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Exploring the hepatic-ophthalmic axis through immune modulation and cellular dynamics in diabetic retinopathy and non-alcoholic fatty liver disease

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Abstract

Background Dysfunctions within the liver system are intricately linked to the progression of diabetic retinopathy (DR) and non-alcoholic fatty liver disease (NAFLD). This study leverages systematic analysis to elucidate the complex cross-talk and communication pathways among diverse cell populations implicated in the pathogenesis of DR and NAFLD.

Methods Single-cell RNA sequencing data for proliferative diabetic retinopathy (PDR) and NAFLD were retrieved from the Gene Expression Omnibus (GEO) database. Differential gene expression analysis was conducted and followed by pseudo-time analysis to delineate dynamic changes in core cells and differentially expressed genes (DEGs). CellChat was employed to predict intercellular communication and signaling pathways. Additionally, gene set enrichment and variation analyses (GSEA and GSVA) were performed to uncover key functional enrichments.

Results Our comparative analysis of the two datasets focused on T cells, macrophages and endothelial cells, revealing SYNE2 as a notable DEG. Notably, common genes including PYHIN1, SLC38A1, ETS1 (T cells), PPFIBP1, LIFR, HSPG2 (endothelial cells), and MSR1 (macrophages), emerged among the top 50 DEGs across these cell types. The CD45 signaling pathway was pivotal for T cells and macrophages, exerting profound effects on other cells in both PDR and NAFLD. Moreover, GSEA and GSVA underscored their involvement in cellular communication, immune modulation, energy metabolism, mitotic processes.

Conclusion The comprehensive investigation of T cells, macrophages, endothelial cells, and the CD45 signaling pathway advances our understanding of the intricate biological processes underpinning DR and NAFLD. This research underscores the imperative of exploring immune-related cell interactions, shedding light on novel therapeutic avenues in these disease contexts.

Keywords Diabetic retinopathy, Non-alcoholic fatty liver disease, Cell-cell communication, Single-cell RNA sequencing, SYNE2

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Introduction

In recent years, the concept of inter-organ communication has gained substantial recognition in medical research, particularly in understanding the systemic nature of metabolic disorders. Emerging evidence from modern molecular biology reveals a clinically significant liver-eye axis, wherein bidirectional interactions between hepatic dysfunction and ocular pathologies exacerbate disease progression. This interdependence is most prominently observed in diabetic retinopathy (DR) and non-alcoholic fatty liver disease (NAFLD), two conditions sharing common etiological pathways rooted in chronic inflammation and microvascular dysfunction [1–3].

DR is a complex and debilitating complication that arises from diabetes and progressively impairs vision. Proliferative diabetic retinopathy (PDR) represents an advanced stage of this condition, characterized by the abnormal growth of blood vessels in the retina [4]. In PDR, the delicate balance of the ocular environment is disrupted by fibrovascular membrane (FVM), leading to potentially severe consequences for vision (vitreous hemorrhage and tractional retinal detachment) [5, 6]. NAFLD, characterized by fat accumulation in the liver, has emerged as a widespread and significant chronic liver condition with a global prevalence, affecting approximately 25% of the global population [7]. Systemic hypertension, insulin resistance and significant diabetes, dyslipidemia are frequently observed in patients with NAFLD [8]. A cohort study involving 3,123 individuals with type 2 diabetes demonstrated that patients with NAFLD had a higher prevalence of retinal microvascular damage compared to control patients without NAFLD [9]. Furthermore, clinical reports indicate that the evaluation of DR through ocular examination may serve as a valuable tool in predicting liver fibrosis progression in patients with NAFLD [10].

Despite these advances, critical knowledge gaps persist. First, existing studies predominantly focus on epidemiological correlations rather than mechanistic linkages. Second, the cellular mediators of liver-eye communication remain poorly characterized– while circulating extracellular vesicles and gut-liver-derived metabolites are hypothesized as potential carriers [11], their specific roles in DR progression lack experimental validation. Third, current therapies for PDR (anti- (vascular endothelial growth factor) VEGF drugs, laser photocoagulation) and NAFLD (lifestyle modification, pioglitazone) primarily target individual organs without addressing systemic immune-metabolic dysregulation, resulting in suboptimal long-term outcomes.

In order to shed light on these complex questions, we employed a comprehensive approach by integrating single-cell RNA sequencing (scRNA-seq) datasets obtained from PDR fibrotic membranes and NAFLD liver tissues (Fig. 1). Our research creatively concentrates on providing a unique opportunity to unravel the cellular heterogeneity and molecular signatures associated with these two conditions. In the analyzed dataset, we identified three pivotal cell types that are commonly implicated in both PDR and NAFLD: T cells, macrophages and endothelial cells. Additionally, SYNE2 was filtrated as highly differentially expressed genes (DEGs) within T cells and endothelial cells, which hold considerable importance in comprehending cell proliferation, migration and fibrosis [12]. Traditionally, metabolic and hemodynamics have been considered as primary factors in patients with PDR and NAFLD [13, 14]. Moreover, recent investigations have demonstrated an augmented infiltration of macrophages and activation of T cells in patients suffering from PDR and NAFLD [15–18]. Surprisingly, we discovered that the CD45 signaling pathway played a crucial role in regulating cell-cell communication between T cells and macrophages in both PDR and NAFLD, known for its involvement in immune cell activation and function. In summary, directing attention towards the immune and inflammatory mechanisms involved in PDR and NAFLD may uncover promising therapeutic targets for the effective management and treatment of these conditions.

Materials and methods

Data acquisition and quality control

The scRNA-seq datasets GSE165784 and GSE189175 were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). Human FVM of PDR samples were collected from six patients without any treatment of preoperative anti-VEGF injections or retinal laser photocoagulation in GSE165784. Using the R (version 4.3.2) package Seurat (version 4.4.0), cells (with nCount_RNA > 500, nFeature_RNA < 5000 and mitochondrial < 30%) were filtered for subsequent analyses. Additionally, human liver tissue isolated from the three patients with NAFLD was acquired in GSE189175. NAFLD datasets were filtered with the quality control (nCount_RNA > 200, nFeature_RNA < 1000, percent. mitochondrial < 5%).

Cell clustering and annotation

All samples were subjected to logarithmic normalization to ensure uniformity and minimize the impact of high expression values. Subsequently, genes with significant expression variability were selected to perform Principal Component Analysis (PCA) for dimensionality reduction. The "FindAllMarkers" function was applied to identify marker genes for each cell cluster, facilitating the characterization of distinct cellular populations. The main cell types were determined based on Cell Markers database along with described markers in the previous literature [19, 20]. In order to maintain consistency

