

Multi-omics integrative analysis reveals shared immunometabolic features between breast cancer and type 2 diabetes through CLEC7A associated myeloid immune reprogramming and the MIF signaling axis

Jia Li

Qilu Medical University

Chundi Gao

Shandong Second Medical University

Minghui Wu

Weihai Municipal Hospital, Shandong University

Yanyan Pan

Qilu Medical University

Xiaoming Zhang

Qilu Medical University

Changgang Sun

zyxyscg@sdsu.edu.cn

Shandong Second Medical University

Research Article

Keywords: breast cancer, type 2 diabetes mellitus, CLEC7A, MIF-CD74/CXCR4, myeloid immunity, single-cell RNA sequencing, multi-omics analysis

Posted Date: September 1st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10535830/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Multi-omics integrative analysis reveals shared immunometabolic features between breast cancer and type 2 diabetes through CLEC7A associated myeloid immune reprogramming and the MIF signaling axis

Jia Li^{1,†}, Chundi Gao^{2,3,†}, Minghui Wu⁴, Yanyan Pan¹, Xiaoming Zhang¹, Changgang Sun^{2,3,*}

¹ Clinical Medical College, Qilu Medical University, Zibo 255300, Shandong, China

² College of Traditional Chinese Medicine, Shandong Second Medical University, Weifang 261000, Shandong, China

³ Department of Oncology, Weifang Traditional Chinese Hospital, Weifang 261000, Shandong, China

⁴ Weihai Municipal Hospital, Cheeloo College of Medicine, Shandong University, Weihai 264200, Shandong, China

† Jia Li and Chundi Gao contributed equally to this work.

*** Correspondence:** Changgang Sun, College of Traditional Chinese Medicine, Shandong Second Medical University, and Department of Oncology, Weifang Traditional Chinese Hospital, No. 7166 Baotong West Street, Weifang 261000, Shandong, China. Email: zyxyscg@sdsu.edu.cn

Running title: CLEC7A-associated myeloid reprogramming in BC and T2DM

Abstract

Background: Breast cancer (BC) and type 2 diabetes mellitus (T2DM) are closely associated at the epidemiological level; however, the potential shared immunometabolic mechanisms between them remain unclear.

Methods: This study integrated publicly available bulk transcriptomic and single-cell RNA sequencing datasets to systematically characterize the shared molecular features and immune microenvironment remodeling mechanisms between breast cancer (BC) and type 2 diabetes mellitus (T2DM) from a cross-disease perspective.

Results: A total of 37 genes exhibiting shared differential expression patterns between BC and T2DM were identified, which were primarily enriched in pathways associated with oxidative stress, glutathione metabolism, and immune responses. Protein–protein interaction (PPI) network analysis further identified 15 candidate hub genes, among which CLEC7A was the only gene showing a consistent upregulated trend in both diseases within the discovery cohorts. Notably, CLEC7A exhibited cell-type-specific expression in myeloid immune populations, particularly monocytes and macrophages. Cell–cell communication analysis revealed that MIF–CD74/CXCR4-associated signaling was significantly activated in both the BC tumor microenvironment and the peripheral immune microenvironment of T2DM, and its activity was positively correlated with CLEC7A

expression. Independent cohort analyses showed that CLEC7A was significantly upregulated in the main BC validation cohort and exhibited a generally increasing expression pattern in T2DM, although the statistical significance varied across cohorts. Its expression was consistently associated with myeloid immune infiltration and MIF receptor-related molecules.

Conclusions: This study suggests that breast cancer (BC) and type 2 diabetes mellitus (T2DM) may share features of myeloid immune reprogramming driven by metabolic dysregulation. The CLEC7A-marked myeloid immune state may participate in cross-disease inflammatory communication and microenvironmental remodeling through the MIF–CD74/CXCR4 signaling axis. These findings provide new insights into the immunometabolic mechanisms underlying T2DM-associated BC and highlight potential therapeutic targets.

Keywords: breast cancer; type 2 diabetes mellitus; CLEC7A; MIF–CD74/CXCR4; myeloid immunity; single-cell RNA sequencing; multi-omics analysis.

1 Introduction

Breast cancer (BC) is one of the most common malignancies among women, with its incidence and disease burden continuing to rise. Type 2 diabetes mellitus (T2DM), characterized by insulin resistance and chronic metabolic dysregulation, has increasingly been recognized as closely associated with the initiation and progression of various cancers [2–10]. Epidemiological studies have demonstrated a modestly increased overall risk of BC in women with T2DM, with a more pronounced association observed in postmenopausal women. Moreover, patients with BC complicated by T2DM often exhibit poorer survival outcomes and more adverse clinicopathological features [2–10]. Among the subtypes of BC, triple-negative breast cancer (TNBC) is of particular interest due to its high aggressiveness, limited therapeutic options, and poor prognosis, highlighting the need to better understand its potential biological association with T2DM [13].

At present, research on the relationship between T2DM and BC has gradually shifted from simple epidemiological associations to mechanistic investigations. Existing studies have shown that the two conditions share multiple risk factors, including obesity, chronic low-grade inflammation, insulin resistance, and metabolic dysregulation [14,15]. However, bidirectional Mendelian randomization analyses have not identified a stable genetic causal relationship between T2DM and the overall risk of BC [16,17]. These findings suggest that the association between T2DM and BC may not be driven by direct genetic factors, but rather by a systemic

inflammatory state arising from long-term metabolic abnormalities, which may promote tumor progression by influencing immune cell function and the tumor microenvironment. Therefore, elucidating the crosstalk mechanisms between these two diseases from the perspective of “metaflammation–immune microenvironment remodeling” has become an important focus of current research.

Myeloid immune cells play a critical bridging role between metabolic dysregulation and tumor progression. T2DM is characterized by a chronic low-grade inflammatory state, in which myeloid cells such as monocytes, macrophages, and neutrophils are prone to functional reprogramming under conditions of hyperglycemia, dyslipidemia, and insulin resistance [18,19]. Meanwhile, within the BC microenvironment, tumor-associated macrophages and other myeloid cells promote immune evasion and tumor progression by releasing inflammatory mediators, recruiting chemokines, and mediating intercellular communication.

Previous studies have demonstrated that the inflammatory signaling axis composed of macrophage migration inhibitory factor (MIF) and its receptors CD74 and CXCR4 plays a pivotal role in inflammation amplification, immune regulation, and tumor progression [20–22]. In addition, CLEC7A (Dectin-1), an important myeloid immune receptor, is closely associated with macrophage polarization and the establishment of an immunosuppressive microenvironment [23,24]. However, in the context of T2DM and BC, it remains unclear whether CLEC7A-positive myeloid cells represent a shared immune reprogramming state and how they are linked to MIF–CD74/CXCR4-mediated inflammatory communication, as systematic investigations on this topic are still lacking.

In recent years, advances in single-cell RNA sequencing (scRNA-seq) and cell–cell communication analysis have provided powerful tools for dissecting immune microenvironment remodeling in complex diseases. Most existing studies have focused on the immune characteristics within either T2DM or BC alone, while integrative cross-disease analyses exploring shared immunometabolic mechanisms remain relatively limited. In particular, at the single-cell level, it remains unclear how T2DM-associated metaflammation drives the remodeling of myeloid cells in BC, and whether persistent inflammatory amplification is mediated through specific ligand–receptor communication networks, as systematic evidence on these aspects is still lacking.

Based on this rationale, the present study integrates publicly available bulk transcriptomic and scRNA-seq datasets to establish a multi-omics analytical framework comprising “shared differentially expressed gene identification–functional enrichment analysis–PPI hub gene screening–single-cell localization–cell–cell communication analysis–immune correlation validation.” Using this framework, we systematically investigate the shared immunometabolic mechanisms between T2DM and BC.

We propose that the association between T2DM and BC is primarily characterized by “metabolic dysregulation-driven myeloid immune reprogramming,” in which CLEC7A-positive myeloid cells and the MIF–CD74/CXCR4 inflammatory signaling axis may serve as key regulatory components. This study aims to elucidate the shared mechanisms of immune microenvironment remodeling between T2DM and BC from a cross-disease multi-omics perspective, and to provide a theoretical basis for future therapeutic strategies targeting myeloid inflammatory communication.

2 Materials and methods

2.1 Overall Study Design

This study was based on publicly available multi-omics datasets and aimed to systematically elucidate the potential shared immunometabolic features between breast cancer (BC) and type 2 diabetes mellitus (T2DM), as well as to identify key immune-related factors with cross-disease relevance.

An integrated strategy combining stepwise screening and multidimensional analyses was employed to construct an analytical framework comprising “candidate gene identification–multidimensional feature screening–cellular source characterization–functional association validation.” The workflow was as follows: first, differentially expressed genes (DEGs) for BC and T2DM were identified separately through bulk transcriptomic analysis, and their intersection was defined as shared DEGs. Subsequently, functional enrichment analysis and protein–protein interaction (PPI) network construction were performed on the shared DEGs, and candidate hub genes were preliminarily screened based on network topological features.

On this basis, a multidimensional prioritization strategy was applied to further evaluate the candidate hub genes. The selection criteria included: (1) consistency of expression patterns across diseases; (2) cell type specificity at the single-cell level; (3) correlation with immune infiltration and myeloid marker genes; and (4) association with inflammation-related signaling pathways, particularly the MIF signaling axis.

The scRNA-seq datasets of BC and T2DM were further integrated to determine the cellular sources of the candidate genes, and cell–cell communication analysis was performed to elucidate their potential intercellular interaction mechanisms. Finally, independent cohort validation, immune infiltration analysis, and pathway correlation analysis were conducted to systematically evaluate the identified key molecules.

This study primarily focuses on immune- and metabolism-related expression patterns that are stably conserved across different tissue contexts, rather than tissue-specific alterations. In addition, the aim is to characterize shared immunometabolic features across diseases rather than to infer direct causal relationships. All data used in this study were obtained from publicly available databases and were fully de-identified, and no new clinical samples were collected; therefore, additional ethical approval was not required.

2.2 Bulk Transcriptomic Data Analysis

2.2.1 Analysis Strategy and Data Sources

Gene expression datasets related to BC and T2DM were obtained from the NCBI Gene Expression Omnibus (GEO) database ([http:// www. ncbi. nlm. nih. gov/ geo/](http://www.ncbi.nlm.nih.gov/geo/)). A total of four datasets based on the GPL570 platform (GSE7014, GSE76894, GSE10810, and GSE42568) were included in this study. Among them, GSE7014 and GSE10810 were used as discovery cohorts, while GSE76894 and GSE42568 served as validation cohorts.

Specifically, GSE7014 included 6 normal controls, 10 patients with type 1 diabetes, and 20 patients with T2DM; only the normal control group and the T2DM group were included in the present analysis. GSE76894 comprised 84 normal controls and 19 T2DM samples. GSE10810 included 27 normal controls and 31 BC samples, whereas GSE42568 contained 17 normal controls and 104 BC samples.

2.2.2 Differential Expression and Functional Enrichment Analysis

The limma package was used for data normalization and differential expression analysis of the expression matrices [17]. Differentially expressed genes (DEGs) were identified using the criteria of adjusted $P < 0.05$ and $|\log_2 \text{fold change (FC)}| \geq 1$. Shared DEGs were determined using the VennDiagram package.

Functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, was performed using the clusterProfiler package [18]. Given that bulk transcriptomic data represent the average expression levels across heterogeneous cell populations, the identified DEGs cannot be directly attributed to specific cellular sources and therefore require further interpretation in conjunction with single-cell analyses.

2.2.3 PPI Network Construction and Hub Gene Identification

To investigate the protein–protein interaction (PPI) relationships among the shared DEGs, these genes were imported into the STRING database (<https://string-db.org/>) to construct a PPI network [19]. To explore potential weak interactions under conditions of a limited number of shared DEGs, a relatively low confidence score threshold of 0.15 was applied in STRING to generate an exploratory PPI network, which was primarily used to obtain a pool of candidate genes.

To mitigate the potential false-positive risk associated with the low threshold, subsequent multidimensional filtering was performed, incorporating expression consistency, single-cell localization, immune relevance, and pathway associations. The PPI network was then imported into Cytoscape software, and topological analysis was conducted using the cytoHubba plugin [20]. The top 15 genes ranked by the Degree algorithm were selected as the candidate hub gene set.

