Y, Wang YN, et al. Oxidative phosphorylation promotes vascular calcification in chronic kidney disease. *Cell Death Dis*. 2022;13 (3):229. doi:10.1038/s41419-022-04679-y
72. Mapuskar KA, Vasquez-Martinez G, Mayoral-Andrade G, Tomanek-Chalkley A, Zepeda-Orozco D, Allen BG. Mitochondrial oxidative metabolism: an emerging therapeutic target to improve CKD outcomes. *Biomedicines*. 2023;11(6):1573. doi:10.3390/biomedicines11061573
73. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubiña P, Lahera V, Luño J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int Suppl*. 2008;111:S4–9. doi:10.1038/ki.2008.516
74. Acharya D, Li XRL, Heineman RE, Harrison RE. Complement receptor-mediated phagocytosis induces proinflammatory cytokine production in murine macrophages. *Front Immunol*. 2020;10:3049. doi:10.3389/fimmu.2019.03049

75. Restrepo BI, Twahirwa M, Rahbar MH, Schlesinger LS. Phagocytosis via complement or Fc-gamma receptors is compromised in monocytes from type 2 diabetes patients with chronic hyperglycemia. *PLoS One*. 2014;9(3):e92977. doi:10.1371/journal.pone.0092977
76. Ferracini M, Martins JO, Campos MR, Anger DB, Jancar S. Impaired phagocytosis by alveolar macrophages from diabetic rats is related to the deficient coupling of LTs to the Fc gamma R signaling cascade. *Mol Immunol*. 2010;47(11–12):1974–1980. doi:10.1016/j.molimm.2010.04.018
77. Jia Y, Xu H, Yu Q, Tan L, Xiong Z. Identification and verification of vascular cell adhesion protein 1 as an immune-related hub gene associated with the tubulointerstitial injury in diabetic kidney disease. *Bioengineered*. 2021;12(1):6655–6673. doi:10.1080/21655979.2021.1976540
78. Wang M, Zhang Y, Zhai Y, Li H, Xie Z, Wen C. The mechanism of Langchuangding in treatment of systemic lupus erythematosus via modulating TLR7-IRF7-IFNα pathway. *Heliyon*. 2024;10(5):e26022. doi:10.1016/j.heliyon.2024.e26022
79. Bao M, Wang Y, Liu Y, et al. NFATC3 promotes IRF7 transcriptional activity in plasmacy–toid dendritic cells. *J Exp Med*. 2016;213(11):2383–2398. doi:10.1084/jem.20160438
80. Di Domizio J, Cao W. Fueling autoimmunity: type I interferon in autoimmune diseases. *Expert Rev Clin Immunol*. 2013;9(3):201–210. doi:10.1586/eci.12.106
81. Owens BM, Moore JW, Kaye PM. IRF7 regulates TLR2-mediated activation of splenic CD11c(hi) dendritic cells. *PLoS One*. 2012;7(7):e41050. doi:10.1371/journal.pone.0041050
82. Kumar S, Jeong Y, Ashraf MU, Bae YS. Dendritic cell-mediated Th2 immunity and immune disorders. *Int J Mol Sci*. 2019;20(9):2159. doi:10.3390/ijms20092159
83. Di Sabatino A, Pickard KM, Gordon JN, et al. Evidence for the role of interferon-alfa production by dendritic cells in the Th1 response in celiac disease. *Gastroenterology*. 2007;133(4):1175–1187. doi:10.1053/j.gastro.2007.08.018
84. Terhune J, Berk E, Czerniecki BJ. Dendritic cell-induced Th1 and Th17 cell differentiation for cancer therapy. *Vaccines*. 2013;1(4):527–549. doi:10.3390/vaccines1040527
85. Hung PH, Hsu YC, Chen TH, Lin CL. Recent advances in diabetic kidney diseases: from kidney injury to kidney fibrosis. *Int J Mol Sci*. 2021;22(21):11857. doi:10.3390/ijms222111857
86. Pichler R, Afkarian M, Dieter BP, Tuttle KR. Immunity and inflammation in diabetic kidney disease: translating mechanisms to biomarkers and treatment targets. *Am J Physiol Renal Physiol*. 2017;312(4):F716–F731. doi:10.1152/ajprenal.00314.2016
87. Zhan X, Yan C, Chen Y, et al. Celastrol antagonizes high glucose-evoked podocyte injury, inflammation and insulin resistance by restoring the HO-1-mediated autophagy pathway. *Mol Immunol*. 2018;104:61–68. doi:10.1016/j.molimm.2018.10.021
88. Hanouneh M, Echouffo Tcheugui JB, Jaar BG. Recent advances in diabetic kidney disease. *BMC Med*. 2021;19(1):180. doi:10.1186/s12916-021-02050-0
89. Bellucci R, Martin A, Buren M, Nguyen H-N, Bommarito D, Ritz J. JAK1 and JAK2 modulate myeloma cell susceptibility to NK cells through the interferon gamma (IFN-γ) pathway. *Blood*. 2011;118(21 Suppl):3960. doi:10.1182/blood.V118.21.3960.3960
90. Yao M, Mao X, Zhang Z, Cui F, Shao S, Mao B. Communication molecules (ncRNAs) mediate tumor-associated macrophage polarization and tumor progression. *Front Cell Dev Biol*. 2024;12:1289538. doi:10.3389/fcell.2024.1289538
91. Swaim CD, Canadeo LA, Huibregtse JM. Approaches for investigating the extracellular signaling function of ISG15. *Methods Enzymol*. 2019;618:211–227. doi:10.1016/bs.mie.2018.12.027
92. Kiripolsky J, Romano RA, Kasperek EM, Yu G, Kramer JM. Activation of Myd88-dependent TLRs mediates local and systemic inflammation in a mouse model of primary Sjögren's syndrome. *Front Immunol*. 2020;10:2963. doi:10.3389/fimmu.2019.02963
93. Mussbacher M, Derler M, Basílio J, Schmid JA. NF-κB in monocytes and macrophages - an inflammatory master regulator in multitalented immune cells. *Front Immunol*. 2023;14:1134661. doi:10.3389/fimmu.2023.1134661
94. Liang S, Wei H, Sun R, Tian Z. IFNalpha regulates NK cell cytotoxicity through STAT1 pathway. *Cytokine*. 2003;23(6):190–199. doi:10.1016/s1043-4666(03)00226-6
95. Fortin C, Huang X, Yang Y. Both NK cell-intrinsic and -extrinsic STAT1 signaling are required for NK cell response against vaccinia virus. *J Immunol*. 2013;191(1):363–368. doi:10.4049/jimmunol.1202714
96. Miyagi T, Gil MP, Wang X, Louten J, Chu WM, Biron CA. High basal STAT4 balanced by STAT1 induction to control type 1 interferon effects in natural killer cells. *J Exp Med*. 2007;204(10):2383–2396. doi:10.1084/jem.20070401
97. Phatarpekar P, Zhu S, Denman CJ, et al. STAT3 activation promotes NK cell proliferation, NKG2D expression, and NK cell antitumor activity. *Blood*. 2010;116(21 Suppl):105. doi:10.1182/blood.V116.21.105.105

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