Archives of Medical Research 56 (2025) 103072

ORIGINAL ARTICLE

Integrative Transcriptomic Analysis of Peripheral Blood Monocytes in Systemic Sclerosis and Shared Pathogenic Pathways in Autoimmune Diseases

Shaoqi Chen,[a] Yu Fan,[b] Qiulin Wu,[a] Guohong Zhang,[b] Yukai Wang,[c] Weiping Li,[a] Shengli Yang,[a,d] Marco Matucci-Cerinic,[e] and Daniel E. Furst[f]

[a] *The First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China*
[b] *Department of Pathology, Shantou University Medical College, Shantou, Guangdong, China*
[c] *Department of Rheumatology and Immunology, Shantou Central Hospital, Shantou, Guangdong, China*
[d] *Shanghai Academician Consulting and Academic Activities Center of Chinese Academy of Engineering, Shanghai, China*
[e] *Unit of Immunology, Rheumatology, Allergy and Rare diseases, San Raffaele Hospital, Milan, Italy*
[f] *Division of Rheumatology, School of Medicine, University of California at Los Angeles, California, USA*

Received for publication March 30, 2024; accepted August 14, 2024 (ARCMED-D-24-00250).

Background. Systemic sclerosis (SSc) is an autoimmune disease (AD), that receives less attention compared to rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and primary Sjögren's syndrome (pSS). This study aims to analyze transcriptional profiles and immune cell composition in peripheral blood mononuclear cells (PBMC) from SSc patients compared to other ADs.

Methods. RNA-seq data from 119 untreated patients (eight with SSc, 42 with RA, 41 with pSS, 28 with SLE) and 20 healthy controls were analyzed. Bioinformatics tools were employed to identify differentially expressed genes (DEGs), biological functions and immune cell profiles unique to SSc and shared with other ADs.

Results. 1,148 DEGs were found in SSc, with upregulated genes associated with megakaryocyte processes and downregulated genes associated with neutrophil function and immune response.
DEGs, including *ALDH1A1* and *MEGF9*, were associated with neutropenia. Upregulated transcription factors (TFs) were linked to embryonic hematopoiesis and downregulated TFs were involved in leukocyte differentiation and immune regulation. Comparative analysis with other ADs revealed common pathogenic pathways, emphasizing megakaryocyte proliferation. Neutrophils count was significantly decreased in ADs (p <0.001) compared to healthy controls. Comparative analysis highlighted common pathways, particularly in megakaryocyte proliferation, and unique genes (*MEGF9, MMP8,* and KRT family members) in SSc, suggesting roles in neutrophil function, skin integrity, and fibrosis.

Conclusions. This study identifies dysregulated gene expression (KRT and *MMP8*) associated with neutrophil function and increased megakaryocytes in SSc, highlighting common patterns across autoimmune diseases. These findings offer new insights into the potential pathogenesis of SSc, and help to explore new targets for the treatment.

Key Words: Systemic sclerosis, Autoimmune diseases, Transcription factors, RNA sequencing, Keratins, Neutropenia.

Address reprint requests to: Shengli Yang, The First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, 515041, China; E-mail: slyang@caeshc.com.cn or wpli818@126.com

https://doi.org/10.1016/j.arcmed.2024.103072

Introduction

Systemic sclerosis (SSc) is a rare and complex autoimmune disease (AD) characterized by autoimmunity, vasculopathy, and fibrosis (1), but its exact pathogenesis remains unclear. Compared to other common ADs such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and primary Sjögren's syndrome (pSS), patients with SSc have a higher premature mortality rate (2). Pulmonary involvements, particularly fibrotic interstitial lung disease and pulmonary arterial hypertension, are the leading causes of death (3). Unfortunately, drugs that can effectively control fibrosis and vascular impairment in patients with SSc are limited (4).

SSc, RA, SLE, and pSS share clinical and autoantibody similarities, including a significant female predominance, familial aggregation tendency, and polyautoimmunity. Differentiating these ADs can be challenging, as joint pain in pSS may lead to misdiagnosis as RA, while hematological abnormalities could mimic SLE. Shared autoantibodies, such as rheumatoid factor in pSS and SLE, and pSS-specific autoantibodies SSA and SSB in SLE suggest a concept of "shared autoimmunity" (5). SSc presents with typical symptoms of other diseases, including antinuclear antibody (ANA) positivity, arthritis, and interstitial lung disease (6–8). However, its characteristic fibrotic and vascular pathological features suggest different molecular mechanisms. Studies have suggested that endothelial injury precedes the development of fibrosis. Raynaud's phenomenon (RP) is a syndrome characterized by impaired digital perfusion that occurs in almost all patients with SSc and is usually the earliest clinical manifestation of the disease. RP may be related to vasomotor dysfunction and vasospasm. Altered microcirculation may be the triggering factor that leads to increased endothelial permeability, vascular leakage and inflammatory cell infiltration (9–11). Fibrosis is a central aspect, with immune-mediated tissue damage leading to excessive fibrosis in the skin and organs. Patients with SSc often develop interstitial lung disease due to inflammation and fibrosis of the alveolar wall and interstitium, resulting in decreased lung function. In this case, researchers have proposed that the combination treatment of immunosuppression and anti-fibrosis may allow multi-target therapy and provide greater benefit to SSc patients (12,13).

