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RAPID COMMUNICATION

Identification of immune cell infiltration pattern in diabetic nephropathy

Diabetic nephropathy (DN) is now the most common cause of end-stage renal disease in the world. Inflammatory and immune responses play an important role in the pathogenesis of DN, but the specific mechanisms responsible for the development of this disease have yet to be fully elucidated. Numerous epidemiological and preclinical studies have shown that inflammatory response and immune response play an important role in the early stages of DN pathogenesis.[1] Still, the exact mechanism for the development of this disease has not been fully elucidated.

In this work, we aim to evaluate the immune characteristics of DN patients (Fig. S1). We attempt to determine whether there are different immune subtypes in the DN population and differences in physiological function between different immune subtypes. Additionally, we look to explore the relationship between different immune characteristics and the severity of DN.

We first downloaded the expression profile data for two DN datasets, GSE96804 (training set) and GSE142025 (validation set). The MCPcounter (http://github.com/ebecht/MCPcounter), xCell (https://xcell.ucsf.edu/), and EPIC (http://epic.gfellerlab.org) algorithms were used to calculate the proportion of immune cells. We found that DN patients were divided into cluster 1 and cluster 2, depending on the unsupervised clustering method (Fig. S2A, B). Patients in cluster 2 had an abundance of immune cells, such as B cells, T cells, dendritic cells, cytotoxic lymphocytes, and $CD8^+$ T cells. By contrast, cluster 1 DN patients had enriched neutrophils and endothelial cells (Fig. 1A). DN patients in the independent validation data set GSE142025 were divided into cluster 1 and cluster 2 by supervised clustering algorithm (Fig. 1B). Similarly, we found that cluster 2 and cluster 1 showed enriched and depleted immune subtype, respectively (Fig. 1B). In the training set GSE96804, we found that cluster 2 exhibited a significantly increased percentage of B cells, $CD8^+$ T cells, $CD4^+$ T cells, and macrophages compared with cluster 1 DN patients (Fig. 1C). These findings were verified by similar results in the validation set GSE142025 (Fig. 1D). The identification of subtypes is crucial for clinicians to select accurate treatments for DN patients. However, direct subtyping of DN patients based on immune cells poses significant challenges in clinical practice. As a result, we constructed immune cell scores (Fig. S3A) by employing multiple machine learning algorithms (random forest/RF, logistic regression/LR, and support vector machine/SVM) in conjunction with the Lasso algorithm on immune cells. In both GSE96804 and GSE142025 datasets, these scores exhibited an excellent discriminatory ability for classifying two subtypes of DN patients according to immune cells scores (GSE96804-AUC: LR = 0.931, RF = 0.949, SVM = 0.969; GSE142025-AUC: LR = 0.849, RF = 0.849, SVM = 0.969; Fig. S3B, C). We then analyzed the correlation between immune cells in the cluster 1 and cluster 2 groups ($P < 0.05$; Fig. S4). The results showed a significant positive correlation between myeloid dendritic cells and B cells in cluster 2 ($P < 0.05$; Fig. S4A). Similarly, there was a significant positive correlation between T cells and B cells in cluster 2 ($P < 0.05$; Fig. S4B), and there was a significant difference between T cells and myeloid dendritic cells in cluster 2 compared with cluster 1 ($P < 0.05$; Fig. S4C).

Next, we compared the immune-related genes expressed between cluster 2 and cluster 1. In the training set (GSE96804), cluster 2 had significantly increased BLK, BTLA, CD79A, CD79B, FCRL1, FCRL3, RALGPS2, GZMA, TNFRSF9, TNFSF8, and CXCR3 (Fig. S4D; $P < 0.05$). These findings were verified ($P < 0.05$) by similar results from the validation set (GSE142025), which are displayed in Figure S4E. Also, we found that there was a significant positive correlation between the immune-related genes expressed in cluster 2 ($R > 0$, $P < 0.05$; Fig. S3F). On the contrary, there was no significant correlation between the multiple immune-related genes expressed in cluster 1 ($P > 0.05$; Fig. S4F).