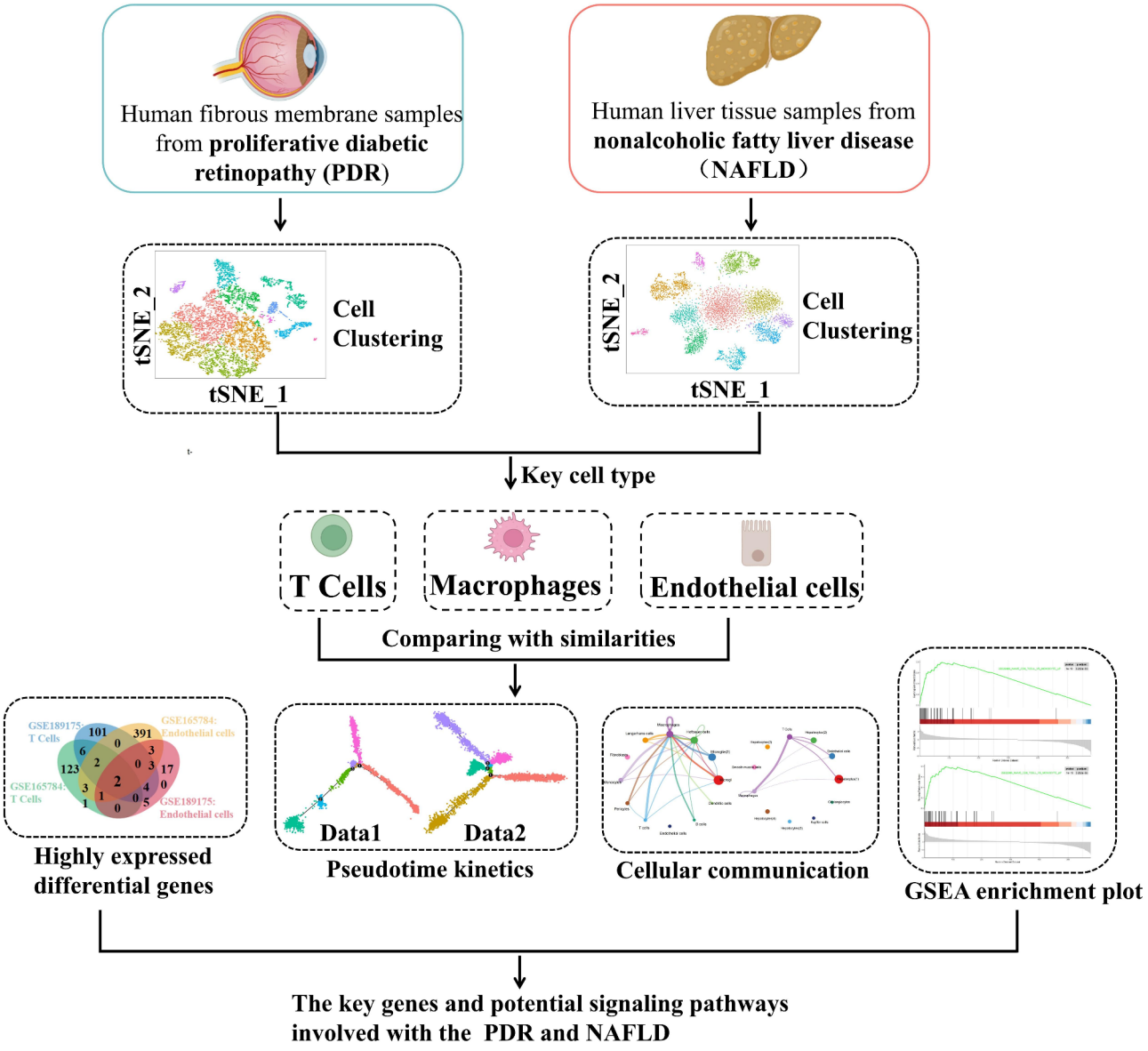

Fig. 1 Flow chart of the study. ScRNA-seq data for PDR and NAFLD were retrieved from the Gene Expression Omnibus (GEO) database. The key cell types were calculated by differential gene expression analysis, following with pseudo-time analysis to delineate dynamic changes in core cells. CellChat was employed to predict intercellular communication and signaling pathways. Additionally, gene set enrichment and variation analyses were performed to uncover key functional enrichments

and facilitate comparability between the two datasets, we employed uniform parameters for analyzing each individual dataset.

Cell composition and differential expressed gene analysis

Following the retrieval of cell cluster labels using the Idents function, "cell. num", "cell. freq" were employed to compute the cell count and proportion in each sample. The FindMarkers function was used to determine differentially expressed genes (DEGs), with the following parameters: only.pos = TRUE, min.pct = 0.25, and logfc. threshold = 0.25. Genes were regarded as statistically significant with a fold change > 0.25 and an adjusted p-value < 0.05.

Functional enrichment analysis

To investigate and observe the biological functionalities of distinct cell populations in PDR and NAFLD disease, the top 200 genes exhibiting the highest expression levels were carefully selected. Subsequently, the gene symbols were converted into corresponding Entrez gene IDs. The R package clusterProfiler (version 4.10.0) was employed to conduct Graphene Oxide (GO) and Kyoto

Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

Pseudo-time analysis

The Seurat object was initially converted into monocle and performed by monocle (version 2.30.0) for pseudo-time trajectory analysis. Then we constructed cell development trajectories by "setOrderingFilter" and "estimateSizeFactors" functions. During pseudo-time processing, the tree on the dataset recognizes the Dimensionality Reduction tree (DDRTree) algorithm to reduce dataset dimensionality. The expression of a specific gene along a developmental trajectory was allowed to be viewed by the "plot_cell_trajectory" function, while the "plot_genes_in_pseudo-time" visualized the expression patterns of specific genes throughout pseudo-time.

Cell–cell communication analysis

Employing the "CellChatDB.human" receptor-ligand database (version 1.6.1), a comprehensive analysis of cellular communication was conducted on preprocessed scRNA-seq datasets. Overexpressed ligands or receptors were identified within cell populations, followed by the projection of gene expression data onto a protein-protein interaction (PPI) network. The communication strength was inferred by calculating the communication probabilities of ligand-receptor interactions associated with each respective signaling pathway. The intercellular communication network demonstrated significant senders, receivers, mediators and influencers, while signaling gene expression distribution was displayed by violin plot.

Gene set enrichment analysis and gene set variation analysis

Gene Set Enrichment Analysis (GSEA) was widely used in genomics research to uncover biological pathways, biological functions or molecular signatures associated with different phenotypes or experimental conditions. We downloaded the immunologic signature gene sets (c7: immunologic signature gene sets) and performed GSEA analysis (version 1.64.0) on all genes within immune cell cluster. On the other hand, Gene Set Variation Analysis (GSVA) provided a better representation of gene set variation across samples, by transforming gene expression data into gene set activity scores without relying on data ranking. The average expression values of genes in each cell cluster were calculated with immune-related gene sets (h: hallmark gene sets) (version 1.50.0). Finally, we visualized the GSVA analysis results by employing a heatmap.

Results

Mapping cell types in DR and NAFLD

Following quality control and cell filtering, we obtained 10,039 cells and 21,437 genes from PDR, as well as 6,923 cells and 29,842 genes from NAFLD for further analysis. After normalization and dimensionality reduction, the cells were categorized into 12 and 11 unique clusters, separately. We performed the t-SNE technique to demonstrate cellular clustering (Fig. 2A, Supplementary Table S1). Upon comparison with the two datasets, we specifically chose to focus on T cells, macrophages and endothelial cells for a deeper analysis. Then a heatmap was constructed to pinpoint marker genes in each cell cluster. As shown in Fig. 2B, T cells were enriched in LTB, CD2, TRBC2, CD96, PYHIN1, IL7R. Macrophages exhibited high expression levels in SELENOP, SLC40A1, IGF1, CD163, CR1, MARCO and endothelial cells were characterized by COL4A1, VWA1, VWF, AKAP12, DNASE1L3, NOSTRIN. The distribution of cell type proportions in each sample reveals that T cells, macrophages and endothelial cells constitute approximately 10–20% of the total cell population in PDR patients, while they make up 20–30% in NAFLD liver tissue (Fig. 2C, Supplementary Table S2). These findings highlighted the significant presence and potential importance of vascular progression and immune regulation.

Identification of highly expressed gene in key cell type between PDR and NAFLD

To investigate similarities between PDR and NAFLD, we performed DEGs analysis between the two datasets within T cells, macrophages and endothelial cells. Then 780 highly DEGs were identified in dataset1, including T cells (165), macrophages (98) and endothelial cells (517). Meanwhile, dataset 2 exhibited a total of 537 deregulated genes, including T cells (125), macrophages (182) and endothelial cells (230). The Venn diagram showed that 9 genes were common between the two datasets in T cells. In addition, 7 and 40 genes were overlapped in macrophages and endothelial cells, separately (Fig. 3A, Supplementary Table S3-S5). This analysis served as a starting point for identifying potential key players in T cells, macrophages and endothelial cells that contribute to both PDR and NAFLD pathogenesis. Furthermore, SYNE2, one identical target genes, were found in T cells and endothelial cells within both datasets (Fig. 3B). The t-Distributed Stochastic Neighbor Embedding (t-SNE) showed that SYNE2 was mainly expressed in T cells and endothelial cells (Fig. 3C). The dot plots demonstrated the top 50 DEGs in T cells, macrophages and endothelial cells, which specifically highlighted the expression of PYHIN1, SLC38A1, ETS1 and SYNE2 in T cells. Similarly, PPFIBP1, LIFR and HSPG2 in endothelial cells stood out prominently and macrophages exhibited