Peripheral blood represents a common pathway for immune cell trafficking to target organs affected by various ADs. Peripheral blood mononuclear cells (PBMCs), which include lymphocytes and monocytes, are essential for immune responses (14). Our previous study of PBMCs from RA, SLE, and SSc patients revealed common pathogenic mechanisms involving megakaryocytes in antigen presentation (5). In addition, PBMCs are crucial for the diagnosis and treatment of various ADs due to their unique cell subsets and activation profiles (15). In SSc, PBMCs consistently exhibit chronic inflammation characterized by elevated cytokines (16–18). Transcription factors (TFs) in PBMCs are critical in regulating cytokine production and immune responses (19–21). However, there remains a limited understanding of TF dysregulation in PBMCs in SSc, particularly when compared to other ADs.

The aim of this study is to uncover the unique and shared cellular and molecular foundations of SSc compared to other common ADs through comprehensive transcriptomic analysis of PBMCs. We used RNA sequencing to assess the gene expression profiles in PBMCs from SSc and AD patients. Through differential gene expression analysis, functional enrichment analysis, PPI network construction, and immune cell composition analysis, we identified key genes and biological pathways closely related to the pathogenesis of the diseases. This research may provide new therapeutic targets and biomarkers for SSc and other ADs, potentially changing disease management and advancing the field of autoimmune disease research.

Materials and Methods

Subjects and Sample Collection

A total of eight patients with SSc, 42 with RA, 41 with pSS, 28 with SLE, and 20 healthy controls were enrolled in this study. None of the enrolled patients were receiving any medication. Patient inclusion criteria were based on the 2002 American-European Consensus Group criteria for pSS (22), the 2010 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for RA, the 2019 EULAR/ACR classification criteria for SLE (23), and the 2013 ACR/EULAR classification criteria for SSc (24). The study was approved by the Ethics Committee of the Shantou Central Hospital (Guangdong, China). All investigations were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants prior to sample collection. Peripheral blood samples were collected from each participant, and PBMCs were isolated using Histopaque-1077 solution (Sigma-Aldrich, St. Louis, MO, USA).

RNA-Seq

Sequencing libraries were generated using the NEBNext® UltraTM RNA Library Prep Kit for Illumina® (San Diego, CA, USA) following the manufacturer's recommendations. Libraries were sequenced on the Hiseq PE150 platform (Illumina) according to the manufacturer's guidelines at the Novogene Bioinformatics Institute (Beijing, China). The raw data, in FASTQ format, underwent initial processing through in-house Perl scripts. In this step, clean reads were obtained by removing adapter-containing reads, those with ploy-N sequences, as well as those of low-quality from the raw data. The raw reads were then aligned to the human

reference genome (hg19). To assess sequencing quality, the sequencing data underwent quality checks using FASTQC. Gene expression levels were estimated using the Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced (FPKM) values.

Differential Gene Expression and Enrichment Analyses

Principal component analysis (PCA) was used to assess the clustering of gene expression patterns among different ADs and to distinguish SSc from the control group. Differential expression analysis was performed using the DESeq2 R package (1.16.1). The *p*-values were adjusted using the Benjamini and Hochberg approach to control the false discovery rate (FDR). The list of human TFs was obtained from http://humantfs.ccbr.utoronto.ca/download.php. Genes with FDR <0.05 and $|\text{fold-change}| > 1$ determined by DESeq2 were considered differentially expressed genes (DEGs). The DEGs and differentially expressed TFs were subjected to enrichment analyses using ChIP-X Enrichment Analysis Version 3 (ChEA3, https://amp.pharm.mssm.edu/ChEA3), MetaScape (https://metascape.org/), Enrichr platform (https://maayanlab.cloud), as well as Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses through DAVID (v6.8) (http://david.abcc.ncifcrf.gov/home.jsp).

PPI Network

PPI enrichment analysis was performed using MetaScape (https://metascape.org/) with databases including STRING, BioGrid, OmniPath, and InWeb_IM.

Immune Cell Assessment

Immune cell proportion was estimated using xCell (https://xcell.ucsf.edu/), ABIS algorithm, and CIBERSORTx (https://cibersortx.stanford.edu/) (25). Cell type-specific signatures were employed to identify and characterize the abundance of specific cell types (26). Cell type enrichment scores were computed using the FPKM gene expression matrix.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.0 software. All data were tested for normality before analysis by the Shapiro-Wilk test. For normally distributed data, a two-tailed Mann-Whitney test was used to compare the differences between the two groups. Exact *p*-values were indicated in the figure legends. All data were presented as mean ± standard deviation (SD). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ were considered statistically significant.