In both the training (GSE96804) and validation (GSE142025) sets, we found that the activation of inflammatory factors, chemokines, and immune cell activation in cluster 2 was significantly higher than in cluster 1 (Fig. 1E,

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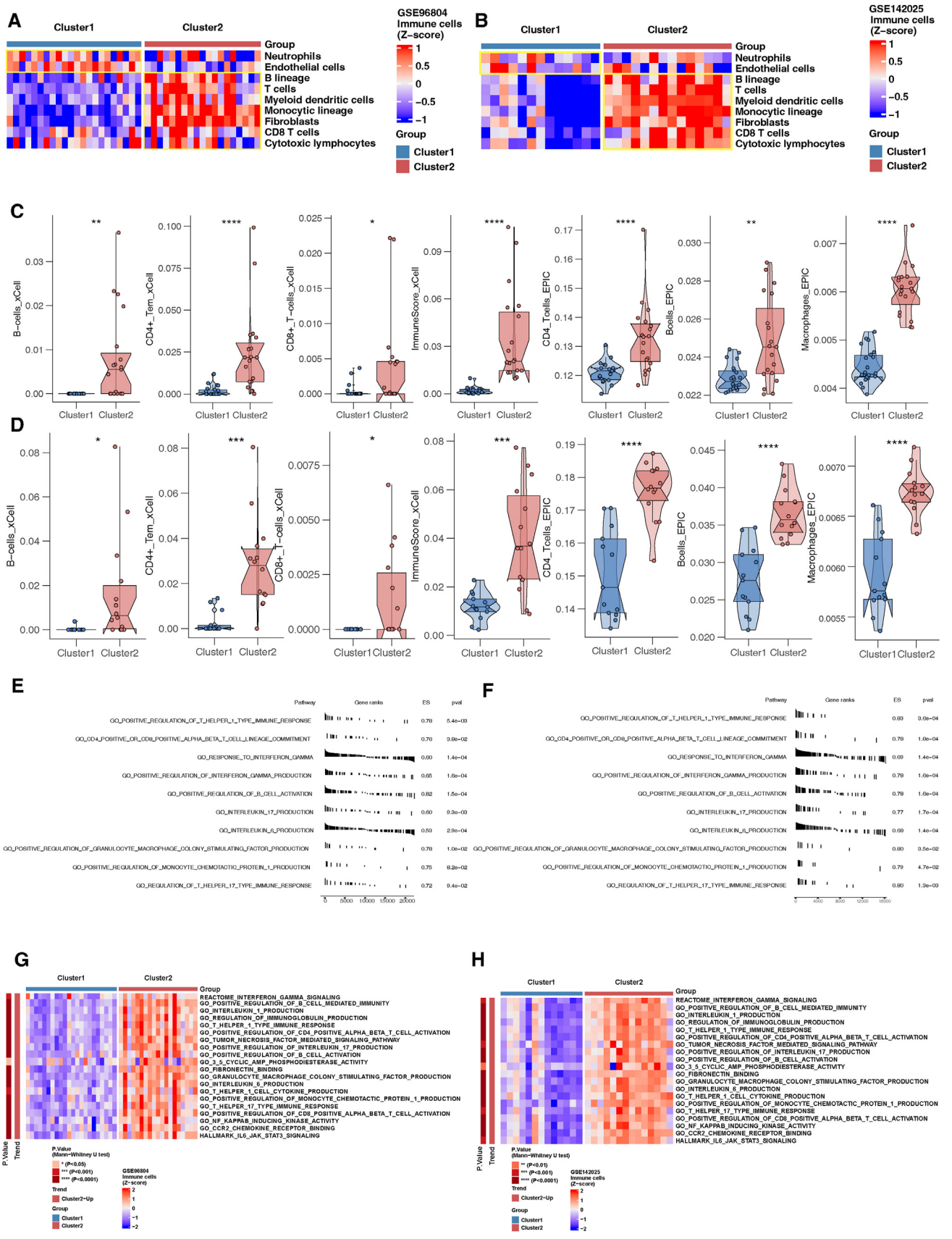

Figure 1 Different immune subtypes in the diabetic nephropathy (DN) population and differences in physiological function between different immune subtypes. **(A, B)** The heatmap indicating immune cells estimated by the MCPcounter algorithm within clusters of training cohort GSE96804 (A) and validation cohort GSE142025 (B). **(C, D)** The differences between the immune cells

F; Fig. S5A, B). Similarly, we used the ssGSEA algorithm to calculate the signal pathway scores for each DN patient and compared the differences in these scores between the cluster 2 and cluster 1 groups (Fig. 1G, H). Both training data set (GSE96804) and validation data set (GSE142025) were found to have significantly up-regulated immune activation signaling pathways (Fig. 1G, H; Fig. S6A, B). Additionally, we summarized the potential differences between cluster 1 and cluster 2 with regard to immune cells, immune-related genes, and immune response-related pathways (Fig. S7).

Immune cells play a vital role in the development of DN. Lei et al[2] found that the proportion of circulating $CD8^+$ T cells in patients with type 2 diabetes mellitus complicated with proteinuria was higher than that in patients without proteinuria, demonstrating the level of $CD8^+$ T cells was positively correlated with urinary protein. $CD4^+$ T cells can organize the release of multiple pro-inflammatory factors (such as chemokines and cytokines).[3] B cells also play a role in the pathogenesis of DN.[4] Xu et al[4] found that in non-obese diabetic mice, the depletion of B cells from using anti-CD20 or anti-CD22 monoclonal antibodies can prevent type 1 diabetes. All the above studies suggest that $CD4^+$ T cells, $CD8^+$ T cells, and B cells are involved in the initial development progression of DN.