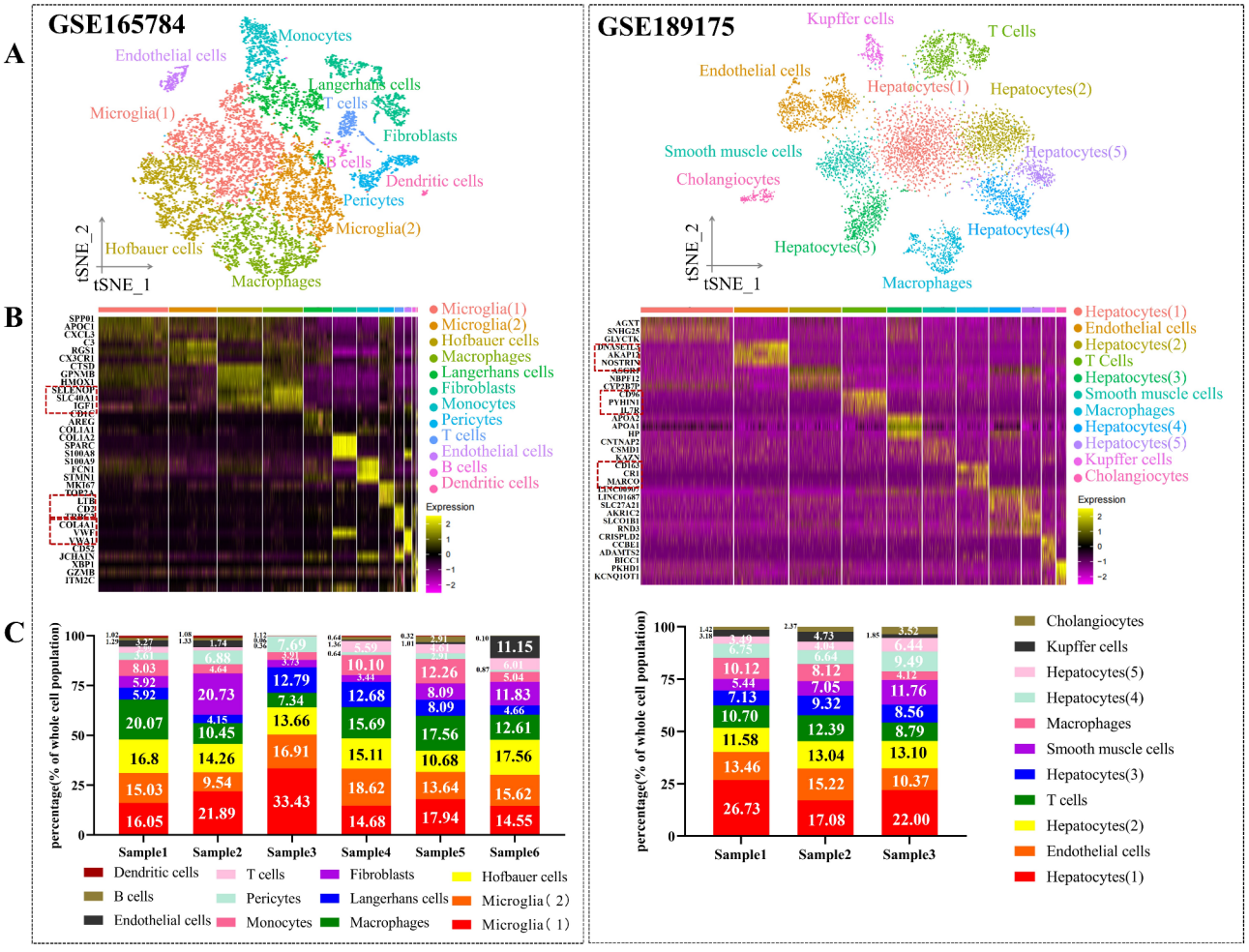

Fig. 2 Atlas of cell types from PDR and NAFLD. (**A**) TSNE plots show 12 cell clusters in GSE165784 (PDR) and 11 unique cell clusters in GSE189175 (NAFLD). (**B**) Heatmap of top three marker genes in each cluster among two datasets. The marker genes in T cells, macrophages and endothelial cell are highlighted. (**C**) Bar plots show the distribution of cell type proportions in PDR and NAFLD

distinctive expression of MSR1 (Fig. 3D). Focusing on these key genes could give insights into their potential roles in the pathogenesis and progression of PDR and NAFLD within these specific cell types.

GO and KEGG analysis of highly expressed genes in T cells, macrophages and endothelial cells

A comprehensive functional analysis was conducted to explore the roles and regulatory mechanisms of DEGs within T cells, macrophages and endothelial cells, as well as their potential involvement networks in PDR and NAFLD (Supplementary Table S6-S7). Regarding the GO analyses performed on two datasets, T cells were significantly enriched in "0050852": T cell receptor signaling pathway ($p = 3.59 * 10^{-4}$, $p = 7.28 * 10^{-5}$), "0043065: positive regulation of apoptotic process" ($p = 0.001$, $p = 7.06 * 10^{-4}$); "0042110": T cell activation ($p = 1.85 * 10^{-6}$, $p = 0.010$) (Fig. 4: BP-T cells). Moreover, T cells were closely associated with "0097421: liver regeneration ($p = 0.004$)" in PDR samples. In Fig. 4: BP-Macrophages, the enrichment of macrophages was observed with "0006898": receptor-mediated endocytosis ($p = 4.91 * 10^{-4}$, $p = 0.001$). Furthermore, there was a strong association between macrophages and T cells in biological processes, which could be confirmed in "0007044": Positive regulation of T cell activation ($p = 2.60 * 10^{-5}$) and "0046633": Alpha-beta T cell proliferation ($p = 0.031$). Next, endothelial cells exhibited a marked enrichment in "0016477": cell migration ($p = 1.25 * 10^{-10}$, $p = 8.51 * 10^{-16}$); "0048041": focal adhesion assembly ($p = 4.32 * 10^{-7}$, $p = 0.004$). Meanwhile, "0032024": positive regulation of insulin secretion ($p = 0.005$) and "0008286": insulin receptor signaling pathway ($p = 0.009$) were closely related to NAFLD patients (Fig. 4: BP-Endothelial cells). These results potentially emphasized insulin dysregulation and signaling in this disease development.

With regard to KEGG analysis, T cells were enriched in "hsa04210": Apoptosis ($p = 4.15 * 10^{-6}$, $p = 0.031$);

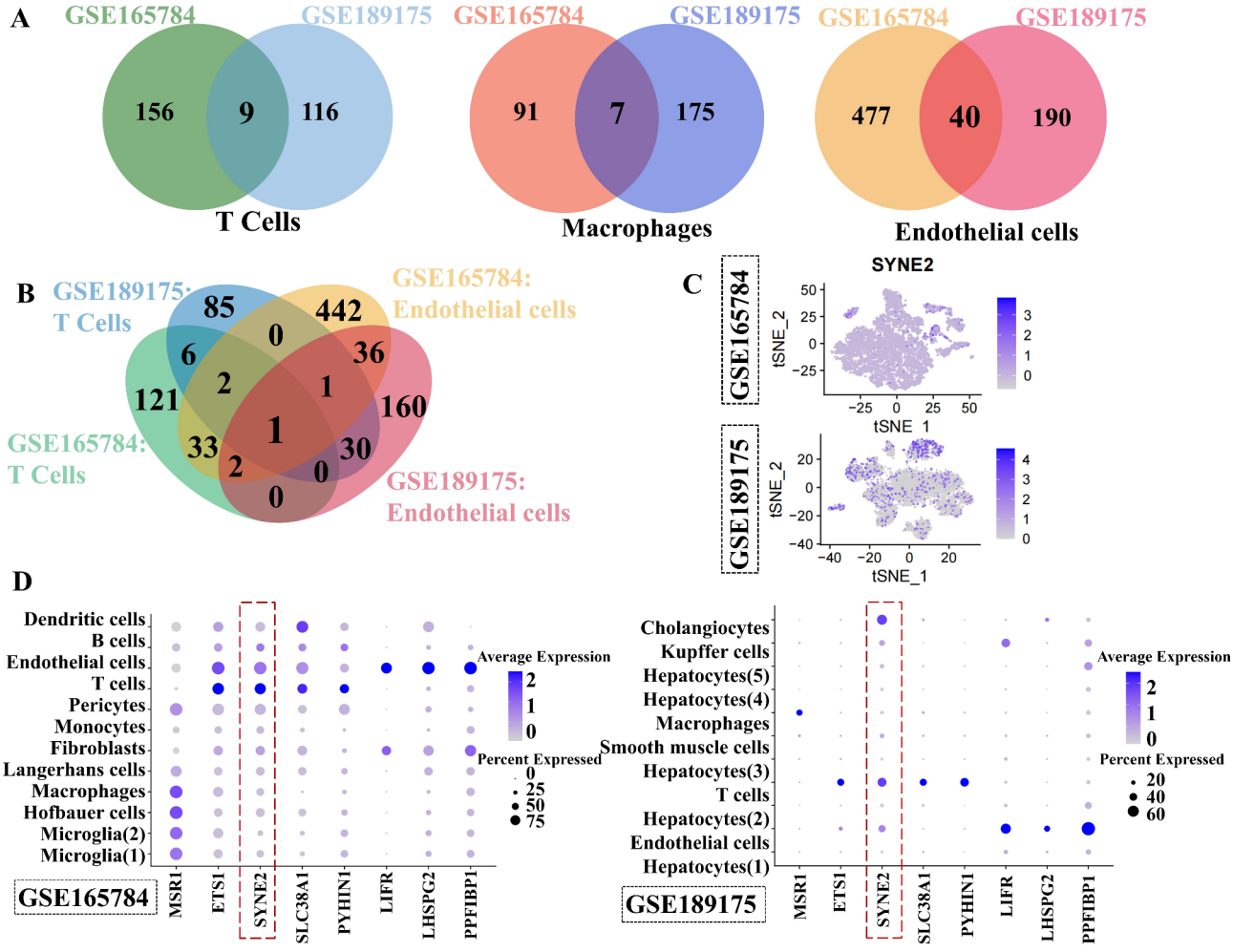

Fig. 3 Identification of highly expressed genes in key cell type between PDR and NAFLD. (**A**) The Venn diagrams show common genes in T cells macrophages and endothelial cells between GSE165784 and GSE189175. (**B**) The Venn diagrams exhibit identical target genes in T cells and endothelial cells within both datasets. (**C**) The t-SNE plots demonstrate the expression of SYNE2 in identified cell clusters. (**D**)The dot plots show the top 50 common DEGs in T cells (PYHIN1, SLC38A1, ETS1, SYNE2), macrophages (MSR1) and endothelial cells (PPFIBP1, LIFR, HSPG2)

"hsa04660": T cell receptor signaling pathway ($p = 0.002$, $p = 0.004$); Cell cycle ($p = 2.15^{*}10^{-6}$, $p = 0.012$) (Fig. 5: KEGG- T cells). As shown in Fig. 5: KEGG-Macrophages, macrophages were predominantly enriched in "hsa04514": Cell adhesion molecules ($p = 0.006$); "hsa05418": Fluid shear stress and atherosclerosis ($p = 0.021$); "hsa04936": Alcoholic liver disease($p = 0.048$) in dataset1. While in dataset2, it was found that macrophage was strongly relevant to "hsa04621": Endocytosis ($p = 0.003$); "hsa04022": cGMP-PKG signaling pathway($p = 0.004$). Then, endothelial cells were predominantly enriched in "hsa04520": Adherens junction ($p = 5.82^{*}10^{-9}$, $p = 0.001$); "hsa04510": Focal adhesion ($p = 6.07^{*}10^{-14}$, $p = 0.001$); "hsa04151": PI3K-AKT signaling pathway ($p = 1.54^{*}10^{-8}$, $p = 0.008$) (Fig. 5: KEGG-endothelial cells). These findings contributed to a deeper understanding of T cells, macrophages and endothelial cells functional implications in the analyzed datasets and underscore their importance in the PDR and NAFLD biological contexts.