2.3 Single-Cell RNA Sequencing Data Analysis

To determine the cellular sources and cross-disease expression patterns of the shared hub genes identified from bulk transcriptomic analysis, scRNA-seq data were incorporated for analysis at single-cell resolution.

2.3.1 Data Acquisition

The scRNA-seq datasets were obtained from the GEO database. The breast cancer dataset GSE161529 includes 69 samples; to minimize inter-individual variability, two pairs of matched samples (adjacent normal tissue and primary tumor tissue from the same patients; a total of four samples) were selected for analysis. The T2DM dataset GSE255566 comprises eight samples, including three normal controls and five patients with T2DM.

Given that only two pairs of matched samples were included in the BC scRNA-seq analysis, this component was primarily used to assist in identifying the cellular sources of candidate genes and to explore potential cell–cell communication patterns. Therefore, the corresponding findings should be interpreted in conjunction with results from bulk transcriptomic cohorts and external databases.

2.3.2 Data Processing and Cell Type Annotation

Single-cell transcriptomic data were processed using the Seurat package (v5.0) for quality control, normalization, batch correction, and clustering analysis [14]. Cells with fewer than 50 detected genes or with a mitochondrial gene proportion exceeding 15% were excluded. Data normalization was performed using the LogNormalize method, followed by data scaling and principal component analysis (PCA). After batch effect correction using Harmony, clustering and uniform manifold approximation and projection (UMAP) dimensionality reduction were conducted based on the top 20 principal components.

Cell type annotation was performed by combining automated annotation using the SingleR package with manual curation based on canonical marker genes. Marker genes for each cell cluster were identified using the FindAllMarkers function (parameters: `only.pos = FALSE`, `min.pct = 0.25`, `logfc.threshold = 0.585`). Differential expression analysis between case and control groups was conducted using the FindMarkers function (parameters: `logfc.threshold = 0.1`, `min.pct = 0.1`), with selection criteria of $|\log_2FC| > 0.585$ and adjusted $P < 0.05$.

2.3.3 Cell–Cell Communication Analysis

Cell–cell communication analysis was performed using the CellChat R package [21]. Based on a curated database of known human ligand–receptor interactions, the communication

probability between different cell types was calculated. Network plots and bubble plots were used to visualize the number and strength of intercellular interactions, as well as the communication patterns of specific ligand–receptor pairs.

2.4 Validation of Hub Genes and Mechanistic Analysis

2.4.1 Comprehensive Evaluation Strategy for Candidate Key Molecules

To identify key immune-related molecules with cross-disease relevance from the candidate hub genes derived from the PPI network, a multidimensional screening framework was established. The evaluation criteria included: (1) network topological features, with gene importance assessed based on connectivity within the PPI network; (2) cross-disease expression consistency, by comparing the direction and significance of gene expression changes in BC and T2DM; (3) single-cell localization characteristics, by evaluating the distribution and specificity of gene expression across different cell types using scRNA-seq data; (4) immune relevance, by analyzing the correlation between gene expression and immune cell infiltration levels as well as myeloid marker genes; and (5) association with inflammatory signaling pathways, particularly by assessing the correlation between candidate genes and key components of the MIF signaling pathway (e.g., CD74 and CXCR4).

2.4.2 Identification of Key Molecules

Following comprehensive evaluation of the 15 candidate hub genes based on the multidimensional screening framework described above, notable heterogeneity was observed across different evaluation criteria. Some genes exhibited high network connectivity but lacked consistent expression trends across diseases; others showed no clear cell type specificity; while certain genes were associated with immune infiltration but did not demonstrate stable correlations with inflammatory signaling pathways.

In contrast, CLEC7A consistently met multiple key criteria: (1) it showed a generally concordant upward expression pattern across BC and T2DM analyses; (2) it was predominantly localized to myeloid immune cells, including monocytes and macrophages, at the single-cell level; (3) it showed significant correlations with immune infiltration and multiple myeloid marker genes; and (4) it exhibited stable associations with key receptor molecules in the MIF

signaling pathway. Therefore, CLEC7A was identified as one of the few candidate genes that satisfied all the multidimensional selection criteria and was selected for subsequent in-depth analysis.

2.4.3 Validation of Hub Genes

GSE76894 and GSE42568 were selected as independent external validation cohorts. Expression profile data from these datasets were preprocessed and analyzed using R software (v4.5.2). Raw probe-level data were converted into gene expression matrices, and when multiple probes corresponded to the same gene, their values were averaged using the *avereps* function in the limma package. The necessity for $\log_2$ transformation was determined based on the quantile distribution of the expression data; if required, values less than 0 were set to 0, followed by $\log_2(x+1)$ transformation. Subsequently, data normalization was performed using the *normalizeBetweenArrays* function in the limma package.

Expression data for the hub gene were extracted, and samples were classified into disease and normal control groups according to clinical annotations. Violin plots with overlaid boxplots were generated using the *ggviolin* function from the *ggpubr* package. Differences in gene expression between groups were assessed using the Wilcoxon rank-sum test via the *stat_compare_means* function, with $P < 0.05$ considered statistically significant.

To further evaluate the robustness of CLEC7A expression patterns, an additional T2DM validation cohort and a paired breast cancer tissue dataset (GSE15852) were included for supplementary analysis. In the T2DM cohort, CLEC7A expression levels were compared between groups using the Wilcoxon rank-sum test. The GSE15852 dataset, which includes 43 pairs of breast cancer tissues and matched normal breast tissues, was analyzed using the paired Wilcoxon signed-rank test to assess differences in CLEC7A expression between tumor and corresponding normal tissues.

2.4.4 Single-Cell-Level Expression Analysis of Hub Genes

The Seurat package (v5.0) was used to analyze the expression patterns of hub genes across different cell populations. The preprocessed Seurat object was loaded, and the list of hub genes was imported using the *read.table* function. Genes with detectable expression in the expression matrix were retained for downstream analysis.

The single-cell expression characteristics of the candidate hub genes were visualized using *FeaturePlot*, *VlnPlot*, and *DotPlot*. These methods were applied to display the spatial distribution of gene expression in UMAP space, compare expression levels across different cell clusters, and illustrate the proportion of expressing cells along with the average expression intensity in each cell population, respectively. Visualization was primarily performed using the *scRNAtoolVis* package.

2.5 Clinical Relevance and Immunological Mechanism Analysis of CLEC7A

2.5.1 Survival Analysis (TCGA-BRCA)

The prognostic value of CLEC7A in breast cancer was evaluated based on the TCGA-BRCA dataset using the GEPIA2 online platform (<http://gepia2.cancer-pku.cn/>). Patients were stratified into high- and low-expression groups according to the median expression level of CLEC7A. Overall survival (OS) curves were generated using the Kaplan–Meier method, and differences between groups were assessed using the log-rank test, with $P < 0.05$ considered statistically significant.

2.5.2 Immune Infiltration Analysis

In BC, the association between CLEC7A expression and immune cell infiltration was analyzed using the TIMER2.0 database (<http://timer.cistrome.org/>) [22]. Spearman correlation analysis was performed to assess the relationships, with tumor purity adjusted using partial correlation.

In the T2DM datasets, immune infiltration analysis was conducted based on GEO expression matrix data. The expression data were preprocessed by removing duplicate genes and performing $\log_2$ transformation when necessary. Single-sample gene set enrichment analysis (ssGSEA) was performed using the GSVA package (version 2.4.4) to calculate enrichment scores for monocytes, neutrophils, and macrophages [23]. Differences in immune cell infiltration scores between the T2DM and control groups were evaluated using the Wilcoxon rank-sum test. Spearman correlation analysis was further applied to assess the association between CLEC7A expression and immune cell enrichment scores.

2.5.3 Correlation Analysis of the MIF Signaling Pathway

Genes associated with the MIF signaling pathway, including MIF, CD74, CXCR4, CD44, and ACKR3, were selected for correlation analysis. In the TCGA-BRCA dataset, gene expression correlation analysis was performed using the GEPIA2 online platform. For the T2DM dataset, correlation analysis was conducted based on GEO expression matrix data. Before analysis, the expression matrices were preprocessed by removing duplicated genes and applying log2 transformation when necessary. The Spearman correlation analysis was used to evaluate the expression correlations between CLEC7A and the selected MIF signaling pathway-related genes. Multiple testing correction was performed using the Benjamini–Hochberg method, and the adjusted P values were reported as the false discovery rate (FDR). A correlation matrix was generated for visualization of the gene expression relationships.

2.5.4 Validation of CLEC7A Expression and Correlation Analysis with Myeloid Marker Genes

CLEC7A expression levels were extracted from the T2DM dataset, and differences in CLEC7A expression between the T2DM and control groups were evaluated using the Wilcoxon rank-sum test. A panel of myeloid-related marker genes, including CD14, LYZ, CD163, CSF1R, and S100A8, was selected for correlation analysis. The expression correlations between CLEC7A and these myeloid marker genes were assessed using Spearman correlation analysis, followed by multiple testing correction using the Benjamini–Hochberg method.

2.5.5 Analysis Using the Human Protein Atlas Database

To validate the expression characteristics of CLEC7A at the protein level, the Human Protein Atlas (HPA) database (<https://www.proteinatlas.org/>) was utilized to analyze its protein expression distribution across normal human tissues. The “Tissue” module was used to obtain protein expression profiles of CLEC7A across different tissue categories, with particular attention paid to immune-related tissues, including bone marrow and lymphoid tissues.

3 Results

3.1 Bulk Transcriptomic Analysis Identified Shared Molecular Features Between BC and T2DM

3.1.1 Identification and Functional Enrichment Analysis of Shared Differentially Expressed Genes

Differential expression analysis was performed using the T2DM discovery cohort GSE7014 (excluding samples from individuals with type 1 diabetes) and the BC discovery cohort GSE10810. A total of 730 and 1,372 differentially expressed genes (DEGs) were identified in T2DM and BC, respectively. Intersection analysis yielded 37 shared DEGs, including 8 commonly upregulated genes and 29 commonly downregulated genes (Figure 1A).

Gene Ontology (GO) enrichment analysis revealed that the shared DEGs were primarily involved in biological processes associated with cellular stress responses and defense mechanisms, including responses to toxic substances, metal ions, and oxidative stress, as well as immune-related processes such as phagocytosis and cytokine production (Figure 1B). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis demonstrated that these shared DEGs were significantly enriched in glutathione metabolism, cytochrome P450-mediated xenobiotic metabolism, and chemical carcinogenesis-related pathways (Figure 1C).

Collectively, these findings indicate that BC and T2DM exhibit a certain degree of molecular convergence at the transcriptomic level, characterized predominantly by shared alterations in oxidative stress, metabolic detoxification, and immune response-related processes. These results suggest the existence of a functional inflammatory–metabolic regulatory network linking the two diseases, which may be driven primarily by non-genetic factors.

3.1.2 Identification of Hub Genes Through PPI Network Analysis

Based on the STRING database, a protein–protein interaction (PPI) network was constructed. After removing isolated nodes, 29 genes were retained, resulting in a network comprising 29 nodes and 58 edges. Topological analysis revealed that PDK4, GPX3, RASD1, PFKFB3, GABARAPL1, IRS2, EPHX1, and CLEC7A exhibited relatively high connectivity and occupied central positions within the network (Figure 1D). Subsequently, the cytoHubba plugin was applied, and the Degree algorithm identified 15 hub genes: PDK4, GPX3, RASD1,

PFKFB3, GABARAPL1, IRS2, EPHX1, MGST1, MT1X, MT1M, ADM, PTX3, CLEC7A, PLA2G2A, and RHOU (Figure 1E).

Among these candidate genes, CLEC7A has a well-established role in myeloid cell-mediated immune responses and was the only commonly upregulated gene identified in the discovery cohort, suggesting that it may play an important role in inflammation- and immune-related processes.