Results

DEGs in SSc are Associated with Neutrophil Function and Histone-Related Transcriptional Regulation

To investigate differential gene expression in SSc, we used bulk RNA-seq data analysis. We observed a clear segregation of SSc patients from healthy controls by PCA (Figure 1A). RNA-seq of PBMCs generated 1148 DEGs, with 724 genes being upregulated and 424 downregulated in SSc patients (Figure 1A–C). The upregulated genes were significantly enriched in biological processes such as *RUNX1,* which regulates genes involved in megakaryocytes, regulation of wound response, and platelet activation (Figure 1D). Conversely, the downregulated genes were notably enriched in processes such as neutrophil degranulation, granulocyte activation, blood vessel development, and inflammatory response (Figure 1E). PPI networks highlighted the involvement of histones, such as *H2AC* and *H2BC*, keratins (KRT), including *KRT2, KRT5, KRT8*, and *KRT74*, as well as chemokines and chemokine receptors such as *CXCL1* and *CXCR1* (Figure 1F). These proteins play critical roles regulating gene transcription (27), maintaining skin integrity (28), and modulating the immune response (29). Notably, the KRT family genes (*KRT2, KRT5,* and *KRT74*) associated with fibrosis and skin integrity (30,31) were observed to be downregulated in SSc, specifically *KRT5*. These data suggest dysregulation of key genes and pathways associated with immune response, wound healing, and maintenance of skin integrity in SSc.

The Downregulated DEGs in SSc Suggest the Presence of Neutropenia

To determine whether the downregulation of specific genes in SSc is related to neutropenia, we conducted a GO enrichment analysis on the 424 downregulated DEGs. Among these genes, significant enrichment was observed in biological processes related to neutrophil degranulation (Figure 2A), nervous system development, and negative regulation of chemotaxis (Figure 2B). Enrichment analysis using cell type-specific signatures showed a reduction in neutrophils, confirming the dysregulated neutrophil signal (Figure 2C). *MEGF9* is expressed in central neutrophils and skin keratinocytes (32). For the first time, we identified *MEGF9* as a downregulated gene in SSc.

Further confirmation of reduced neutrophil numbers was obtained by cell type enrichment analysis and absolute deconvolution of immune cells using xCell and ABIS algorithms. These results consistently demonstrated a decrease in neutrophil numbers in SSc (Figure 2D). Consistent with the deconvolution results, the downregulated DEGs specific to SSc, including *ALDH1A1, ASIC1, CASP5, IL1RL1, IL13RAI,* and *MMP8*, were associated with the immune