Convergent patterns and robustness of pseudo-time analysis in PDR and NAFLD

In order to reveal the underlying biological processes and regulatory networks that govern their differentiation, activation and functional states, we reconstructed the three key cell types developmental trajectories. Seven states in cell clusters were calculated by pseudotime (Fig. 6A), which exhibited commonality and stability across both datasets. In Fig. 6B, endothelial cells were found to be predominantly located in the early phase and T cells were primarily presented in a phase of development or differentiation. Macrophages were situated in both early and late stages in dataset 1, while were mainly in the late period in dataset 2. Further analysis of gene expression patterns and macrophages phenotypic

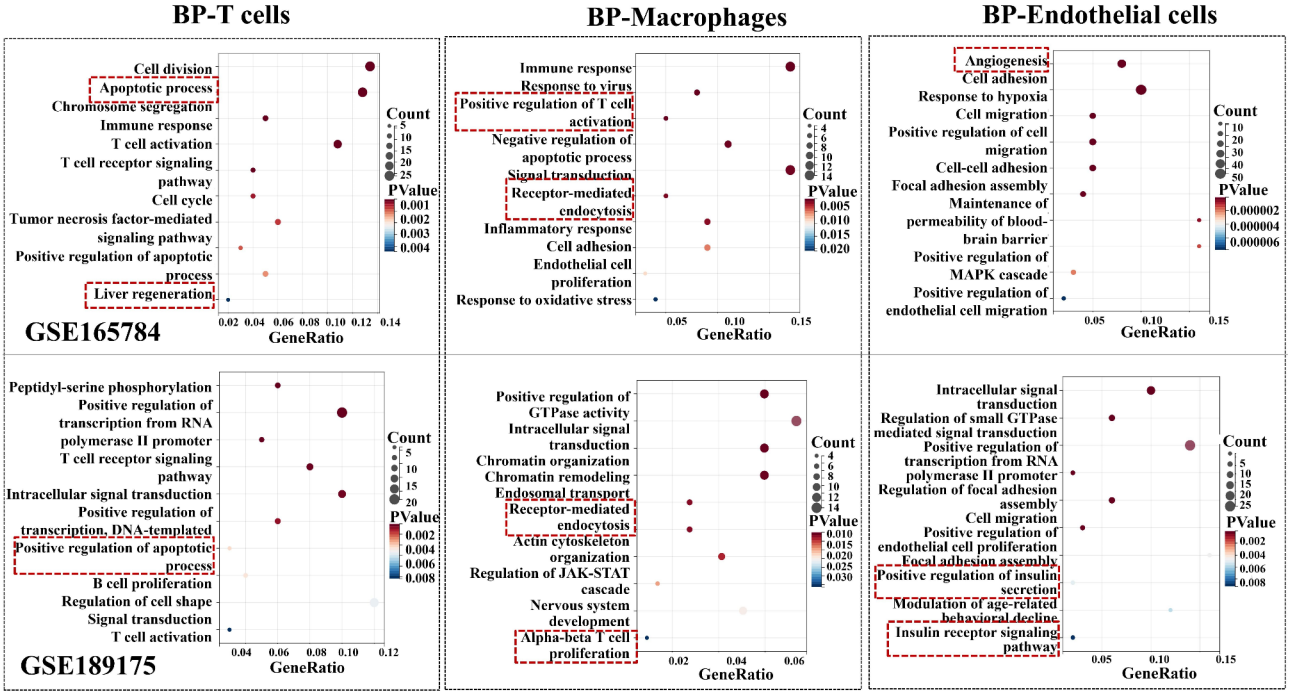

Fig. 4 GO analysis of highly expressed genes in T cells, macrophages and endothelial cells. The upper panel is GSE165784 and the below panel represents GSE189175

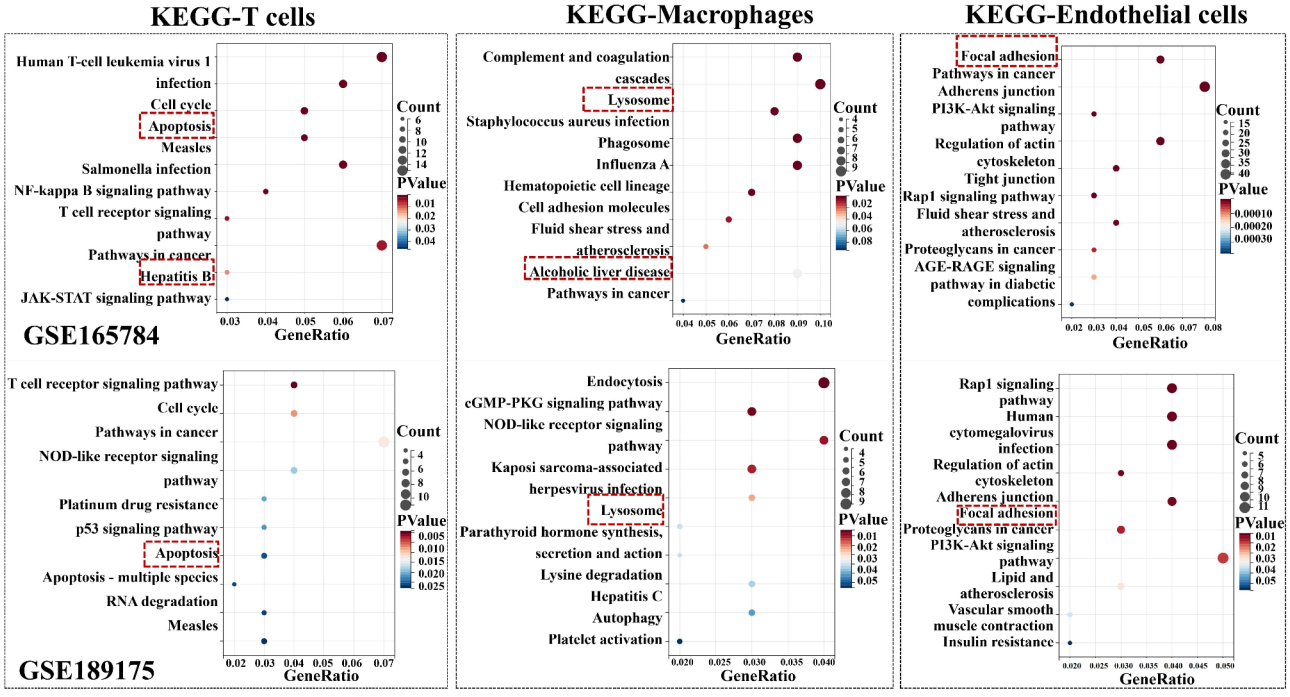

Fig. 5 KEGG analysis of highly expressed genes in T cells, macrophages and endothelial cells. The upper panel is GSE165784 and the below panel represents GSE189175

changes at different stages in both datasets need to be performed, which could provide a deeper understanding of their features and regulatory mechanisms. Moreover, the scatter plots visually represented the dynamic gene expression patterns across pseudo-time values (Fig. 6C). SYNE2 gene exhibited a low expression at the beginning of PDR and NAFLD, but as the diseases progressed, there was a notable upregulation of SYNE2 expression in