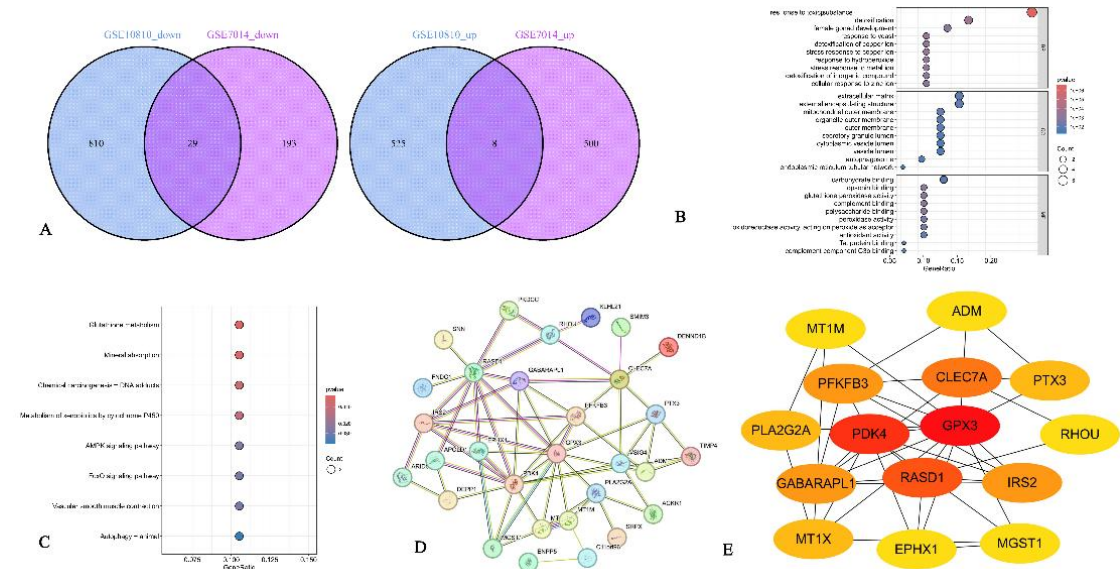


Figure 1. Identification, functional enrichment, and protein-protein interaction analysis of shared differentially expressed genes between breast cancer and type 2 diabetes mellitus. (A) Venn diagrams showing differentially expressed genes shared between the breast cancer (BC) discovery cohort GSE10810 and the type 2 diabetes mellitus (T2DM) discovery cohort GSE7014. The left diagram shows 29 genes commonly downregulated in both diseases, whereas the right diagram shows eight genes commonly upregulated. (B) Gene Ontology (GO) enrichment analysis of the 37 shared differentially expressed genes, including the biological process (BP), cellular component (CC), and molecular function (MF) categories. Dot size represents the number of enriched genes, and dot color indicates statistical significance. (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the shared differentially expressed genes. Dot size represents the number of enriched genes, and dot color indicates statistical significance. (D) Protein-protein interaction (PPI) network of the shared differentially expressed genes constructed using the STRING database. A confidence score threshold of 0.15 was applied. After removal of isolated nodes, the network contained 29 nodes and 58 edges. (E) Network of the top 15 candidate hub genes identified using the Degree algorithm in the cytoHubba plugin of Cytoscape. The candidate genes were PDK4, GPX3, RASD1, PFKFB3, GABARAPL1, IRS2, EPHX1, MGST1, MT1X, MT1M, ADM, PTX3, CLEC7A, PLA2G2A, and RHOU. Node colors ranging from red to yellow indicate decreasing degrees of connectivity.

3.2 Single-Cell Analysis Reveals the Cellular Origin of Shared Hub Genes

3.2.1 Single-Cell Atlas of BC and Expression Characteristics of Hub Genes

To characterize the cellular carriers of the shared molecular features identified above, single-cell RNA sequencing (scRNA-seq) analysis was performed on breast cancer (BC) samples. Following quality control, dimensionality reduction, data integration, and unsupervised clustering, a total of 14 cell clusters were identified and subsequently annotated into six major cell types, including epithelial cells, B cells, endothelial cells, fibroblasts, macrophages, and tissue stem cells (Figure 2A). UMAP visualization demonstrated distinct separation among the identified cell clusters. Epithelial cells represented the predominant cell population and were distributed across multiple clusters, suggesting substantial transcriptional heterogeneity.

Compared with adjacent normal tissues, the cellular composition of BC tissues exhibited marked alterations. Quantitative analysis revealed increased numbers of B cells and macrophages, whereas endothelial cells, fibroblasts, and tissue stem cells were markedly reduced in BC tissues (Figure 2B). Consistently, proportional analysis demonstrated an increased proportion of B cells and decreased proportions of endothelial cells, fibroblasts, and tissue stem cells in BC tissues (Figure 2C). The expression profiles of selected marker genes further demonstrated group-associated transcriptional differences between adjacent normal and BC tissues (Figure 2D), indicating substantial remodeling of the tumor microenvironment accompanied by pronounced alterations in immune cell composition.

The hub genes displayed heterogeneous expression patterns across the BC single-cell atlas, exhibiting varying degrees of enrichment within distinct regions of the UMAP embedding. Notably, CLEC7A expression was predominantly enriched in immune cell-associated regions, particularly within myeloid cell populations, which is consistent with the active immunoregulatory role of macrophages in the BC tumor microenvironment.

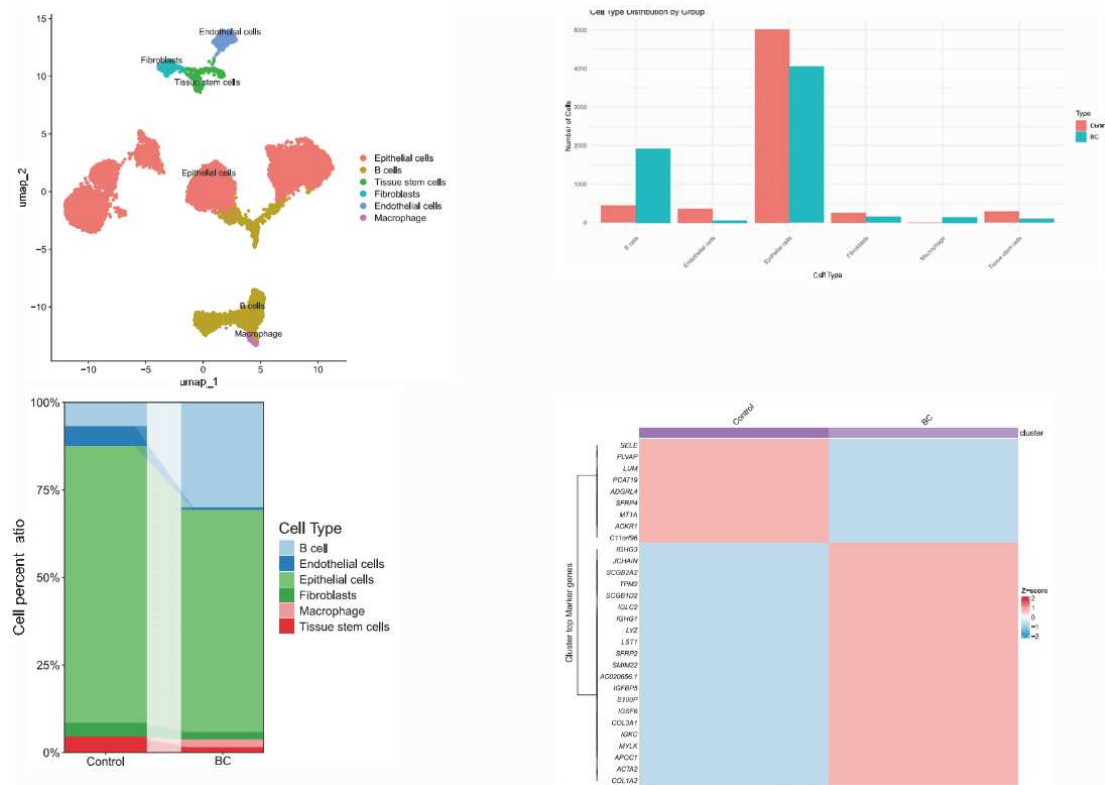


Figure 2. Single-cell transcriptomic landscape and cell-type composition of breast cancer tissues. Single-cell RNA sequencing data were obtained from GSE161529 and included two paired samples of primary breast cancer tissues and adjacent non-tumor breast tissues from the same patients. (A) Uniform manifold approximation and projection (UMAP) plot of the analyzed cells. Following clustering and cell-type annotation, six major cell types were identified: epithelial cells, B cells, endothelial cells, fibroblasts, macrophages, and tissue stem cells. (B) Comparison of the absolute numbers of each cell type between the control and BC groups. (C) Stacked bar plot showing the relative proportions of each cell type in the control and BC groups. (D) Heatmap showing the average expression levels of representative marker genes in the control and BC groups. Colors indicate gene-wise standardized expression values expressed as Z-scores.

3.2.2 Single-Cell Atlas of T2DM and Expression Characteristics of Hub Genes

To determine whether the shared hub genes exhibited similar cell type preferences within the peripheral immune microenvironment of T2DM, single-cell RNA sequencing (scRNA-seq) data from patients with T2DM were further analyzed. Following quality control, dimensionality reduction, data integration, and unsupervised clustering, a total of 23 cell clusters were identified and subsequently classified into eight major immune cell populations, including neutrophils, monocytes, CD4⁺ T cells, CD8⁺ T cells, natural killer (NK) cells, B cells, dendritic cells, and HSC G-CSF-like cells (Figures 3A–B). UMAP visualization demonstrated clear separation among the identified cell populations, with myeloid cells (monocytes and

neutrophils) and lymphoid cells occupying relatively distinct clustering regions (Figures 3A–B).

Compared with the healthy control group, the proportion of neutrophils was reduced in patients with T2DM, whereas the proportions of monocytes, CD4⁺ T cells, CD8⁺ T cells, NK cells, and B cells were increased (Figure 3C). The expression profiles of canonical marker genes further validated the accuracy of cell type annotation (Figure 3D). At the global transcriptomic level, differentially expressed genes (DEGs) in the T2DM group were predominantly enriched in biological processes related to metabolic regulation, immune activation, and effector functions. Cell type-specific analysis further revealed extensive transcriptional remodeling in myeloid cell populations, particularly monocytes and neutrophils. In addition, B cells exhibited transcriptional features indicative of maturation or post-class-switch differentiation, whereas T cells and NK cells displayed enhanced effector activity. By contrast, dendritic cells showed reduced expression of genes associated with antigen presentation. Collectively, these findings indicate that the peripheral immune microenvironment of T2DM is characterized by inflammation-associated transcriptional remodeling, with alterations in myeloid cells representing a central feature (Figure 3E). The complete global and cell type-specific differential expression results are provided in Supplementary Tables S1 and S2.

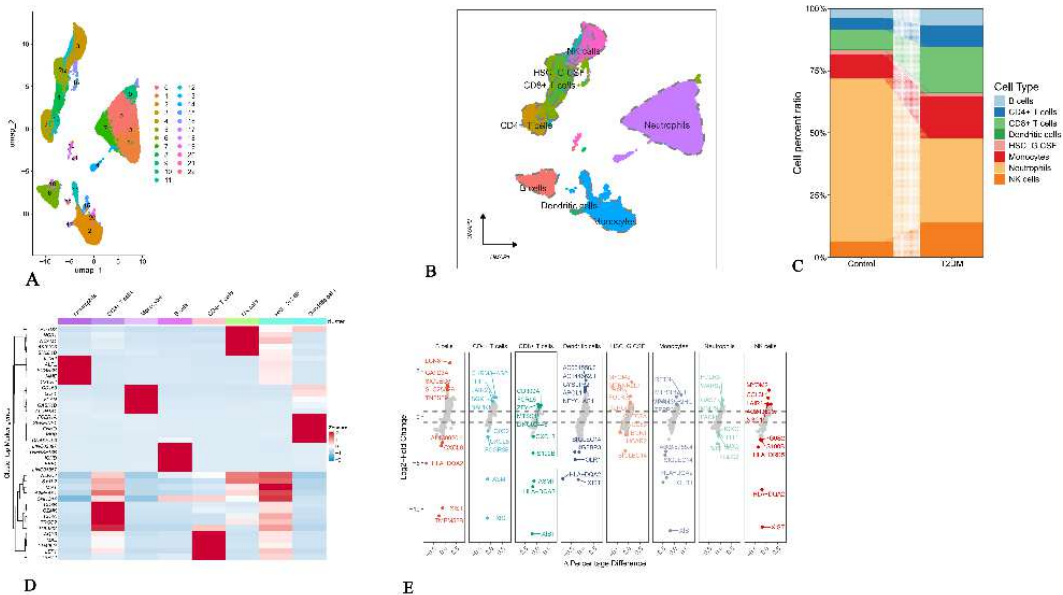
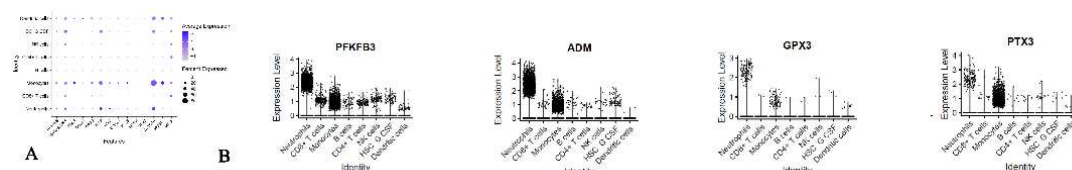