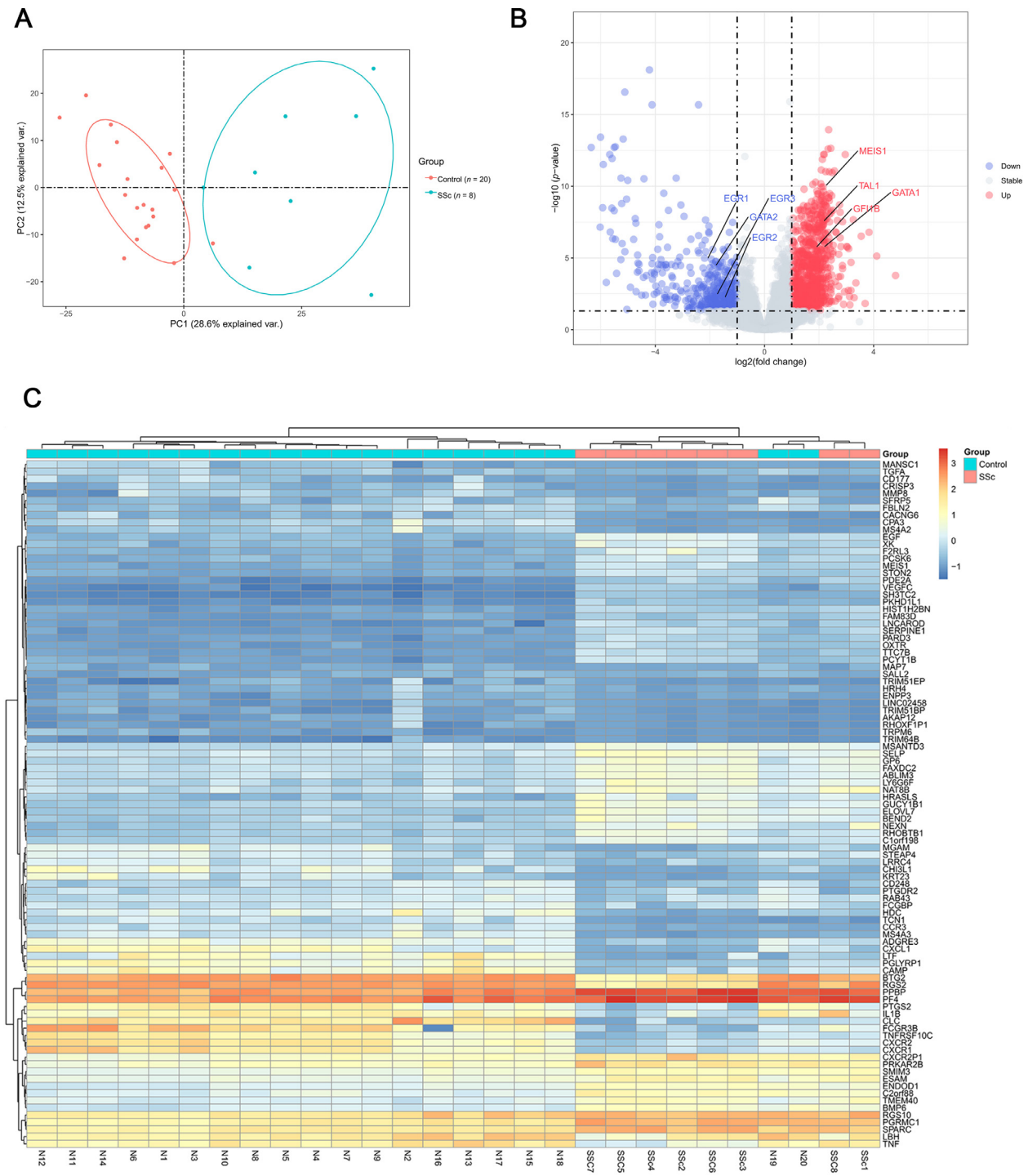

Figure 1. Differential gene expression and functional enrichment in systemic sclerosis (SSc) compared to healthy controls. A. Principal component analysis (PCA) distinguishes SSc patients from healthy controls based on gene expression patterns in peripheral blood mononuclear cells (PBMCs). B. Volcano plot shows differentially expressed genes (DEGs) with specific upregulated genes such as *MEIS1, TAL1, GATA1,* and *GFI1B*, and downregulated genes including *EGR1, EGR2,* and *EGR3* highlighted. C. Heatmap illustrates the expression levels of the most