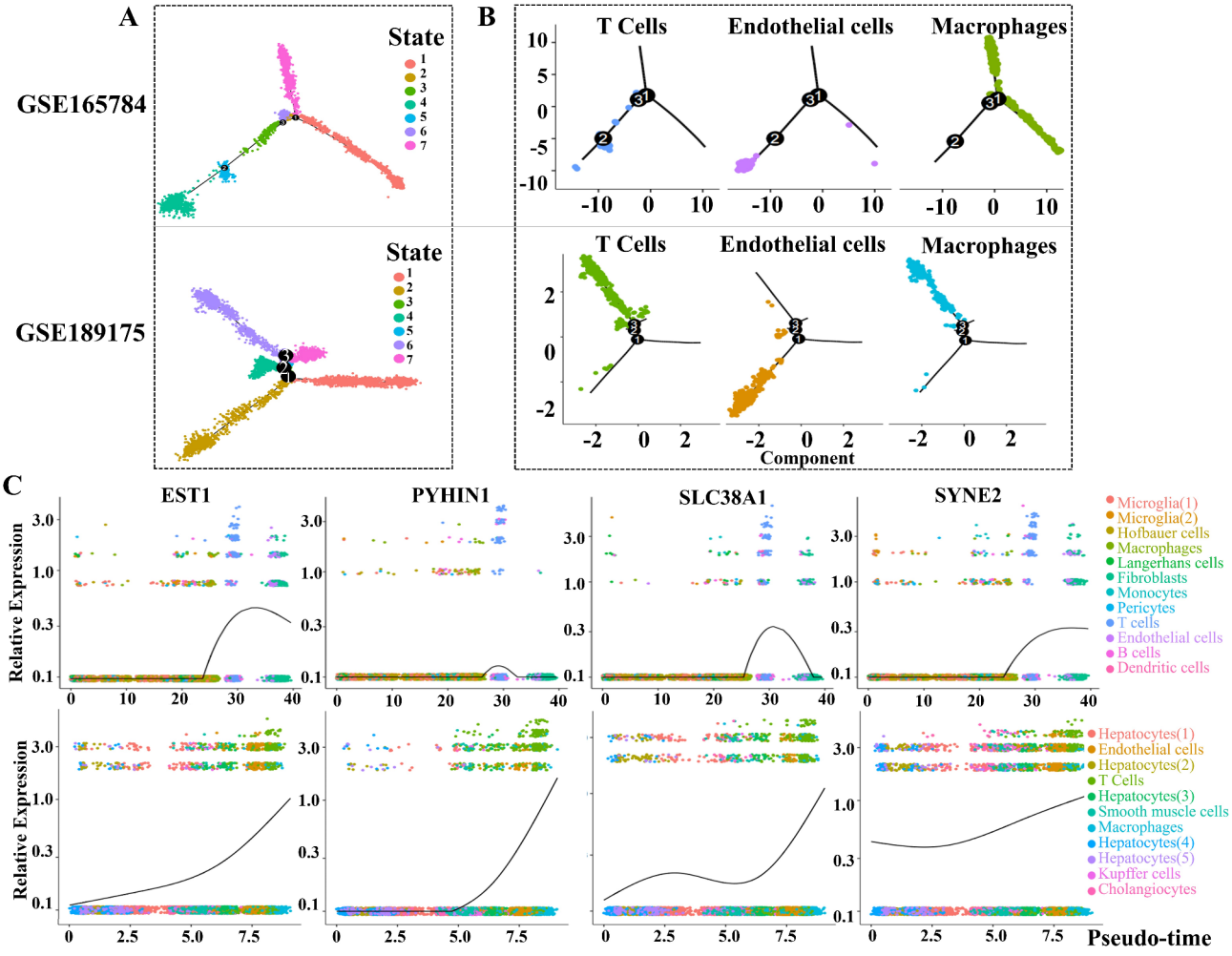

Fig. 6 Convergent Patterns and Robustness of Pseudo-time Analysis in PDR and NAFLD. (**A**) Trajectory state plots show common seven states in cell clusters between GSE165784 and GSE189175. (**B**) Trajectory separated cell types in T cells and endothelial cells in both datasets. (**C**) Pseudo-time kinetics of the top 50 common DEGs in T cells (EST1, PYHIN1, SLC38A1, SYNE2). The dynamic changes of macrophages (MSR1) and endothelial cells (PPFIBP1, LIFR, HSPG2) are shown in Supplementary Figure S1

T cells and endothelial cells. The expression of EST1 and SLC38A1 was found to be consistent with SYNE2.

Additionally, PYHIN1 displayed low expression levels in the initial stages, but its expression increased specifically in T cells during the late stage of NAFLD. Conversely, the change in PYHIN1 expression was not as pronounced in cells affected by PDR. PPFIBP1 and HSPG2 genes expression increased in endothelial cells within the two datasets. LIFR showed no significant change due to low expression in PDR and increased expression in endothelial cells in later period of NAFLD. MSR1 was highly expressed in macrophages at the initial stage of PDR. However, there was a significant decrease in MSR1 expression, exhibiting an expression pattern that was contrary to that observed in NAFLD (Supplementary Fig S1).

Unveiling the intricacies of the CD45 signaling pathway in harmonizing T cells and macrophages in FVM of PDR

Figure 7A revealed an interesting observation regarding the CD45 signaling pathway. While paracrine signaling predominantly mediated intercellular interactions within this pathway, it was noteworthy that T cells and macrophages exhibited significant autocrine signaling. Analysis of the network centrality in the inferred CD45 signaling (PTPRC – MRC1) demonstrated that T cells primarily served as senders, exerting their influence on macrophages (Fig. 7B). As shown in Fig. 7C, PTPRC was highly expressed in T cell function and signaling, while MRC1 was involved in macrophage-related processes. Furthermore, the PTPRC-MRC1 mediated CD45 signaling originating from T cells and macrophages, which was observed to be received by most cell populations in the FVM of PDR (Fig. 7D). Notably, CD45 signaling emerged as one of the prominently expressed pathways

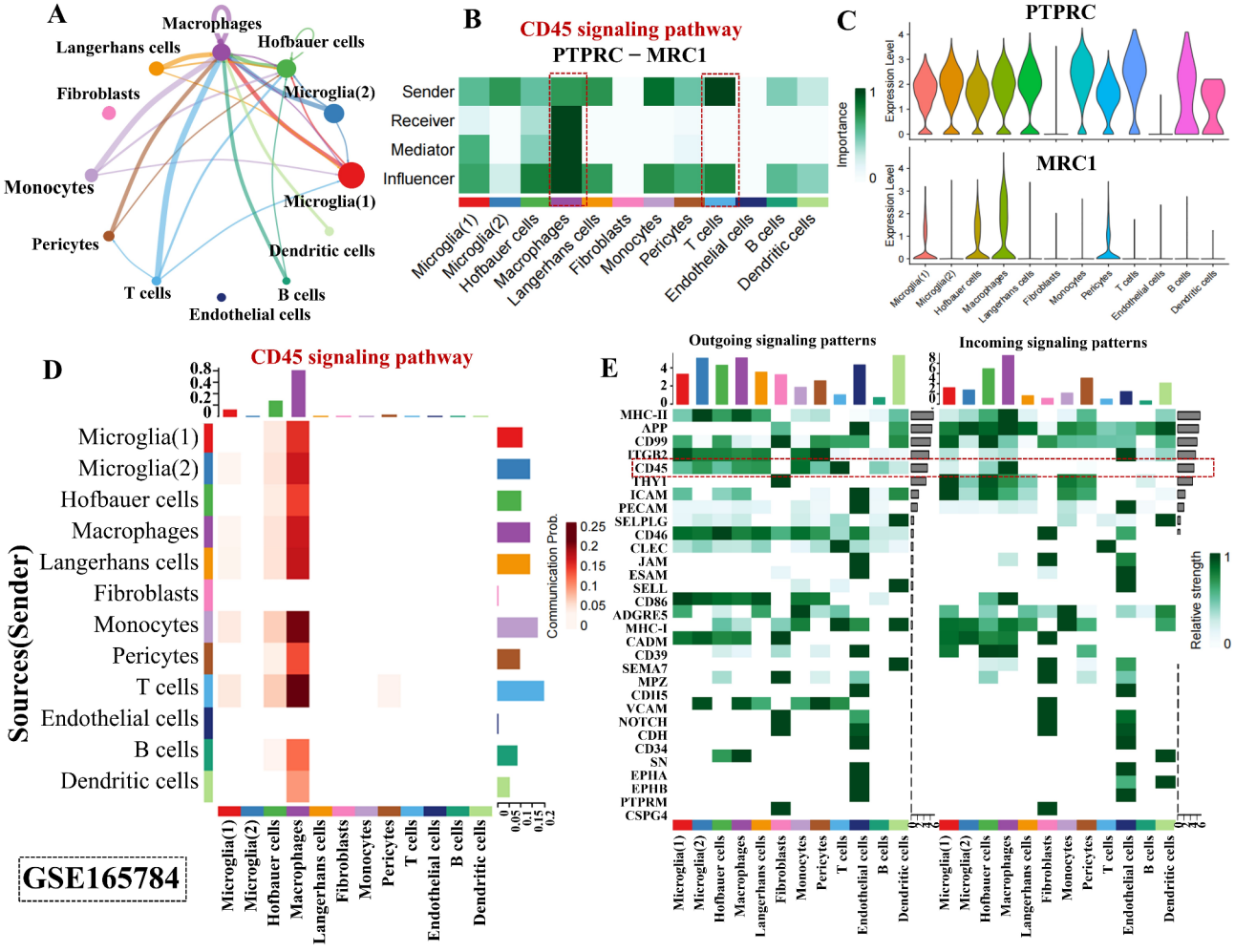

Fig. 7 Cell–cell communication inferred by CD45 signaling pathway in PDR. (**A**) Circle plot demonstrated intercellular communication network associated with the CD45 signaling pathway in PDR. The thickness of the line and color represents the communication signal strength. (**B**) Heatmap showed the sender, receiver, mediator and influencer of CD45 signaling pathway network. (**C**) Expression level of the CD45 signaling ligand and receptor (PTPRC-MRC1) in each cell population. (**D**) Heatmap of the CD45 signaling pathway–mediated intercellular communication intensity. (**E**) Outgoing and incoming signal strength of each signaling pathway in each cell population in PDR

for signaling source and reception in PDR (Fig. 7E). In summary, these comprehensive findings strongly indicated that T cells and macrophages established intricate cellular communication by CD45 signaling network contributing to the occurrence and progression of PDR.