Figure 3. Single-cell transcriptomic landscape, cellular composition, and cell-type-specific transcriptional alterations in type 2 diabetes mellitus. Single-cell RNA sequencing data were obtained from GSE255566 and included samples from three healthy controls and five patients with T2DM. (A) UMAP and t-distributed stochastic neighbor embedding (t-SNE) plots of 23 cell clusters identified after quality control, batch correction, and clustering. Different colors represent distinct cell clusters. (B) UMAP plot showing cell-type annotations based on canonical marker genes. Eight immune cell types were identified: neutrophils, monocytes, CD4⁺ T cells, CD8⁺ T cells, natural killer cells, B cells, dendritic cells, and HSC G-CSF-like cells. (C) Stacked bar plot showing the relative proportions of each cell type in the control and T2DM groups. (D) Heatmap showing the expression of representative marker genes across the annotated cell types. Colors indicate standardized expression levels expressed as Z-scores. (E) Distribution of differentially expressed genes between the T2DM and control groups within each cell type. The x-axis represents the difference in the proportion of cells expressing each gene between the two groups, and the y-axis represents the log₂ fold change. Colors denote different cell types, and selected representative genes are labeled.

3.2.3 CLEC7A Is Predominantly Expressed in Myeloid Immune Cells

The single-cell expression patterns of the hub genes were further examined in the T2DM dataset to characterize their cell type-specific distribution. PLA2G2A was excluded from subsequent analyses because its expression was negligible across all cell populations. The remaining hub genes exhibited heterogeneous expression patterns with distinct cell type preferences. Specifically, PFKFB3, ADM, GPX3, and PTX3 were predominantly expressed in neutrophils, whereas MGST1 and CLEC7A were preferentially expressed in monocytes. In contrast, PDK4 and IRS2 were mainly distributed across both neutrophils and monocytes (Figure 4).

The preferential expression of CLEC7A in monocytes is consistent with the myeloid cell-dominated inflammatory remodeling observed in T2DM. Likewise, its immune cell-associated expression pattern in the BC scRNA-seq dataset is in agreement with the active immunoregulatory role of macrophages within the breast cancer tumor microenvironment. Among the candidate hub genes, CLEC7A was the only gene that consistently exhibited a myeloid cell-biased expression pattern across both diseases, whereas the remaining genes displayed either more dispersed expression profiles or weaker cell type specificity. Collectively, these findings indicate that the shared hub genes are predominantly enriched in myeloid immune cells, suggesting that myeloid cells may represent the principal cellular carriers of the shared transcriptional signatures linking BC and T2DM.



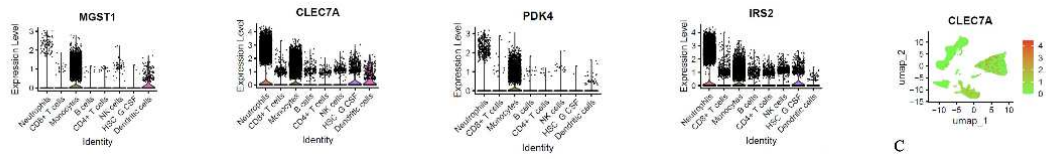


Figure 4. Single-cell expression patterns of candidate hub genes in type 2 diabetes mellitus. (A) Dot plot showing the expression of candidate hub genes across eight immune cell types. Dot size represents the percentage of cells expressing each gene, and dot color indicates the average expression level. PLA2G2A was excluded because of its extremely low expression across all cell populations. (B) Violin plots showing the expression distributions of PFKFB3, ADM, GPX3, PTX3, MGST1, CLEC7A, PDK4, and IRS2 across the annotated cell types. (C) UMAP feature plot showing the cellular distribution of CLEC7A expression. Increasing color intensity indicates higher expression. CLEC7A was predominantly expressed in monocytes and was also detectable in subsets of neutrophils and other myeloid-related cell populations.

3.3 Cell–Cell Communication Analysis Suggests That MIF-Related Inflammatory Signaling May Link BC and T2DM

3.3.1 Active MIF-Related Communication in BC

Cell–cell communication within the BC tumor microenvironment was comprehensively characterized using CellChat to infer ligand–receptor interactions. The analysis revealed a highly intricate intercellular communication network in BC tissues, with the most extensive interactions occurring among epithelial cells, fibroblasts, macrophages, and endothelial cells (Figure 5A). Quantitative analysis of communication strength further demonstrated that the interactions between epithelial cells and macrophages were the most prominent, whereas robust communication was also observed between epithelial cells and fibroblasts, as well as with other stromal cell populations (Figure 5B).

At the signaling pathway level, multiple secreted signaling pathways exhibited high activity, including MIF–(CD74+CXCR4), MIF–ACKR3, and CXCL12–CXCR4/ACKR3. Among these, MIF-associated signaling was broadly distributed across multiple cell types, suggesting a pivotal role in regulating inflammation, immune remodeling, and the reprogramming of intercellular communication within the BC tumor microenvironment (Figure 5C).

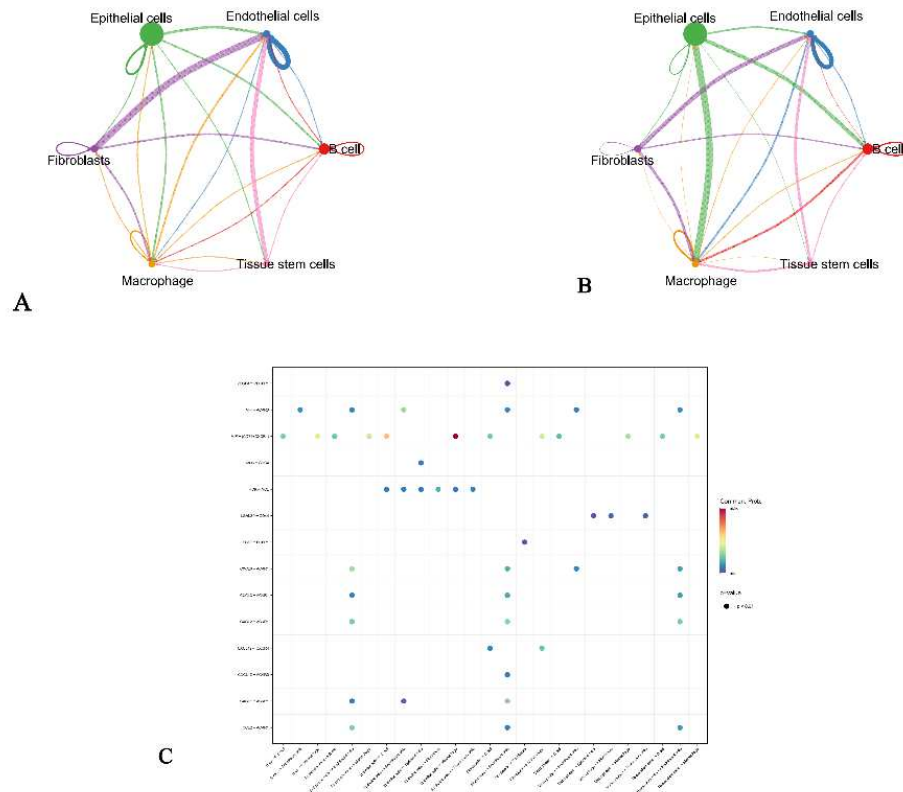


Figure 5. Intercellular communication networks and major ligand–receptor interactions in the breast cancer microenvironment. Cell–cell communication in the BC single-cell transcriptomic dataset was inferred using CellChat. (A) Network showing the inferred number of interactions among different cell types. Nodes represent cell types, and edge thickness indicates the number of inferred interactions between cell populations. (B) Network showing the overall interaction strength among different cell types. Edge thickness represents the inferred communication strength. (C) Bubble plot showing significant ligand–receptor interactions in the BC microenvironment. The x-axis represents sender–receiver cell-pair combinations, and the y-axis represents ligand–receptor pairs. Color indicates the inferred communication probability, and dots denote statistically significant interactions. Representative inflammatory signaling axes included MIF–(CD74+CXCR4), MIF–ACKR3, and CXCL12–CXCR4/ACKR3.

3.3.2 MIF-Related Communication in the T2DM Peripheral Immune Network

Cell–cell communication among peripheral immune cells in T2DM was systematically characterized using CellChat. The analysis revealed a highly interconnected communication network among distinct immune cell populations. Monocytes and dendritic cells participated in a large number of inferred interactions, whereas strong aggregate signaling involved several immune cell populations, particularly CD8⁺ T cells, NK cells, monocytes, and dendritic cells (Figures 6A–B).

Analysis of communication categories showed that cell–cell contact accounted for the largest proportion of inferred interactions in terms of both interaction number and aggregate

communication probability (Supplementary Tables S4 and S5). Nevertheless, secreted signaling also constituted a substantial component of the communication network and involved multiple inflammation-related pathways, indicating that cytokine- and chemokine-mediated crosstalk remains an important feature of the T2DM peripheral immune microenvironment.

At the intercellular ligand–receptor interaction level, MIF–(CD74+CD44) and MIF–(CD74+CXCR4) interactions were widely detected across multiple sender–receiver cell pairs, whereas MIF–(CD74+CXCR2) interactions showed a more restricted distribution. Several chemokine-related interactions, including CXCL8–CXCR1/2, CCL3–CCR1, and CCL5–CCR1, were also detected, with CXCL8-related signaling showing a broader distribution than CCL3- and CCL5-related signaling. In addition, NAMPT–INSR and RETN–TLR4 interactions were identified, suggesting potential links between metabolic dysregulation and immune-cell communication (Figure 6C; Supplementary Table S3).

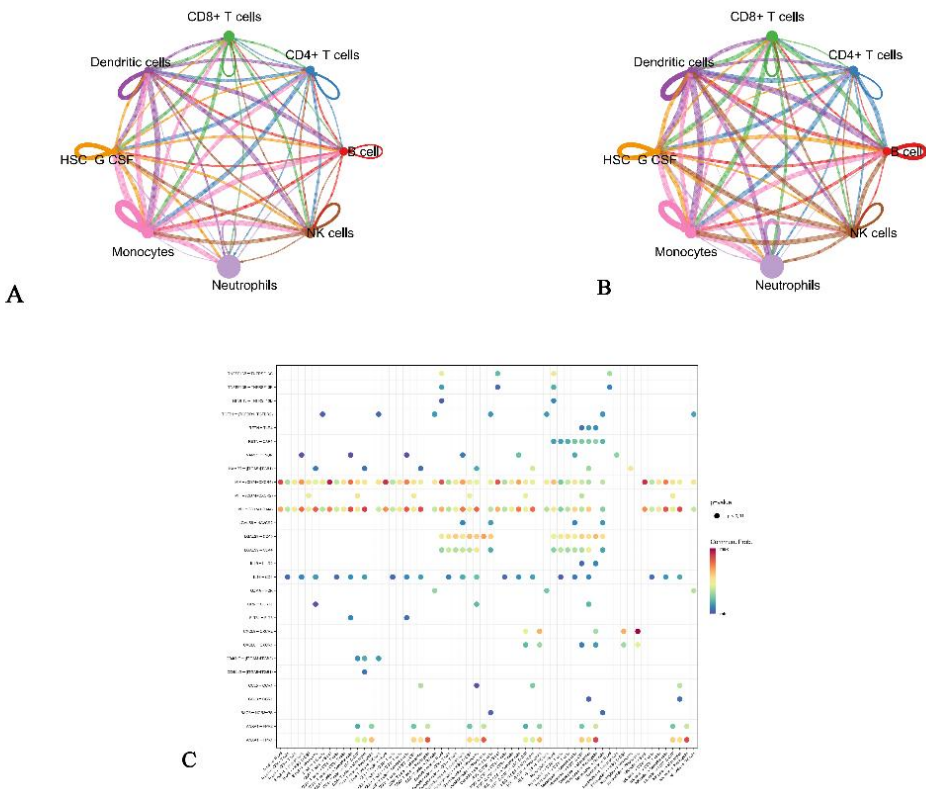


Figure 6. Intercellular communication networks and representative ligand–receptor interactions in type 2 diabetes mellitus. Cell–cell communication in the T2DM single-cell transcriptomic dataset was inferred using CellChat. (A) Network showing the inferred number of interactions among different immune cell types. Nodes represent cell types, and edge thickness indicates the number of inferred interactions. (B) Network showing the aggregate interaction strength among different immune cell types. Edge thickness represents the inferred communication strength. (C) Bubble plot showing significant ligand–receptor interactions among peripheral immune cells in T2DM. The x-axis represents sender–receiver cell-pair combinations, and the y-axis represents ligand–receptor pairs. Color indicates the inferred communication probability, and dots indicate interactions with $P < 0.01$. Representative interactions include MIF–(CD74+CD44), MIF–(CD74+CXCR2), MIF–(CD74+CXCR4), CXCL8–CXCR1/2, CCL3–CCR1, CCL5–CCR1, NAMPT–INSR, and RETN–TLR4.