significant DEGs between SSc patients and healthy controls. D, and E. Gene ontology (GO) network analysis. Each node represents a gene, and the edges indicate the biological processes in which they are involved. F. Protein-protein interaction (PPI) network identifies three hub modules of DEGs.

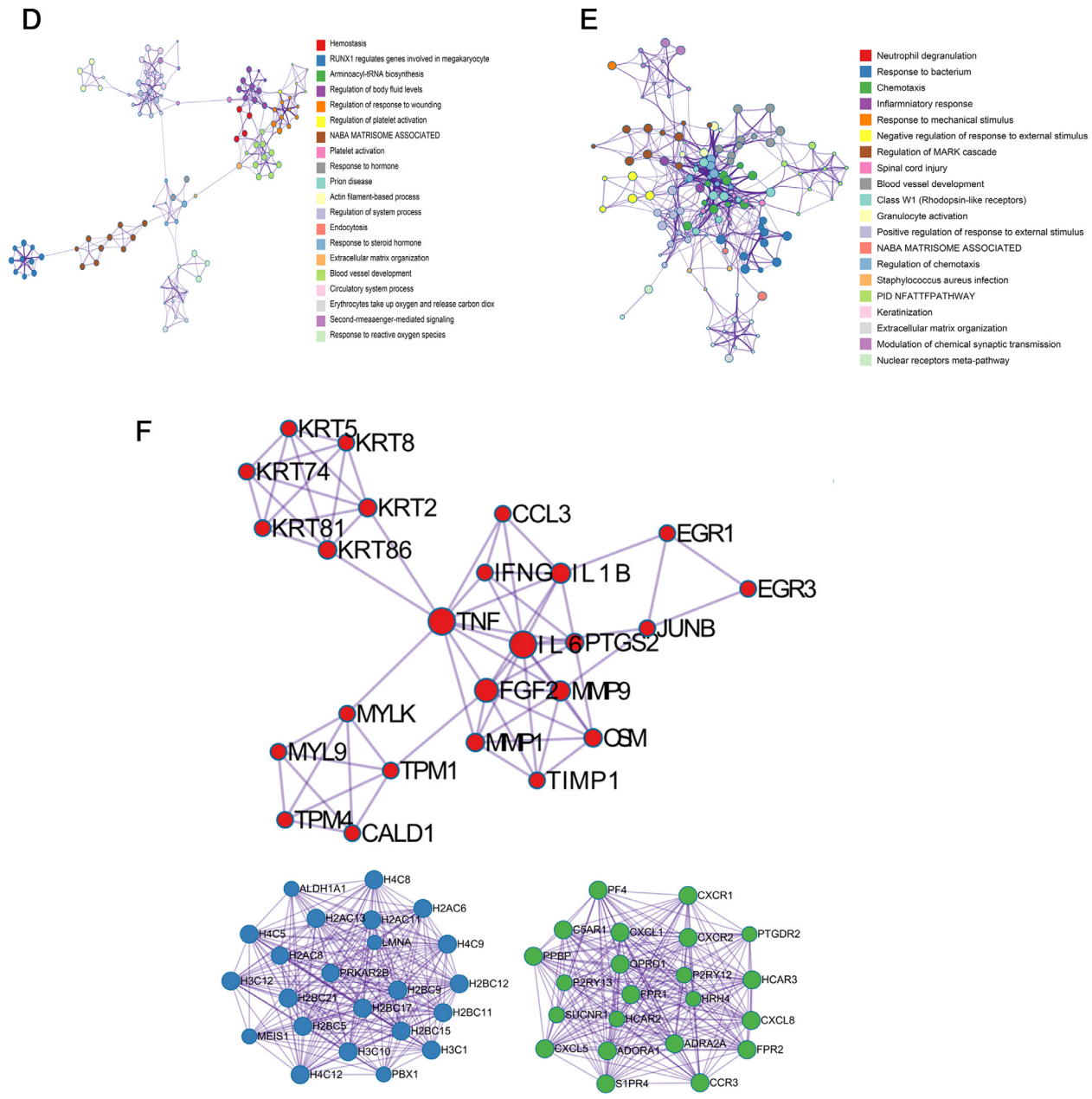

Figure 1. Continued

response (Figure 2E). *MMP8*, which is mainly produced by neutrophils and macrophages (33), was particularly affected. These findings suggest that downregulated DEGs in SSc are associated with neutropenia and immune response alterations.