Regulation of CD45 signaling pathway complexity involving T cells and macrophages in NAFLD

To gain insights into the communication network between cells in NAFLD liver tissue and to compare it with the FVM of PDR, we explored the intercellular interactions and signaling pathways. As shown in Fig. 8A, T cells transmit signals to macrophages, endothelial cells and hepatocytes by paracrine means. Importantly, macrophages acted as the principal receivers and mediators, whereas T cells assumed predominant roles as the primary transmitters and mediators, collectively governing the intricate communication dynamics within the CD45 signaling pathway (Fig. 8B). PTPRC demonstrated exclusive expression in T cells and macrophages, while MRC1 exhibited high expression levels in macrophages (Fig. 8C). Furthermore, T cells emerged as the primary driving source of CD45 signaling pathway in macrophages within liver tissues of NAFLD (Fig. 8D). Meanwhile, the Fig. 8E exhibited that the CD45 signaling pathway played a critical role in NAFLD. These findings shed light on the complex molecular dynamics associated with NAFLD, emphasizing the importance of understanding cell communication throughout these diseases.

Unique biological functions and interactions of T cells and macrophages in PDR and NAFLD

To further elucidate the immune correlation between T cells and macrophages in PDR and NAFLD, we

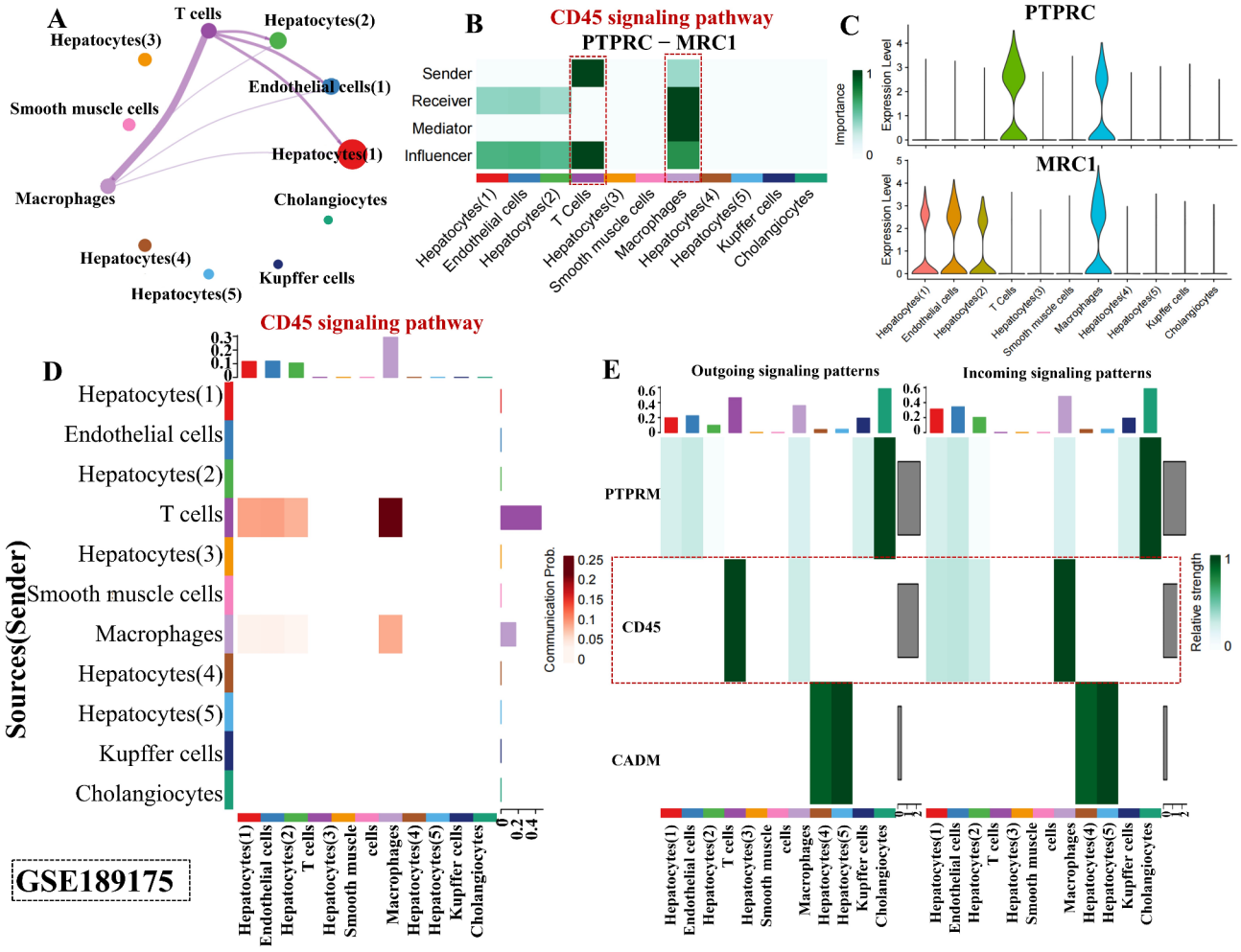

Fig. 8 Cell–cell communication inferred by CD45 signaling pathway in NAFLD. (**A**) Circle plot demonstrated intercellular communication network associated with the CD45 signaling pathway in NAFLD. The thickness of the line and color represents the communication signal strength. (**B**) Heatmap showed the sender, receiver, mediator and influencer of CD45 signaling pathway network. (**C**) Expression level of the CD45 signaling ligand and receptor (PTPRC-MRC1) in each cell population. (**D**) Heatmap of the CD45 signaling pathway–mediated intercellular communication intensity. (**E**) Outgoing and incoming signal strength of each signaling pathway in each cell population in NAFLD

performed GSEA and GSVA. Interestingly, our analysis revealed a noteworthy correlation between the gene sets enriched in the two datasets. Specifically, we found that out of the 51 gene sets significantly enriched in the NAFLD dataset, 42 gene sets (82.3%) were also significantly enriched in the PDR dataset. This shared enrichment suggested potential common molecular pathways or biological functions that may play a role in both PDR and NAFLD (Supplementary Table S8). As shown in Figs. 9A and 10A, GSE22886: T cells VS macrophages_up (p_adj = 3.02e-08, 3.47e-05), GSE22886: T cells VS macrophages_dn (p_adj = 2.96e-05, 8.47e-03). The upregulated gene sets exhibited upregulation specifically in T cells, accompanied by downregulation in macrophages.

Conversely, the downregulated gene sets were significantly downregulated in T cells and upregulated in macrophages. These differences may reflect distinct functions and interactions between T cells and macrophages in these two diseases. Furthermore, T cells and macrophages demonstrated significant enrichment in protein secretion, immune response mediated by interferon-gamma, MYC-targeted gene regulation and oxidative phosphorylation (Fig. 9B, Supplementary Table S9). This suggested their involvement in cellular communication, immune modulation and energy metabolism. Meanwhile, T cells and macrophages displayed enrichment in mitotic spindle function, TGF-beta signaling pathway, PI3K/AKT/mTOR signaling pathway (Fig. 10B). These enrichments implied their roles in cell division, signaling pathways associated with growth and development. Overall, these analyses would provide valuable insights into the involvement of these immune cell populations in the pathogenesis of the respective diseases and facilitate comparisons between the two conditions.