3.3.3 The MIF–CD74 Axis Is Consistent with the Myeloid Expression Pattern of CLEC7A

MIF signaling exhibited consistently high activity in both BC and T2DM and was closely associated with myeloid immune cells in both disease contexts. In BC, MIF-mediated signaling primarily contributed to intercellular communication among epithelial cells, macrophages, and stromal cells within the tumor microenvironment. In contrast, in T2DM, MIF-associated communication was predominantly characterized by a broadly distributed peripheral immune communication network involving both myeloid and lymphoid cell populations.

Integration of the core gene expression profiling results further demonstrated that CLEC7A was predominantly enriched in myeloid immune cell populations, particularly monocytes and macrophages. Notably, MIF-mediated intercellular communication likewise prominently involved myeloid immune cells, suggesting that the myeloid immune state represented by CLEC7A may be functionally linked to the MIF–CD74-associated inflammatory communication network.

3.4 Validation of the Shared Immunological Features of CLEC7A Using Independent Cohorts and Clinical Immune Analyses

3.4.1 Validation of Hub Gene Expression in Independent Bulk Transcriptomic Cohorts

The expression profiles of the 15 hub genes were further validated using independent bulk transcriptomic datasets. In the BC validation cohort (GSE42568), all 15 hub genes exhibited significant differential expression. Among them, CLEC7A was significantly upregulated, whereas the remaining hub genes were all significantly downregulated (Figure 7). In the T2DM validation cohort GSE76894, the concordance of the candidate gene expression patterns was comparatively limited. Based on nominal, unadjusted Wilcoxon P values, CLEC7A,

MGST1, and RHOA showed significantly higher expression in patients with T2DM, whereas PDK4 and RASD1 showed significantly lower expression. The remaining ten candidate hub genes did not exhibit statistically significant differences between the T2DM and healthy control groups (Figure 8). Because no correction for multiple testing was applied, these validation findings should be interpreted as exploratory.

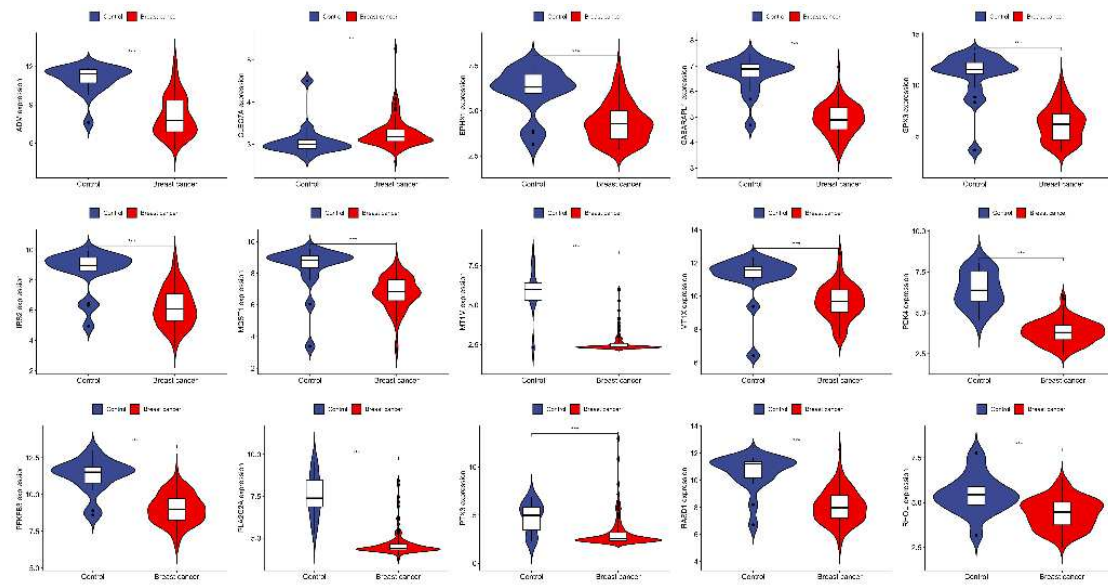


Figure 7. Expression of 15 candidate hub genes in the independent breast cancer validation cohort GSE42568. Violin plots combined with box plots compare the expression levels of 15 candidate hub genes between 17 normal breast tissue samples and 104 breast cancer tissue samples. From left to right and top to bottom, the genes are ADM, CLEC7A, EPHX1, GABARAPL1, GPX3, IRS2, MGST1, MT1M, MT1X, PDK4, PFKFB3, PLA2G2A, PTX3, RASD1, and RHOA. Between-group differences were assessed using the Wilcoxon rank-sum test. Boxes represent the interquartile range, and horizontal lines indicate the median. CLEC7A expression was significantly higher in BC tissues, whereas the other 14 candidate genes were significantly downregulated. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

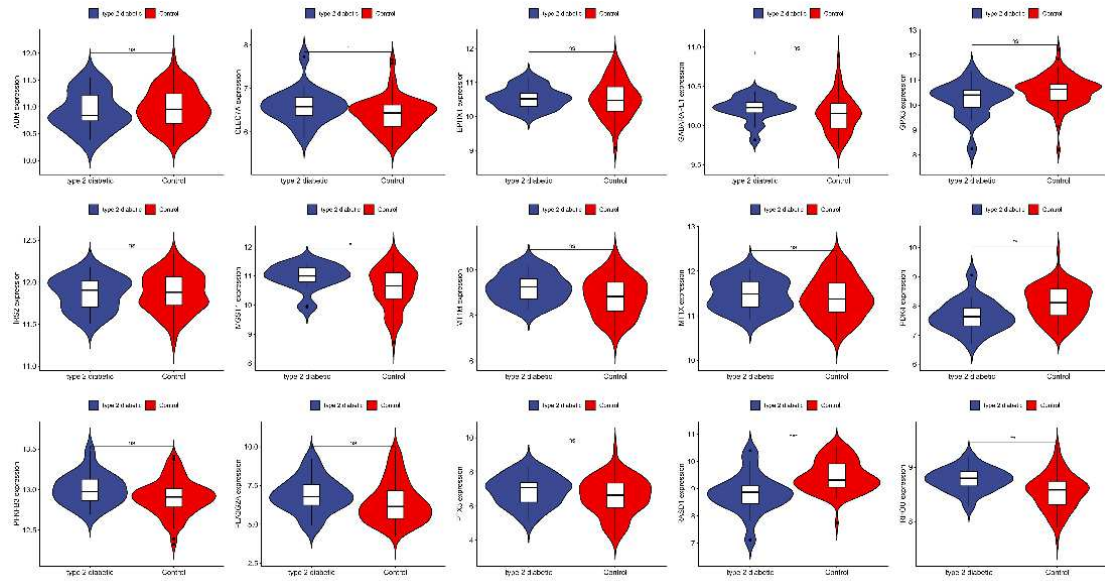


Figure 8. Expression of 15 candidate hub genes in the independent type 2 diabetes mellitus validation cohort GSE76894. Violin plots with overlaid boxplots compare the expression levels of the 15 candidate hub genes between 19 patients with T2DM and 84 healthy controls. From left to right and top to bottom, the genes are ADM, CLEC7A, EPHX1, GABARAPL1, GPX3, IRS2, MGST1, MT1M, MT1X, PDK4, PFKFB3, PLA2G2A, PTX3, RASD1, and RHOA. Between-group differences were assessed using a two-sided Wilcoxon rank-sum test. Boxes represent the interquartile range, and horizontal lines indicate the median. Statistical significance symbols are based on nominal, unadjusted P values, and no correction for multiple testing was applied. ns, nominal $P \geq 0.05$; *nominal $P < 0.05$; **nominal $P < 0.01$; ***nominal $P < 0.001$.

The expression patterns and cross-cohort validation results of the 15 candidate hub genes are summarized in Table 1.

Table 1. Summary of Expression Patterns and Validation Consistency of the 15 Hub Genes Across BC and T2DM Cohorts

Gene	BC Discovery Cohort	BC Validation Cohort	T2DM Discovery Cohort	T2DM Validation Cohort
PDK4	↓	↓	↓	↓
GPX3	↓	↓	↓	NS
RASD1	↓	↓	↓	↓
PFKFB3	↓	↓	↓	NS
GABARAPL1	↓	↓	↓	NS
IRS2	↓	↓	↓	NS
EPHX1	↓	↓	↓	NS
MGST1	↓	↓	↑	↑

Gene	BC Discovery Cohort	BC Validation Cohort	T2DM Discovery Cohort	T2DM Validation Cohort
MT1X	↓	↓	↓	NS
MT1M	↓	↓	↓	NS
ADM	↓	↓	↓	NS
PTX3	↓	↓	↓	NS
CLEC7A	↑	↑	↑	↑
PLA2G2A	↓	↓	↓	NS
RHOU	↓	↓	↑	↑

↑ and ↓ indicate significantly higher and lower expression in the disease group relative to the corresponding control group, respectively. In the discovery cohorts, statistical significance was determined using the limma criteria of adjusted $P < 0.05$ and $|\log_2FC| \geq 1$. In the independent validation cohorts, statistical significance was determined using a nominal, unadjusted Wilcoxon P value < 0.05 . No correction for multiple testing was applied in the exploratory validation analysis of the predefined 15 candidate hub genes. NS indicates no statistically significant difference according to the corresponding cohort-specific criterion.

Collectively, CLEC7A showed a more consistent direction of expression change than most of the other candidate genes across the discovery and validation analyses. CLEC7A was upregulated in both discovery cohorts and showed nominally significantly higher expression in the GSE76894 T2DM validation cohort. However, the magnitude and statistical significance of its expression differences varied across additional independent cohorts, indicating a degree of cohort heterogeneity. When integrated with the single-cell localization and immune association analyses, these findings support CLEC7A as a candidate marker of a shared myeloid immune-related state rather than a uniformly differentially expressed disease-specific biomarker.

In the additional validation analysis, CLEC7A exhibited an upward trend in the T2DM cohort (GSE25724) compared with the control group; however, the difference did not reach statistical significance (Wilcoxon rank-sum test, $P = 0.23$) (Figure 9A). In the independent paired breast cancer cohort (GSE15852), no significant difference in CLEC7A expression was observed between tumor tissues and their matched adjacent normal tissues (paired Wilcoxon test, $P = 0.77$), suggesting that its expression in breast cancer may be influenced by cohort heterogeneity.

and the local immune infiltration status. These findings further support CLEC7A as a myeloid immune-related state marker rather than a breast cancer-specific marker (Figure 9B).