Differential Expression of TFs

As histones can epigenetically modulate the accessibility of TF binding sites on DNA, we sought to investigate the differential expression of TFs in SSc. Our transcriptomic analysis identified 27 upregulated and 22 downregulated TFs in SSc (Figure 3A). To understand potential interactions among these TFs, we examined their correlations and observed a coordinated expression pattern between *MEIS1, GRHL1, TAL1, HESX1, GATA1, GFI1B, THRB, PBX1,* and *EBF1* (Figure 3B). Further analysis using MetaScape revealed that upregulated TFs were significantly enriched in biological processes such as embryonic hematopoiesis, suggesting involvement in transcriptional regulation of megakaryocytes (Figure 3C). Conversely, downregulated TFs were enriched in biological processes related to leukocyte differentiation, suggesting their potential role in regulating hematopoietic differentiation and the immune system (Figure 3D).

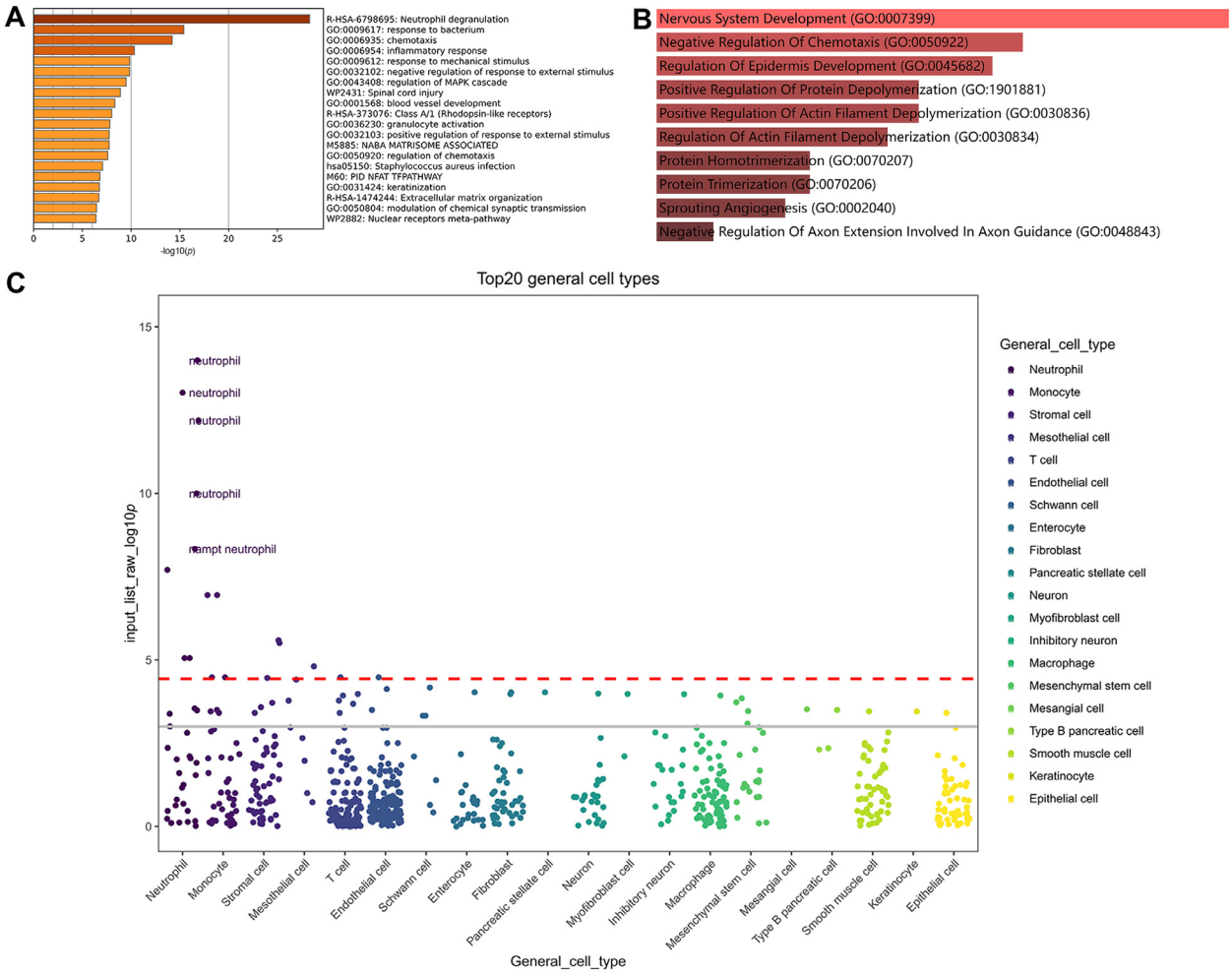

Figure 2. GO enrichment analysis of SSc-specific downregulated genes. A. GO enrichment analysis of SSc-specific downregulated genes in biological processes via MetaScape. B. GO enrichment analysis of SSc-specific downregulated genes in biological processes using the Enrichr platform. C. Enrichment analysis across different cell type-specific signatures. D. Enrichment scores for neutrophils derived from xCell and scores for low-density neutrophils determined by the ABIS algorithm (p <0.001, Kruskal-Wallis test). E. Expression profiles of genes downregulated in SSc, including *ALDH1A1, ASIC1, CASP5, IL1RL1, IL13RAI,* and *MMP8*, compared to controls.

Comparative Transcriptomic Analysis Reveals Shared Pathogenic Pathways in ADs

To understand the commonalities and differences in gene expression patterns between SSc and other ADs, we used bulk RNA-seq data obtained from PBMCs of RA, SLE, pSS, and SSc patients. PCA revealed clustering of these ADs, indicating transcriptional similarities (Figure 4A). Compared to healthy controls, these ADs exhibited parallel transcriptional profiles with shared DEGs, suggesting common pathogenic pathways (Figure 4B). The cross-disease analysis identified 369 upregulated and 179 downregulated genes common to all ADs studied (Figure 4C). Upregulated genes, such as *IFI27* involved in SLE (5), were predominantly associated with biological processes such as wound healing, blood coagulation, and platelet activation (Figure 4D, left panel). Downregulated genes, including chemokines and receptors like *CXCR1, CXCR2, CXCL8,* and *CSF3R*, were enriched for processes involving granulocyte activation, neutrophil activation, and cytokine receptor binding (Figure 4D, right panel). GO enrichment analysis further revealed ‘platelet activation’ as the most significantly upregulated pathway (Figure 4E, left panel), whereas ‘viral protein interaction with cytokine and cytokine receptor’ emerged as the most downregulated pathway (Figure 4E, right panel). These findings are consistent with the GO analysis and PPI network results of SSc-related DEGs (Figure 1D–F), suggesting a common emphasis on megakaryocyte-related processes, neutrophil activation, and chemokine signaling in various ADs.

Next, we sought to identify common differentially expressed TFs in ADs. We found 15 commonly upregulated and nine downregulated TFs across the four ADs