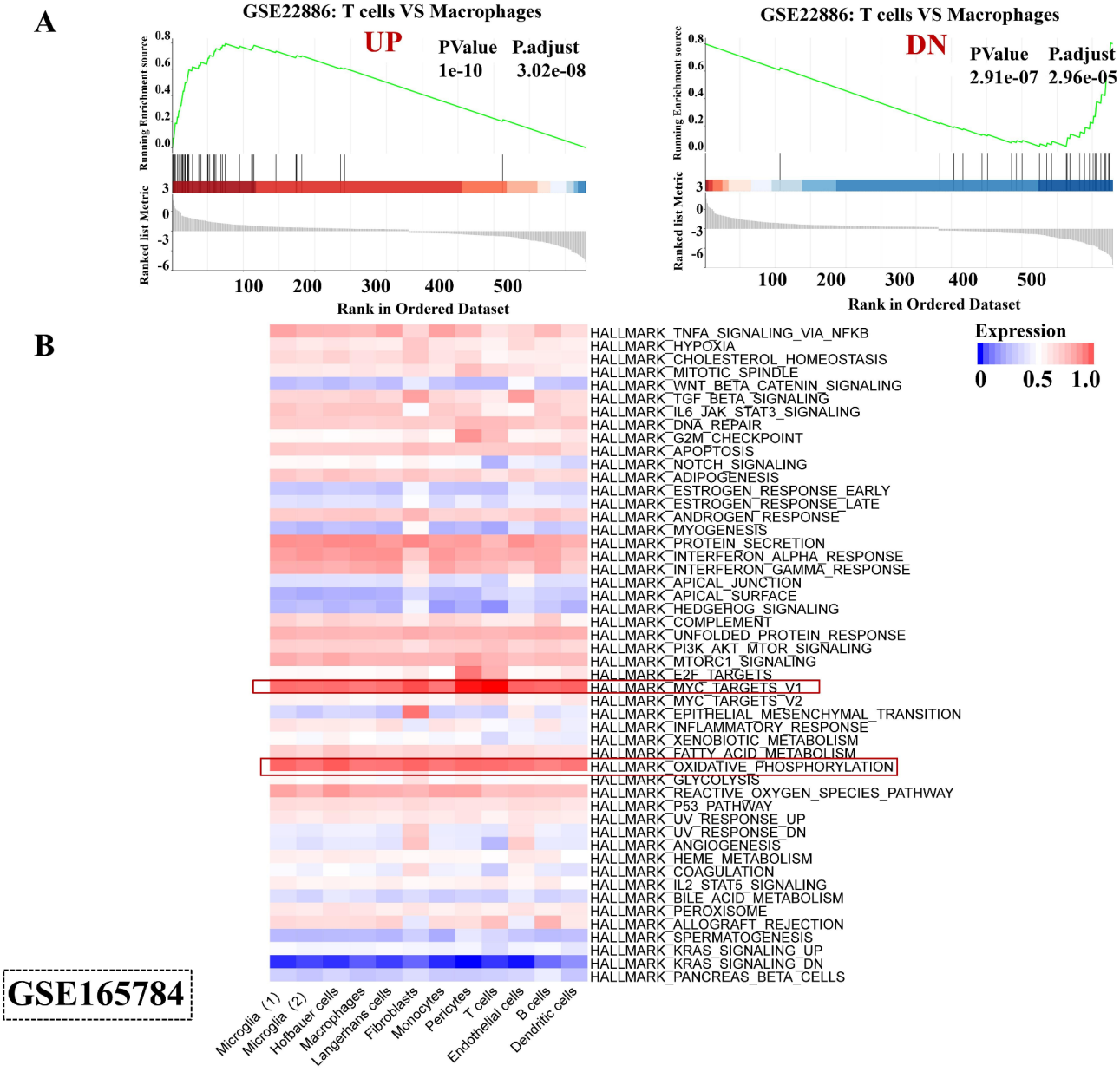

Fig. 9 Unique biological functions and interactions of T cells and macrophages in PDR. (**A**) GSEA analysis in PDR. The left panel shows the up-regulated GSEA enrichment by T cells vs. macrophages, and the right panel represents the down-regulated GSEA enrichment by T cells vs. macrophages in GSE22886. (**B**) Heatmap demonstrates immune-related GSVA analysis result in PDR. Different shades of color represent different intensities of expression

Discussion

Metabolism regulation and fat processing is consistent with NAFLD pathogenesis, a condition characterized by abnormal fat accumulation in the liver in the absence of excessive alcohol consumption. On the other hand, recent studies have reported that DR was a risk factor associated with the development of hepatocellular carcinoma in NAFLD [21]. NAFLD was independently associated with an increased prevalence of PDR. Interestingly, a cross-sectional study showed that the prevalence of NAFLD was 60.8% in patients with DR, while the prevalence of DR was lower in patients with moderate (14.3%) and severe (46.6%) [22]. Moreover, the relationship between DR and NAFLD may stem from common underlying factors, such as chronic inflammation, oxidative stress and metabolic dysregulation, which influence both the eyes and the liver [23, 24]. Modern medical knowledge provides a holistic perspective that can enhance our understanding of the complex interplay between PDR and NAFLD. However, further research is warranted to explore and validate the biological correlations in the context of PDR and NAFLD. Thus,

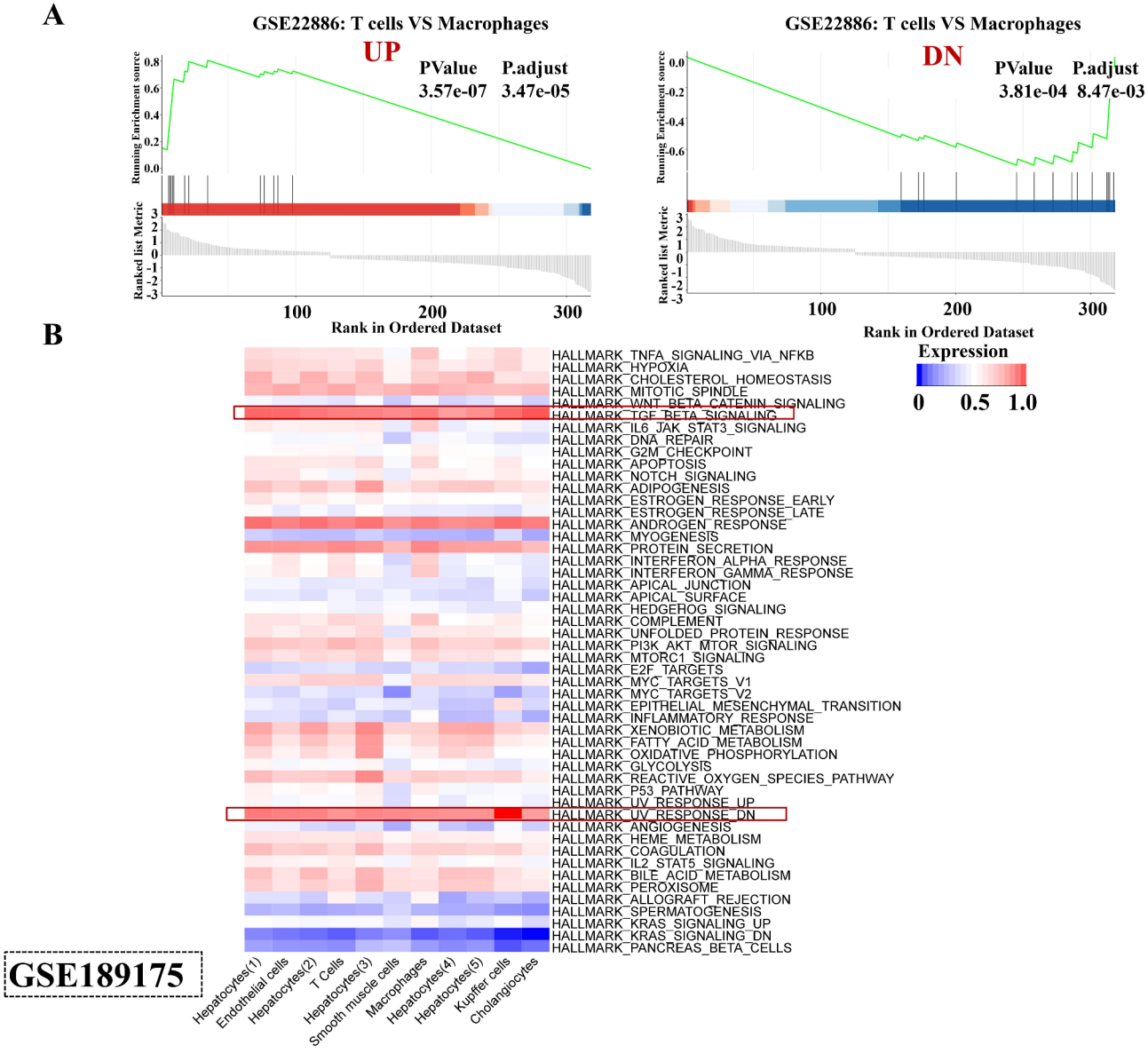

Fig. 10 Biological functions and interactions of T cells and macrophages in NAFLD. (**A**) GSEA analysis in NAFLD. The left panel shows the up-regulated GSEA enrichment by T cells vs. macrophages, and the right panel represents the down-regulated GSEA enrichment by T cells vs. macrophages in GSE22886. (**B**) Heatmap demonstrates immune-related GSVA analysis results in NAFLD. Different shades of color represent different intensities of expression

in this study, we combined the scRNA-seq data from GSE165784, GSE189175 and uncovered potential cross-talk and communication between T cells, macrophages and endothelial cells involved in the pathogenesis of these diseases (Fig. 11).