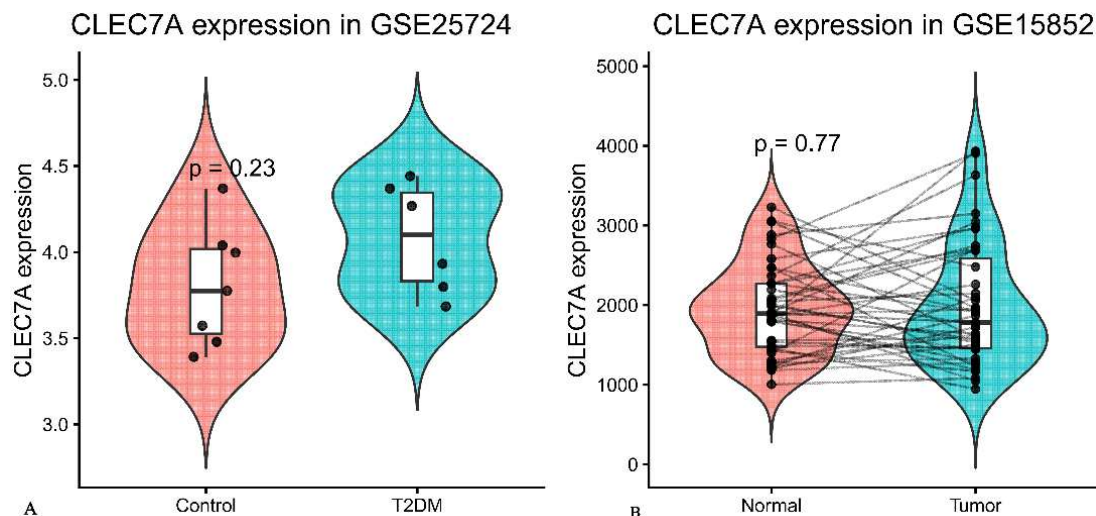


Figure 9. Validation of CLEC7A expression in additional type 2 diabetes mellitus and paired breast cancer cohorts. (A) Comparison of CLEC7A expression between the control and T2DM groups in the GSE25724 cohort. Between-group differences were assessed using the Wilcoxon rank-sum test ($P = 0.23$). (B) Comparison of CLEC7A expression between 43 paired breast cancer and adjacent normal breast tissue samples in the GSE15852 cohort. Lines connect paired samples obtained from the same patient. Differences were assessed using the paired Wilcoxon signed-rank test ($P = 0.77$). Violin plots show the expression distributions, box plots indicate the median and interquartile range, and black dots represent individual samples.

3.4.2 CLEC7A Expression Is Not Significantly Associated with Overall Survival in Breast Cancer

In the TCGA-BRCA cohort, patients were stratified into high- and low-CLEC7A expression groups according to the median CLEC7A expression level. Kaplan–Meier survival analysis revealed no significant difference in overall survival between the two groups (log-rank $P = 0.78$, $HR = 1$), suggesting that CLEC7A may not serve as a direct prognostic biomarker in breast cancer. Instead, its biological significance may be more closely associated with the regulation of the tumor immune microenvironment (Figure 10).

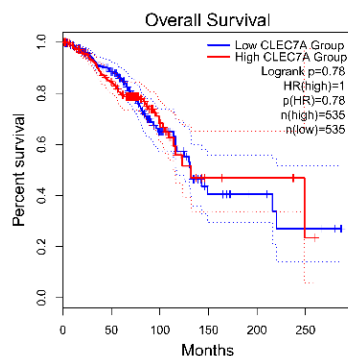


Figure 10. Association between CLEC7A expression and overall survival in the TCGA-BRCA cohort.

Patients in the TCGA-BRCA cohort were stratified into high- and low-CLEC7A-expression groups according to the median expression level, with 535 patients in each group. Kaplan–Meier analysis was performed to compare overall survival between the two groups, and differences were evaluated using the log-rank test. No significant difference in overall survival was observed between the high- and low-expression groups (log-rank $P = 0.78$; hazard ratio = 1.00). Solid lines represent estimated survival probabilities, dotted lines indicate confidence intervals, and tick marks denote censored observations.

3.4.3 CLEC7A Is Associated with Myeloid Immune Infiltration in BC and T2DM

In BC, TIMER2.0 analysis demonstrated that CLEC7A expression was significantly negatively correlated with tumor purity ($\text{cor} = -0.346$) and positively correlated with the infiltration levels of multiple immune cell types, including macrophages (partial $\text{cor} = 0.441$), neutrophils (partial $\text{cor} = 0.780$), and dendritic cells (partial $\text{cor} = 0.712$). These findings suggest that CLEC7A expression primarily reflects the composition of the tumor immune microenvironment and is particularly associated with the infiltration of myeloid immune cells (Figure 11A).

Consistently, CLEC7A also exhibited prominent immune-related characteristics in the T2DM dataset (Figure 11B–C). Correlation analysis revealed that CLEC7A expression was significantly positively correlated with the infiltration scores of monocytes ($R = 0.578$), macrophages ($R = 0.493$), and neutrophils ($R = 0.378$) (Figure 11D–F). Furthermore, CLEC7A expression showed significant positive correlations with several myeloid cell marker genes, including *CD163* ($R = 0.620$), *CSF1R* ($R = 0.540$), and *S100A8* ($R = 0.440$), further supporting its close association with the myeloid immune cell compartment.

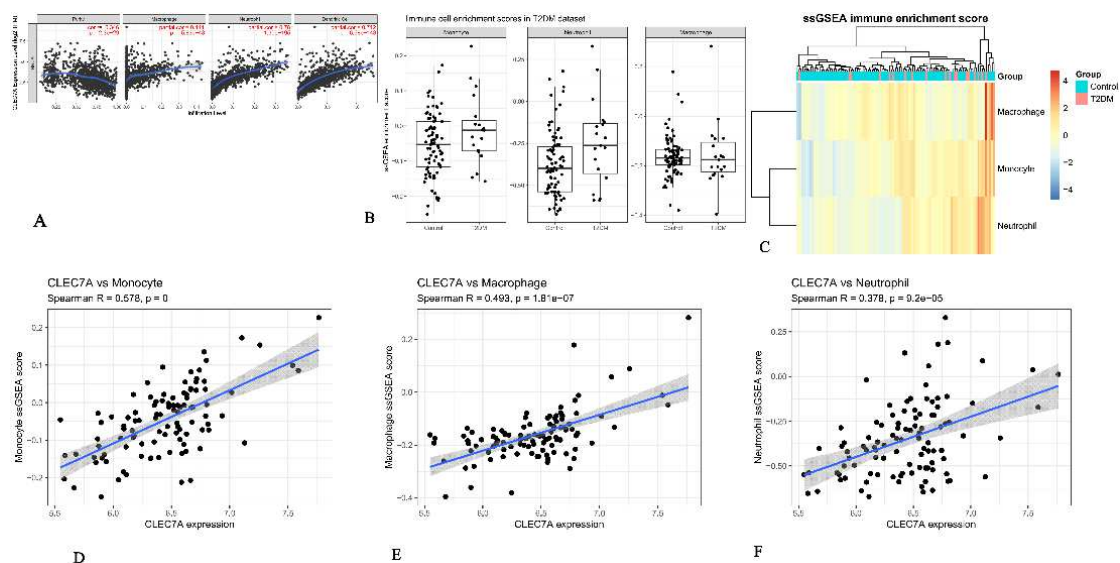


Figure 11. Associations between CLEC7A expression and myeloid immune-cell infiltration in breast cancer and type 2 diabetes mellitus. (A) TIMER2.0 analysis of the associations between CLEC7A expression, tumor purity, and immune-cell infiltration in the TCGA-BRCA cohort. CLEC7A expression was negatively correlated with tumor purity (correlation coefficient = -0.346) and positively correlated with the infiltration of macrophages (partial correlation coefficient = 0.441), neutrophils (partial correlation coefficient = 0.780), and dendritic cells (partial correlation coefficient = 0.712). Immune-cell correlations were adjusted for tumor purity. (B) Distribution of single-sample gene set enrichment analysis (ssGSEA) scores for monocytes, neutrophils, and macrophages in the T2DM and control groups. Black dots represent individual samples. (C) Hierarchical clustering heatmap of ssGSEA scores for monocytes, neutrophils, and macrophages across individual samples. The top annotation indicates sample group, and colors ranging from blue to red represent low to high standardized enrichment scores. (D–F) Spearman correlation analyses between CLEC7A expression and ssGSEA scores for monocytes, macrophages, and neutrophils, respectively. The corresponding correlation coefficients were $R = 0.578$, $R = 0.493$, and $R = 0.378$. Blue lines represent fitted trends, and shaded areas indicate 95% confidence intervals.

3.4.4 CLEC7A Is Correlated with MIF Receptor-Related Molecules

Given that cell–cell communication analysis revealed active MIF signaling in both breast cancer (BC) and type 2 diabetes mellitus (T2DM), we further investigated the correlations between CLEC7A and key components of the MIF signaling pathway. In BC, CLEC7A expression was significantly positively correlated with the canonical MIF receptor CD74 ($R = 0.310$) and its co-receptor CXCR4 ($R = 0.200$), showed a weak positive correlation with CD44, and exhibited a weak negative correlation with the MIF ligand itself ($R = -0.150$) (Figure 12A–E). In T2DM, CLEC7A was strongly positively correlated with CD74 ($R = 0.480$, $P < 0.001$) and moderately positively correlated with ACKR3 ($R = 0.240$, $P < 0.05$), whereas its correlations with CXCR4 and CD44 were weak and not statistically significant. Similar to BC, CLEC7A also showed a weak negative correlation with the MIF ligand ($R = -0.097$) (Figure 12F–J).

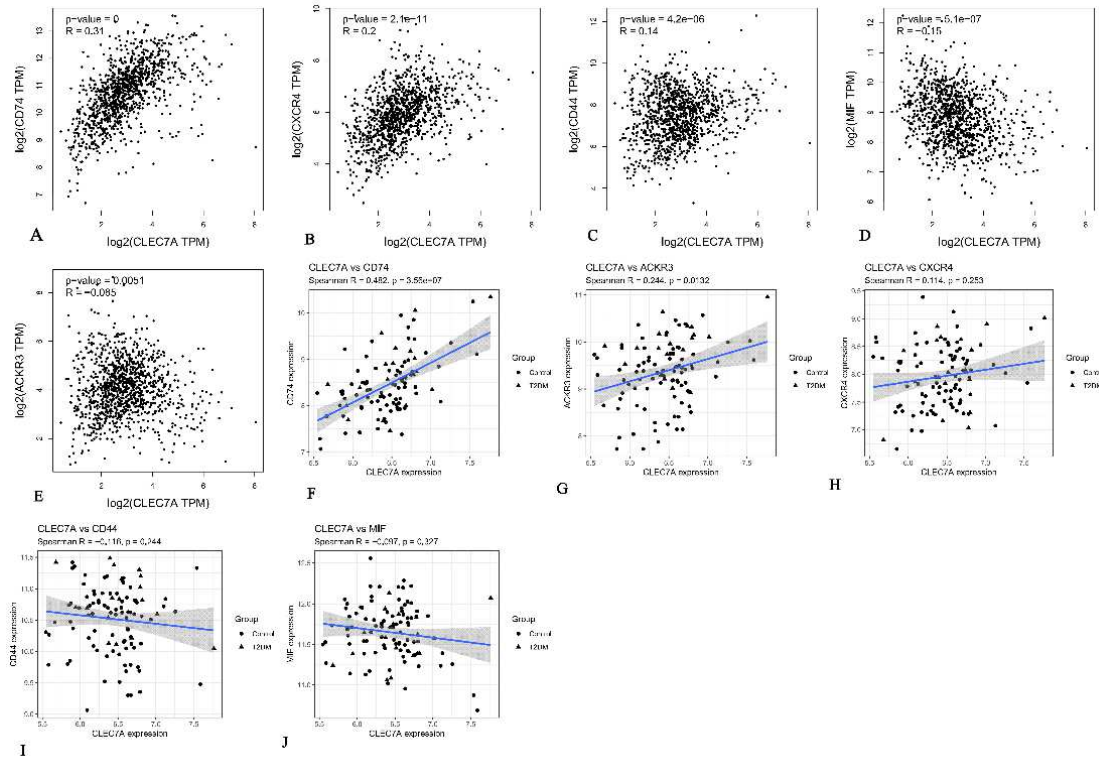


Figure 12. Correlations between CLEC7A and key components of the MIF signaling pathway. (A–E) Correlations of CLEC7A expression with CD74, CXCR4, CD44, MIF, and ACKR3 expression, respectively, in the TCGA-BRCA cohort. Each dot represents an individual sample. The x-axis indicates CLEC7A expression, and the y-axis indicates the expression of the corresponding MIF pathway-related gene. (F–J) Correlations of CLEC7A expression with CD74, ACKR3, CXCR4, CD44, and MIF expression, respectively, in the T2DM dataset. Circles represent control samples, and triangles represent T2DM samples. Blue lines indicate fitted trends, and shaded areas represent 95% confidence intervals. Spearman correlation coefficients and corresponding P values are shown. P values were adjusted for multiple testing using the Benjamini–Hochberg method. CLEC7A showed a consistent positive correlation with CD74 in both disease contexts.