Pro-inflammatory mediators and immune response pathways play a critical role in the initial development and progression of DN.[3] Pro-inflammatory cytokines secreted by T cells can directly activate adjacent macrophages or indirectly stimulate mesangial cells to produce colony-stimulating factor-1 and monocyte chemotactic protein-1 (MCP-1). Once activated, macrophages release nitric oxide, reactive oxygen species, interleukin (IL)-1, and tumor necrosis factor (TNF)-α. The level of serum IL-6 in DN patients was increased. IL-6 had a direct effect on glomeruli and infiltrating cells. JAK-STAT activation was a critical renal injury mechanism in DN42. In terms of cytokines, $CD8^+$ T cells secreted IFN-γ and TNF-α as part of their cytotoxic activity.

Historically, molecular heterogeneity is a key reason why characteristic DN patients cannot be accurately classified. However, the treatment response and clinical outcome of DN patients who have similar clinical stages or pathological characteristics are quite different. The results demonstrate that there are different immune subtypes in DN patients, with different levels of immune response and inflammatory characteristics. The significant differences between cluster 2 and cluster 1 in immune cells, proinflammatory mediators, and immune response-related pathways may provide more accurate treatment for DN patients, depending on their immune type. Currently, the treatment of DN focuses on the regulation of inflammatory pathways, including proinflammatory factors and signaling pathways.[5] Infliximab works against TNF-α by reducing expression and has been shown to improve the prognosis of diabetic mice.[5] Additionally, TNF-α inhibitor (SKF86002) can significantly reduce the level of TNF-α in glomeruli and improve renal function in DN patients. MCP-1/CCL2 inhibitor (NOX-E36) significantly reduced glomerulosclerosis and has improved glomerular filtration rate in DN mice.[5] The metal chelation drug, pyrrolidine dithiocarbamate, inhibits NF-kB (by decreasing its activation) as well as decreases the levels of MCP-1, TNF-α, IL-1, and IL-6, thereby slowing down and reducing renal damage in DN patients.[5] A phase II clinical study showed that baricitinib (JAK1/JAK2 selective inhibitor) can reduce proteinuria in patients with DN.[5]

In this study, we found that patients with DN can be divided into two distinct immune subtypes. Compared with cluster 1 DN patients, cluster 2 DN patients had significantly enriched immune cell infiltration, increased immune-related gene expression, and up-regulated immune activation pathway activity. Interestingly, after using this classification, it was clear that patients with more advanced DN were more likely to be found in cluster 2. These findings may enable us to improve the existing classification of DN immune subtypes, which would be useful in guiding the identification of DN patients who may benefit from specific treatments.

Ethics declaration

We have obtained consent to publish this paper from all the participants of this study.

Author contributions

MZ, YGL, and XYL initiated and supervised the research. YGL and XYL performed most of the data analyses. DL, SZ, JZ, JMH, GRL, JL, ZFG, YZL, SQY, SCL, HC, YG, ML, LPF, and LYL provided crucial suggestions. YGL and XYL prepared the manuscript. All authors reviewed and approved the final version of this manuscript.

Conflict of interests

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.101197.

estimated by multiple algorithms (xCell and EPIC) in cluster 1 and cluster 2 of GSE96804 (C) and GSE142025 (D). **(E, F)** Enriched immune-related signaling pathways annotated by the GSEA algorithm between the cluster 2 and cluster 1 subgroups of GSE96804 (E) and GSE142025 (F). **(G, H)** The heatmap indicating signaling pathway scores estimated by ssGSEA within the clusters of the training cohort GSE96804 (G) and validation cohort GSE142025 (H).

References

1. 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 Ren Physiol*. 2017;312(4):F716—F731.
2. Lei L, Mao Y, Meng D, et al. Percentage of circulating $CD8^+$ T lymphocytes is associated with albuminuria in type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 2014;122(1):27—30.
3. Wu CC, Sytwu HK, Lin YF. Cytokines in diabetic nephropathy. *Adv Clin Chem*. 2012;56:55—74.
4. Xiu Y, Wong CP, Bouaziz JD, et al. B lymphocyte depletion by CD20 monoclonal antibody prevents diabetes in nonobese diabetic mice despite isotype-specific differences in Fc gamma R effector functions. *J Immunol*. 2008;180(5):2863—2875.
5. Chen J, Liu Q, He J, Li Y. Immune responses in diabetic nephropathy: pathogenic mechanisms and therapeutic target. *Front Immunol*. 2022;13:958790.

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