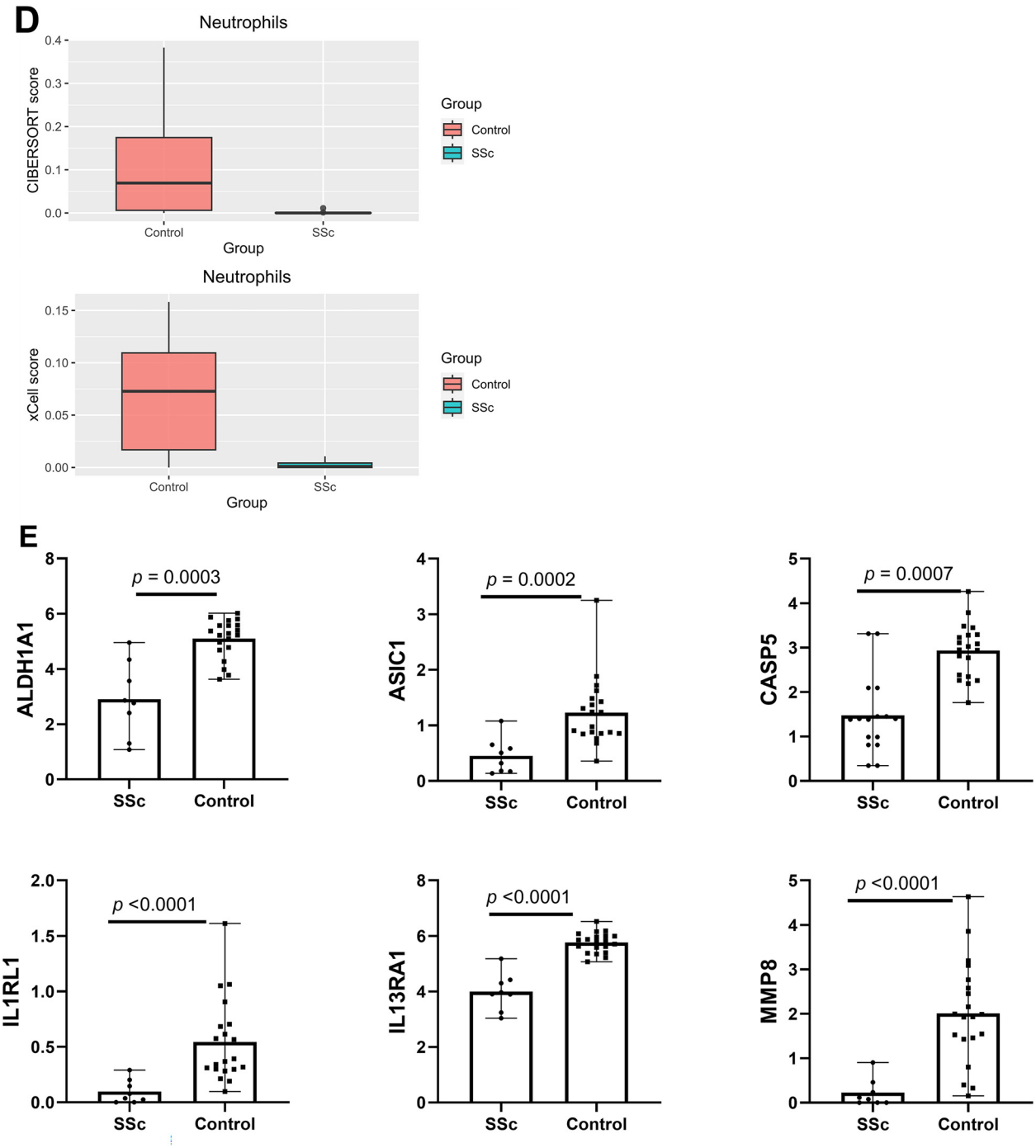

Figure 2. Continued

(Figure 5A), which were mainly enriched in the regulation of hematopoiesis. Among the downregulated TFs *EGR1, EGR2, EGR3,* and *CEBPE* were observed (Figure 5B). Using the CIBERSORTx algorithm, we observed a significant decrease in neutrophil numbers in all ADs (p <0.001; Figure 5C). Enrichment analysis of cell type-specific signatures confirmed this reduction (Figure 5D). Further analysis showed a significant downregulation of *CSF3R, CXCL8,* and *CXCL1*, which are essential components involved in the recruitment and regulation of neutrophils and other immune cells in inflammatory processes (34), when comparing ADs with controls, suggesting that these downregulated genes are associated with the reduction in neutrophils across ADs (Figure 5F).