For a direct comparison between the two disease contexts, the correlation coefficients, directions, and FDR-adjusted P values are summarized in Table 2. Notably, the most consistent finding across BC and T2DM was the positive association between CLEC7A and CD74, whereas the associations with the other MIF-related molecules varied in direction or magnitude between the two diseases.

Table 2. Correlation Analysis Between CLEC7A and Key Molecules in the MIF Signaling Pathway

Disease	Molecule	Related Direction	R value	FDR-adjusted P value
BC	CD74	positive correlation	0.310	< 0.001
	CXCR4	positive correlation	0.200	< 0.001
	CD44	positive correlation	0.140	< 0.001
	MIF	negative correlation	-0.150	< 0.001
	ACKR3	negative correlation	-0.085	0.005
T2DM	CD74	moderately positively correlated	0.482	< 0.001
	ACKR3	weak positive correlation	0.244	0.033
	CXCR4	positive correlation	0.114	0.316
	CD44	negative correlation	-0.116	0.316
	MIF	negative correlation	-0.097	0.327

Taken together, the findings from both diseases demonstrate that CLEC7A is consistently positively associated with MIF receptor-related molecules, particularly CD74, whereas its correlation with the MIF ligand itself is weak or negative. These results suggest that CLEC7A may primarily participate in, or serve as a marker of, the receptor-mediated response to MIF signaling rather than directly regulating MIF ligand expression.

3.4.5 Validation Using the Human Protein Atlas Database

Protein expression of CLEC7A in normal human tissues was analyzed using the Human Protein Atlas (HPA) database. Human Protein Atlas data showed that CLEC7A RNA expression was highest in bone marrow and lymphoid tissues, while the accompanying protein-expression data supported its predominant association with immune-related tissues. This expression pattern is consistent with the well-established myeloid cell-specific expression profile of CLEC7A, further supporting the notion that CLEC7A is more likely to reflect the immune cell status rather than the intrinsic expression characteristics of tumor cells.

4 Discussion

This study addressed the question of whether T2DM and BC share common features of immunometabolic reprogramming. By integrating bulk transcriptomic analysis, protein–protein interaction network analysis, single-cell localization, cell–cell communication analysis, and immune correlation analysis, we established a cross-disease, multi-omics evidence framework. The results revealed a certain degree of molecular convergence between BC and T2DM at the transcriptomic level, with the shared genes primarily enriched in processes related to oxidative stress, xenobiotic metabolism, glutathione metabolism, and immune responses.

Further analyses showed that CLEC7A occupied a central position within the PPI network of shared genes and was predominantly expressed in myeloid immune cells, including monocytes and macrophages. Cell–cell communication analysis further indicated that MIF–CD74/CXCR4-related signaling was highly active in both the BC tumor microenvironment and the peripheral immune environment of T2DM.

Taken together, these findings suggest that the association between T2DM and BC may be more closely related to altered immune states and enhanced inflammatory communication under conditions of metabolic dysregulation, rather than to a single direct genetic causal relationship.

Previous studies have shown that T2DM and BC share several risk factors, including obesity, insulin resistance, chronic low-grade inflammation, and metabolic dysfunction [32–34]. Epidemiological evidence supports an association between the two diseases; however, Mendelian randomization studies have not consistently demonstrated a clear genetic causal relationship [16,17]. Therefore, their connection may be more likely to arise at the level of systemic metabolic inflammation and the local tumor microenvironment.

In the present study, the shared DEGs were predominantly enriched in pathways related to oxidative stress, defense responses, and glutathione metabolism. These findings are consistent with the persistent metabolic stress observed in T2DM and the enhanced oxidative stress characteristic of the BC tumor microenvironment. Previous studies have demonstrated that hyperglycemia, hyperinsulinemia, and dysregulated lipid metabolism can promote cellular stress responses and tumor initiation and progression through the accumulation of reactive oxygen species (ROS) and the release of proinflammatory mediators [35]. In addition,

endocrine alterations associated with metabolic syndrome, including imbalances in insulin, estrogen, and adipokines, may facilitate breast cancer progression through proliferative and proinflammatory mechanisms [32].

Accordingly, the shared transcriptional features identified in this study may primarily reflect a common pathological background characterized by the sequence of metabolic stress, oxidative stress, and inflammatory responses.

Building on these findings, the present study further interprets the observed associations within the framework of systemic immune reprogramming driven by metaflammation. T2DM is characterized not only by metabolic abnormalities but also by a persistent state of low-grade inflammation, which can systemically regulate immune function and cell fate decisions. During this process, metabolic signals, such as hyperglycemia and hyperinsulinemia, not only provide energetic and growth advantages but may also enhance tumor cell adaptation and invasiveness by modulating key signaling pathways, including HIF-1 α [36]. These signals may further induce alterations in specific gene expression profiles, thereby establishing a metabolically reprogrammed state with tumor-promoting properties [37]. Therefore, the shared DEGs identified in this study may represent the transcriptional manifestation of coordinated immunometabolic reprogramming in the context of metaflammation.

Among the candidate hub genes, CLEC7A exhibited a pronounced association with myeloid immunity. CLEC7A encodes Dectin-1, which is predominantly expressed in myeloid immune cells, including monocytes, macrophages, and dendritic cells. In the present study, CLEC7A was consistently upregulated in BC and was primarily localized to immune cell-associated regions at the single-cell level. In T2DM, however, CLEC7A showed an upward expression trend without reaching statistical significance, suggesting that it should not be regarded simply as a disease-specific diagnostic biomarker.

Previous studies have linked CLEC7A to macrophage polarization and the establishment of an immunosuppressive microenvironment, with elevated CLEC7A expression generally associated with enhanced M2-like macrophage activity [23]. In addition, metabolic interventions, including glucose-lowering agents, may modulate CLEC7A expression and thereby influence macrophage-mediated inflammatory responses [19]. From a systems-level perspective, CLEC7A may therefore be better interpreted as a marker of a metaflammation-

driven myeloid immune state, reflecting the functional reprogramming of the immune system under chronic metabolic stress.

From a mechanistic perspective, CLEC7A may participate in myeloid inflammatory responses under conditions of metabolic stress; however, the present study does not provide evidence of a direct regulatory role. Previous studies have shown that CLEC7A/Dectin-1 can regulate inflammatory mediator release and immune cell function through the Syk/CARD9–NF- κ B signaling pathway. Within the tumor microenvironment, its expression has also been associated with immunosuppressive states and unfavorable clinical outcomes. Although the findings of the present study are broadly consistent with these observations, they should be interpreted as correlative evidence. Thus, CLEC7A is more likely to reflect alterations in immune status than to act as a direct driver of these changes.

Cell–cell communication analysis further suggested that MIF-related signaling may serve as an important link between metaflammation and the immune microenvironment. In BC, the MIF–(CD74+CXCR4) axis and related signaling pathways participated in complex interactions among tumor cells, immune cells, and stromal cells. In T2DM, MIF–CD74-associated interactions were broadly distributed across the T2DM peripheral immune network and involved both myeloid and lymphoid cell populations.

Previous studies have demonstrated that the MIF–CD74 axis plays an important role in both diabetes-associated inflammation and tumor immune regulation, and that its inhibition can attenuate inflammatory responses and ameliorate related pathological conditions [20]. In tumors, this pathway also contributes to the establishment of an immunosuppressive microenvironment and the promotion of immune evasion [38]. In the present study, CLEC7A showed only a weak correlation with the MIF ligand itself but was positively correlated with receptor-related molecules such as CD74 and CXCR4. These findings suggest that CLEC7A may be more closely associated with the responsiveness of myeloid cells to inflammatory signaling rather than with the initiation of MIF signaling.

Based on the above findings, we propose a “systemic priming–local amplification” model of immunometabolic crosstalk. T2DM-associated metabolic abnormalities may induce functional reprogramming of myeloid immune cells at the systemic level through chronic metaflammation, with CLEC7A serving as a representative marker of this state. Once these

reprogrammed immune cells or immune states are recruited into the tumor microenvironment, they may be further amplified through signaling axes such as MIF–CD74/CXCR4, thereby promoting inflammatory activation, the establishment of an immunosuppressive niche, and tumor progression. This model provides a plausible biological framework for explaining why T2DM and BC exhibit an epidemiological association despite the lack of a consistently demonstrated genetic causal relationship.

From a broader perspective, the findings of this study can be interpreted within the current paradigm of immunometabolism. Metabolic status not only determines cellular energy availability but also plays a fundamental role in disease initiation and progression by regulating immune cell function, inflammatory responses, and intercellular communication networks. In cancer, metabolic reprogramming is closely coupled with remodeling of the immune microenvironment. Similarly, chronic inflammation and immune dysregulation constitute central pathological features of T2DM. From a cross-disease perspective, the present study provides multi-omics evidence supporting the existence of shared immunometabolic regulatory mechanisms between T2DM and BC.

The principal innovations of this study are as follows: (1) a stepwise multi-omics strategy was employed to systematically characterize the shared immunometabolic features of T2DM and BC from a cross-disease perspective; (2) CLEC7A was identified as a molecule associated with myeloid immune states rather than merely as a diagnostic or prognostic biomarker; and (3) the expression characteristics of CLEC7A were integrated with the MIF–CD74/CXCR4 communication axis, leading to the proposal of a theoretical model in which metaflammation drives immune reprogramming.

This study has several limitations. First, the data were obtained from public databases, and heterogeneity existed across cohorts in terms of sample sources, detection platforms, and the availability of clinical information. Second, the single-cell datasets were derived from different disease contexts and therefore could not fully recapitulate the actual microenvironment of breast cancer complicated by T2DM. Third, the present study was primarily based on computational inference and lacked functional experimental validation. Accordingly, the findings should be regarded as hypothesis-generating evidence rather than direct mechanistic conclusions.

Future studies should validate the spatial colocalization of CLEC7A with the MIF/CD74/CXCR4 axis in clinical samples from patients with coexisting T2DM and breast cancer by integrating spatial omics and protein-level analyses. Functional experiments are also required to further clarify the potential roles of this signaling network in macrophage polarization and tumor progression.

5 Conclusion

In conclusion, this study suggests that T2DM and BC may share a common biological basis centered on metaflammation-driven immune reprogramming. CLEC7A may serve as a representative marker of the myeloid immune state, whereas the MIF–CD74/CXCR4 axis may contribute to intercellular communication and microenvironmental remodeling within this immunological context. These findings provide a new conceptual perspective for understanding the progression of T2DM-associated breast cancer and offer a potential direction for future investigations into combined immunometabolic intervention strategies.

Abbreviations

BC, breast cancer; T2DM, type 2 diabetes mellitus; TNBC, triple-negative breast cancer; DEG, differentially expressed gene; PPI, protein–protein interaction; scRNA-seq, single-cell RNA sequencing; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; MIF, macrophage migration inhibitory factor; HPA, Human Protein Atlas; ssGSEA, single-sample gene set enrichment analysis; OS, overall survival; HR, hazard ratio; FDR, false discovery rate.

Supplementary information

Supplementary Tables S1–S5 accompany this article.

Declarations

Acknowledgements

The authors acknowledge the contributors and maintainers of the Gene Expression Omnibus, The Cancer Genome Atlas, GEPIA2, TIMER2.0, STRING, and the Human Protein Atlas for providing publicly available data and analysis resources.