In the two datasets, the presence of T cells, macrophages and endothelial cells implied their active participation in disease progression and highlighted their potential as therapeutic targets. Endothelial cells form the inner layer of blood vessels and served vital functions in maintaining vascular integrity and regulating blood flow [25]. DR is commonly considered as a neurovascular disease and is primarily characterized by endothelial dysfunction [26]. The disruption blood-retinal barrier (BRB) ultimately led to pathological manifestations such as hemorrhages, exudation and neovascularization [27]. The development of NAFLD and liver fibrosis were attributed to the alteration of hepatic endothelium, particularly the unique subtype of endothelial cells known as liver sinusoidal endothelial cells (LSECs) [28]. T cells were essential in regulating adaptive immune responses and involved in recognizing and eliminating foreign pathogens or abnormal cells [29]. Macrophages, on the other hand, are immune cells that engulf and digest cellular

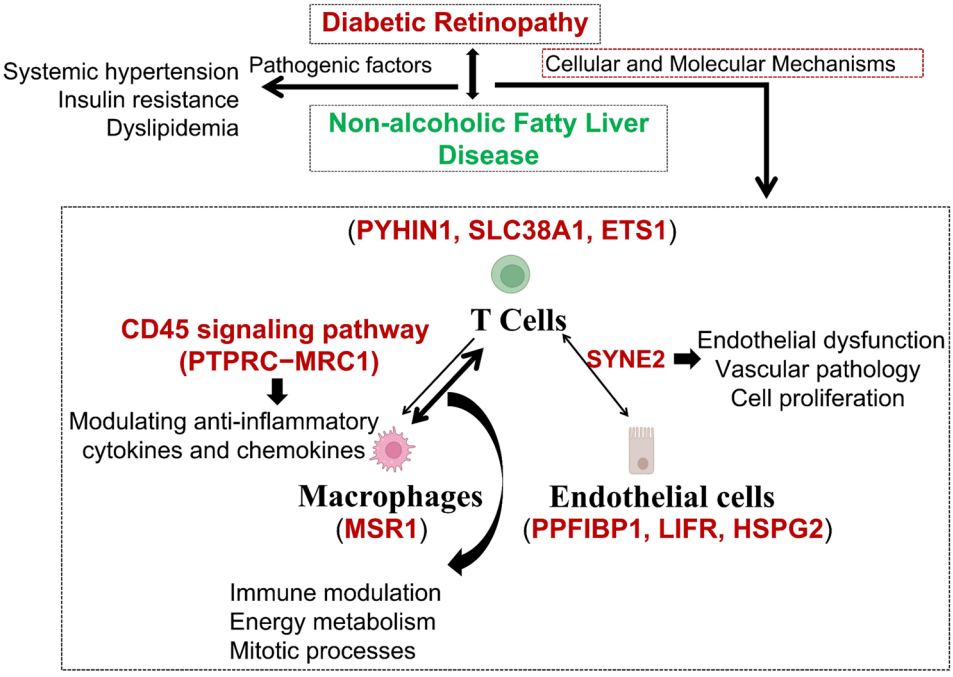

Fig. 11 Cellular and molecular mechanisms involved in the PDR and NAFLD pathogenesis

debris, pathogens and foreign substances [30]. T cells and macrophages directly or indirectly influenced the progression of PDR and NAFLD by regulating inflammatory responses, cytokine production and immune cell activation [31–34]. Understanding the three cell types specific roles and interactions within the vascular progression and immune regulation could provide valuable insights for developing targeted interventions and potential treatment strategies.

By comparing and analyzing DEGs within the two datasets, we could reveal associations, similarities and overlaps between DR and NAFLD. Notably, SYNE2 are expressed in T cells and endothelial cells, suggesting their potential involvement in the pathogenesis of two disease. SYNE2 is a protein involved in cell nucleus and endoplasmic reticulum structures. As was well known that SYNE2 contributes to endothelial dysfunction, vascular pathology and cell proliferation [35, 36]. While there was limited direct research on the relationship between SYNE2, DR and NAFLD, it is possible to explore potential connections based on the known functions and pathways associated with SYNE2. Then, we accidentally identified the common genes in T cells (PYHIN1, SLC38A1, ETS1), endothelial cells (PPFIBP1, LIFR, HSPG2) and macrophages (MSR1) among the top 50 DEGs.

PYHIN1, known as Interferon Gamma Inducible Protein 16, was involved in innate immune responses and had been associated with the inflammatory pathways [37]. SLC38A1 played a crucial role in glutamine transport and metabolism, potentially triggering cells proliferation, migration, invasion and senescence [38]. ETS1 was a transcription factor that regulated various cellular processes, including T cell development, differentiation, activation and new blood vessels formation [39, 40]. PPFIBP1 is a protein coding gene and participates in cell adhesion and migration through inflammatory pathway [41]. LIFR, a receptor for leukemia inhibitory factor, may contribute to growth factor binding activation and ciliary neurotrophic factor receptor activity [42]. HSPG2 encoded a perlecan protein, which was a major component of the extracellular matrix and involved in cell-matrix interactions and angiogenesis [43]. MSR1 was a macrophage scavenger receptor, which played a crucial role in macrophage-mediated immune responses, inflammation. It could also ameliorate pathological deposition of cholesterol in blood vessel walls [44]. Further analysis and functional studies are necessary to understand the specific roles of these genes in T cell biology, endothelial cell function and macrophage-mediated immune responses.

The pseudo-time analysis demonstrated that the calculated cell clusters exhibited common characteristics and stable developmental trajectories across the two datasets. This suggested that there were underlying similarities in the cellular dynamics between the two disease states. However, it was important to note that cell types were also in dynamic changes during development in different disease states, which accounted for complex distribution pattern of macrophage. Furthermore, our findings showed up-regulated expression of SYNE2,

EST1, PPFIBP1, and HSPG2 with PDR and NAFLD progression. On the other hand, SLC38A1, PYHIN1, HSPG2 and LIFR exhibited different expression patterns, which could be attributed to multi-factors, including different disease states or time points, variations in sample sources or experimental conditions, data noise and variability.

Ligand-receptor interactions are pivotal for revealing intercellular communication in essential signaling pathways. The analysis of cell communication revealed that T cells exhibited a stronger interaction with macrophages compared to other cell types in PDR and NAFLD datasets. This finding was consistent with the pathogenesis of PDR with NAFLD, indicating that immune cells may serve as the primary source of signaling molecules that can impact the behavior of other cells. Our study identified the CD45 signaling pathway as the most important pathway and PTPRC-MRC1 interaction was the major ligand-receptor contributor in both datasets. PTPRC was a transmembrane protein tyrosine phosphatase and predominantly expressed on immune cells, including T cells and macrophages [45]. The FVM of PDR was characterized by the accumulation of abundant myofibroblasts, which exhibited infiltrating fibroblasts through PTPRC co-localized expression [46]. In addition, upregulated PTPRC expression was observed in the liver of NAFLD mice and simultaneously decreased the release of IL-2, IL-13, IL-17 and TGF-β inflammatory factors [47, 48]. MRC1 was a receptor primarily expressed on macrophages and modulated phagocytosis, antigen presentation and immune responses. Previous studies had demonstrated that MRC1 receptor agonists exerted a protective effect on experimental DR by modulating anti-inflammatory cytokines and chemokines [49]. MRC1 was associated with resolving inflammation and promoting tissue homeostasis in NAFLD. Relevant research had showed that MRC1 could downregulate the production of pro-inflammatory cytokines, such as IL-1β, TNF-α and IL-6 [50].

In this study, the GSVA and GSEA analyses provided additional evidence to support the biological correlation between T cells and macrophages. Meanwhile, these findings strengthened comprehension between T cells and macrophages by identifying shared enriched gene sets and highlighting their involvement in cellular communication, immune modulation, energy metabolism, mitotic processes and signaling pathways. In addition, our research has some limitations. For example, the identified DEGs, associated pathways, and modules were only generated from bioinformatics analysis. It is important to note that further research is still needed to fully elucidate the intricate relationship between the liver and the eyes in different physiological and pathological contexts. Nonetheless, these findings emphasize the significance of considering the liver-eye axis in clinical practice and highlight the potential for integrated approaches that bridge the gap between hepatology and ophthalmology.

Conclusions

In conclusion, this study sheds significant light on the cellular and molecular dynamics underpinning the development of PDR and NAFLD. We have specifically concentrated on the roles of T cells, macrophages, and endothelial cells within the retinal environment. The CD45 signaling pathway was identified as a key facilitator of cell-cell communication, essential for understanding disease mechanisms. By exploring the cellular diversity and molecular profiles associated with these conditions, this research paves the way for more precise and effective therapeutic interventions. Ongoing research in this area is poised to enhance disease management and foster the development of tailored treatment options for individuals suffering from PDR and NAFLD.

Supplementary Information

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Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8
Supplementary Material 9
Supplementary Material 10

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Author contributions
Shuyan Zhang: Writing– original draft, Reviewing and Editing, Visualization, Conceptualization, Methodology, Software, Data Curation. Jiajun Wu: Supervision. Leilei Wang: Supervision. Cheng Zhang: Supervision. Yinjian Zhang: Review and project administration. Yibin Feng: Review and project administration. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval

The data used in this study were sourced from the GEO database, are publicly available and anonymized, and therefore do not require ethical review according to the institution's guidelines.

Consent for publication

This manuscript was published with the approval of all authors, and there was no conflict of interest in the submission.

Competing interests

The authors declare no competing interests.

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