Funding

The National Natural Science Foundation of China (Grant No. 82405171; 82574820); Shandong Provincial Natural Science Foundation (Grant No. ZR2023QH095); The Youth Innovation Team Development Program for Higher Education Institutions in Shandong

Province (Grant No. 2024KJJ037); Taishan Scholars Program for Young Experts of Shandong Province (NO.tsqn202507392)

Author contributions

Jia Li: Conceptualization, Methodology, Software, Formal analysis, Data curation, Visualization, Writing – original draft, and Writing – review and editing. Chundi Gao: Conceptualization, Methodology, Software, Formal analysis, Validation, Visualization, Writing – original draft, and Writing – review and editing. Minghui Wu: Methodology, Investigation, Data curation, Formal analysis, and Writing – review and editing. Yanyan Pan: Validation, Formal analysis, Data curation, Visualization, and Writing – review and editing. Xiaoming Zhang: Investigation, Resources, Data interpretation, and Writing – review and editing. Changgang Sun: Conceptualization, Supervision, Project administration, Funding acquisition, Resources, and Writing – review and editing. All authors read and approved the final manuscript. Jia Li and Chundi Gao contributed equally to this work.

Competing interests

The authors declare no competing interests.

Ethics approval

Not applicable

Consent for publication

All authors have read the manuscript and provided their consent for the submission..

Data availability

The datasets analyzed in this study are publicly available from the NCBI Gene Expression Omnibus (GEO) under the following accession numbers:

GSE7014: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7014>

GSE76894: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76894>

GSE10810: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10810>

GSE42568: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42568>

GSE161529: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE161529>

GSE255566: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE255566>

GSE25724: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE25724>

GSE15852: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15852>

Additional online resources used for analysis and validation include:

STRING (protein–protein interaction network): <https://string-db.org/>

GEPIA2 (gene expression profiling and survival analysis): <http://gepia2.cancer-pku.cn/>

TIMER2.0 (immune infiltration analysis): <http://timer.cistrome.org/>

Human Protein Atlas (protein expression profiling): <https://www.proteinatlas.org/>

All datasets used are publicly available and de-identified; no new clinical samples were collected. The analysis code is available from the corresponding author upon reasonable request.

Code availability

The analysis code is available from the corresponding author on reasonable request.

Ethical statement

Not applicable.

Consent to participate

Not applicable.

References

1. Scott L, Truong LL, Houlden RL, Wijeratne DT. Screening and management recommendations for type 2 diabetes in women with breast cancer. *Can J Diabetes*. 2024;48(1):66–72. doi: 10.1016/j.cjcd.2023.07.008.
2. Tsilidis KK, Kasimis JC, Lopez DS, Ntzani EE, Ioannidis JPA. Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. *BMJ*. 2015;350:g7607. doi: 10.1136/bmj.g7607.
3. Pearson-Stuttard J, Papadimitriou N, Markozannes G, *et al*. Type 2 diabetes and cancer: an umbrella review of observational and Mendelian randomization studies. *Cancer Epidemiol Biomarkers Prev*. 2021;30(6):1218–1228. doi: 10.1158/1055-9965.EPI-20-1245.
4. Xiong F, Dai Q, Zhang S, *et al*. Diabetes and incidence of breast cancer and its molecular subtypes: a systematic review and meta-analysis. *Diabetes Metab Res Rev*. 2024;40(1):e3709. doi: 10.1002/dmrr.3709.
5. Redaniel MTM, Jeffreys M, May MT, Ben-Shlomo Y, Martin RM. Associations of type 2 diabetes and diabetes treatment with breast cancer risk and mortality: a population-based cohort study among British women. *Cancer Causes Control*. 2012;23(11):1785–1795. doi: 10.1007/s10552-012-0057-0.
6. Luo J, Virnig B, Hendryx M, *et al*. Diabetes, diabetes treatment and breast cancer prognosis. *Breast Cancer Res Treat*. 2014;148(1):153–162. doi: 10.1007/s10549-014-3146-9.
7. Fernández-Arce L, Robles-Rodríguez N, Fernández-Feito A, Llaneza-Folgueras A, Encinas-Muñiz AI, Lana A. Type 2 diabetes and all-cause mortality among Spanish women with breast cancer. *Cancer Causes Control*. 2022;33(2):271–278. doi: 10.1007/s10552-021-01526-x.
8. Zhang F, de Haan-Du J, Sidorenkov G, *et al*. Type 2 diabetes mellitus and clinicopathological tumor characteristics in women diagnosed with breast cancer: a systematic review and meta-analysis. *Cancers (Basel)*. 2021;13(19):4992. doi: 10.3390/cancers13194992.
9. Overbeek JA, van Herk-Sukel MPP, Vissers PAJ, *et al*. Type 2 diabetes, but not insulin (analog) treatment, is associated with more advanced stages of breast cancer: a national linkage of cancer and pharmacy registries. *Diabetes Care*. 2019;42(3):434–442. doi: 10.2337/dc18-2146.
10. Liu L, Gao Y, Liu P, Hui R, Zhang J. Association of type 2 diabetes mellitus with histopathological features of non-metastatic breast cancer in Chinese women: a retrospective cross-sectional study. *Sci Rep*. 2025;15(1):28645. doi: 10.1038/s41598-025-14077-x.
11. Kaplan MA, Pekkolay Z, Kucukoner M, *et al*. Type 2 diabetes mellitus and prognosis in early stage breast cancer women. *Med Oncol*. 2012;29(3):1576–1580. doi: 10.1007/s12032-011-0109-4.
12. Mu L, Zhu N, Zhang J, Xing F, Li D, Wang X. Type 2 diabetes, insulin treatment and prognosis of breast cancer. *Diabetes Metab Res Rev*. 2017;33(1):e2823. doi: 10.1002/dmrr.2823.
13. Matou-Nasri S, Aldawood M, Alanazi F, Khan AL. Updates on triple-negative breast cancer in type 2 diabetes mellitus patients: from risk factors to diagnosis, biomarkers and therapy. *Diagnostics (Basel)*. 2023;13(14):2390. doi: 10.3390/diagnostics13142390.
14. Sankofi BM, Valencia-Rincón E, Sekhri M, Ponton-Almodovar AL, Bernard JJ, Wellberg EA. The impact of poor metabolic health on aggressive breast cancer: adipose tissue and tumor metabolism. *Front Endocrinol (Lausanne)*. 2023;14:1217875. doi: 10.3389/fendo.2023.1217875.

15. Vona-Davis L, Rose DP. Type 2 diabetes and obesity metabolic interactions: common factors for breast cancer risk and novel approaches to prevention and therapy. *Curr Diabetes Rev.* 2012;8(2):116–130. doi: 10.2174/157339912799424519.
16. Zhang L, Liu S, Yue G, *et al.* The causality between type 2 diabetes and breast cancer: a bidirectional two-sample Mendelian randomization study. *Future Oncol.* 2024;20(18):1267–1274. doi: 10.2217/fon-2023-0708.
17. Wu W, Huang GL, Cui J. Type 2 diabetes mellitus and cancer: a systematic review and meta-analysis of Mendelian randomization studies. *Front Endocrinol (Lausanne).* 2026;17:1713815. doi: 10.3389/fendo.2026.1713815.
18. Kong H, Pan M, Sun L. Identification and analysis of metabolic reprogramming-related genes in triple-negative breast cancer. *Clin Exp Med.* 2025;25:332. doi: 10.1007/s10238-025-01870-1.
19. Arefin A, Gage MC. Metformin, empagliflozin, and their combination modulate *ex vivo* macrophage inflammatory gene expression. *Int J Mol Sci.* 2023;24(5):4785. doi: 10.3390/ijms24054785.
20. Xing Y, Ye S, Chen Y, Fan A, Xu Z, Jiang W. MIF/CD74 axis is a target for metformin therapy in diabetic podocytopathy—real world evidence. *Endokrynol Pol.* 2018;69(3):264–268. doi: 10.5603/EP.a2018.0028.
21. Mangano K, Diamantopoulos A, Vallianou NG, *et al.* Serum and urinary levels of MIF, CD74, DDT and CXCR4 among patients with type 1 diabetes mellitus, type 2 diabetes and healthy individuals: implications for further research. *Metabol Open.* 2024;24:100320. doi: 10.1016/j.metop.2024.100320.
22. Li Y, Wang Y, Zhang H, *et al.* The joint impact of elevated homocysteine and type 2 diabetes on non-healing wounds and all-cause mortality: integrated clinical and multi-omics analyses. *Int J Surg.* 2025;111(12):8966–8989. doi: 10.1097/JS9.0000000000003715.
23. Wang J, Li X, Wang K, *et al.* CLEC7A regulates M2 macrophages to suppress the immune microenvironment and implies poorer prognosis of glioma. *Front Immunol.* 2024;15:1361351. doi: 10.3389/fimmu.2024.1361351.
24. Xiao Z, Li J, Fan S, Wang Y, Jin Q. Transcription factor SP1 drives the malignant progression of oral squamous cell carcinoma and M2 macrophage polarization through transcription activation-mediated upregulation CLEC7A. *Cytotechnology.* 2025;77(4):123. doi: 10.1007/s10616-025-00787-7.
25. 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.
26. Wu T, Hu E, Xu S, *et al.* clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation (Camb).* 2021;2(3):100141. doi: 10.1016/j.xinn.2021.100141.
27. Szklarczyk D, Kirsch R, Koutrouli M, *et al.* The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* 2023;51(D1):D638–D646. doi: 10.1093/nar/gkac1000.
28. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol.* 2014;8(Suppl 4):S11. doi: 10.1186/1752-0509-8-S4-S11.

29. Stuart T, Butler A, Hoffman P, *et al.* Comprehensive integration of single-cell data. *Cell*. 2019;177(7):1888–1902.e21. doi: 10.1016/j.cell.2019.05.031.
30. Jin S, Guerrero-Juarez CF, Zhang L, *et al.* Inference and analysis of cell-cell communication using CellChat. *Nat Commun*. 2021;12(1):1088. doi: 10.1038/s41467-021-21246-9.
31. Li T, Fu J, Zeng Z, *et al.* TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res*. 2020;48(W1):W509–W514. doi: 10.1093/nar/gkaa407.
32. Vona-Davis L, Howard-McNatt M, Rose DP. Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer. *Obes Rev*. 2007;8(5):395–408. doi: 10.1111/j.1467-789X.2007.00396.x.
33. Rose DP, Haffner SM, Baillargeon J. Adiposity, the metabolic syndrome, and breast cancer in African-American and White American women. *Endocr Rev*. 2007;28(7):763–777. doi: 10.1210/er.2006-0019.
34. Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. *Am J Clin Nutr*. 2007;86(3):823S–835S. doi: 10.1093/ajcn/86.3.823S.
35. Sciacca L, Vigneri R, Tumminia A, *et al.* Clinical and molecular mechanisms favoring cancer initiation and progression in diabetic patients. *Nutr Metab Cardiovasc Dis*. 2013;23(9):808–815. doi: 10.1016/j.numecd.2013.05.006.
36. Wang L, Fan J, Yan CY, Ling R, Yun J. Activation of hypoxia-inducible factor-1 α by prolonged *in vivo* hyperinsulinemia treatment potentiates cancerous progression in estrogen receptor-positive breast cancer cells. *Biochem Biophys Res Commun*. 2017;491(2):545–551. doi: 10.1016/j.bbrc.2017.03.128.
37. Ahmed SBM, Radwan N, Amer S, *et al.* Assessing the link between diabetic metabolic dysregulation and breast cancer progression. *Int J Mol Sci*. 2023;24(14):11816. doi: 10.3390/ijms241411816.
38. Wang S, Wang Y, Bu Y, *et al.* ISLR knockdown enhances radiotherapy-induced anti-tumor immunity by disrupting the Treg-mregDC-lymphoid niche in triple-negative breast cancer. *Int Immunopharmacol*. 2025;161:114988. doi: 10.1016/j.intimp.2025.114988.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)
- [Table2.docx](#)
- [SupplementaryTableS5.txt](#)
- [SupplementaryTableS4.txt](#)
- [fihuirelengends14.txt](#)
- [Supplementarylegends.txt](#)
- [05.T2DMCD8Tcells.diffGene.txt](#)
- [05.T2DMDendriticcells.diffGene.txt](#)
- [05.T2DMHSCGCSF.diffGene.txt](#)
- [05.T2DMNKcells.diffGene.txt](#)
- [05.T2DMMonocytes.diffGene.txt](#)
- [05.T2DMNeutrophils.diffGene.txt](#)
- [05.T2DMCD4Tcells.diffGene.txt](#)
- [05.T2DMBcells.diffGene.txt](#)