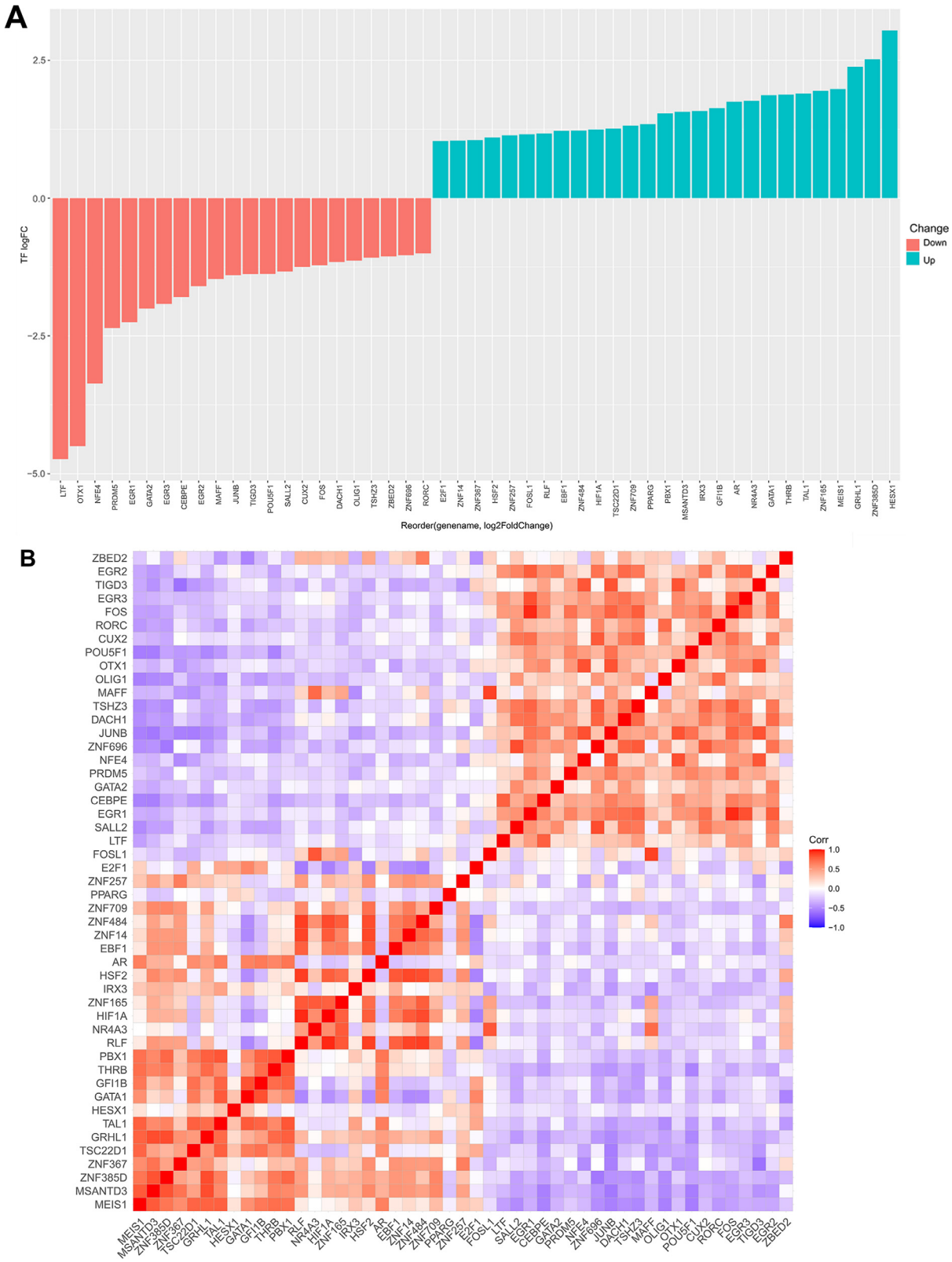

Figure 3. Transcriptional regulation of hematopoiesis and leukocyte differentiation in SSc patients compared to healthy controls. A. Differential expression of transcription factors (TFs) identified from the DEGs. B. Correlation heatmap of differentially expressed TFs. C, and D. Network of GO terms related to biological processes significantly enriched among upregulated TFs C, and downregulated TFs D. Each node represents a GO term, and the edges indicate shared genes between the terms.

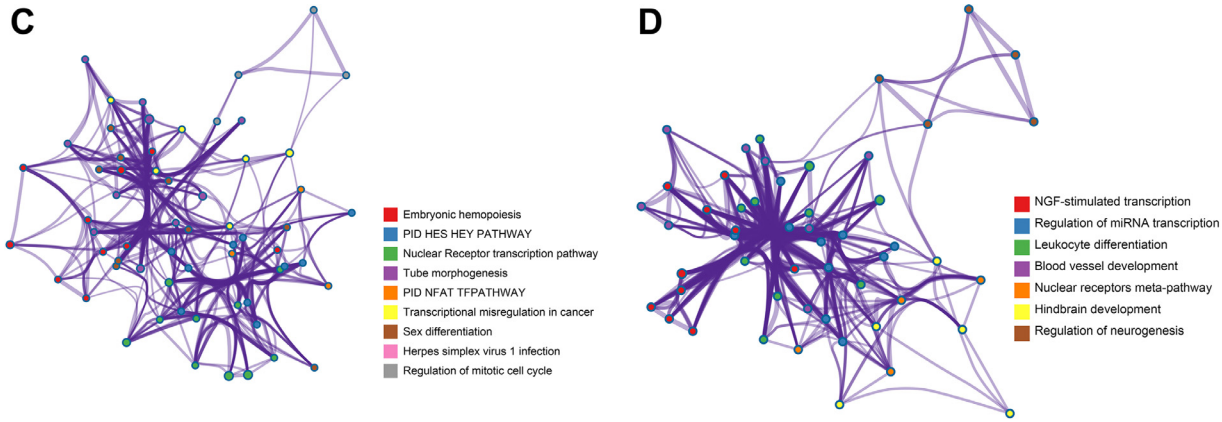

Figure 3. Continued

Discussion

SSc is characterized by fibrosis, vascular abnormalities, and immune dysregulation, leading to skin and internal organ involvement. In this study, we systematically evaluated SSc and its common and unique features compared to other ADs from the perspective of differential gene expression, transcription factors, and immune cell composition. We found that SSc-related DEGs were associated with neutrophil number and function. Skin integrity genes, including *KRT2, KRT74, KRT5,* and *MEGF9* (30,32), were downregulated in SSc, a finding not previously reported. Of note, *KRT2* and *KRT74* were specifically downregulated in SSc. Differentially expressed transcription factors in SSc have potential implications for leukocyte differentiation. In addition, comparative transcriptomic analysis across ADs revealed shared pathogenic pathways and highlighted commonalities in gene expression patterns related to megakaryocyte proliferation and neutropenia in these conditions. These findings provide valuable insights into the molecular mechanisms underlying SSc and its relationship to other ADs.

Megakaryocytes, derived from hematopoietic stem cells, are responsible for the production of blood platelets. These cells harbor several growth factors, including fibroblast growth factor, transforming growth factor, and platelet-derived growth factors, which are critical in fibrotic diseases (35). The presence of these growth factors in megakaryocytes and platelets suggests their potential involvement in wound healing during inflammatory processes, but also in bone marrow fibrosis (36), myelofibrosis (37), and lung fibrosis (38). This evidence highlights the expansion of megakaryocytes in PBMCs in treatment-naive SSc.

In our study, PPI networks highlighted the association of the *KRT* family genes with immune cells. Notably, *KRT2* and *KRT74,* along with *MEGF9* and *MMP8,* were specifically downregulated in SSc compared to other ADs. KRTs are structural proteins expressed in keratinocytes that are crucial for cytoskeletal changes during keratinocyte activation in response to wound healing and various pathological conditions (39). While *KRT5, KRT6, KRT16,* and *KRT17* have been previously recognized for their involvement in skin immunity and disease pathogenesis (40,41). Our study introduces a novel finding by identifying *KRT2* and *KRT74* as associated with immune cell activity, broadening the understanding of KRTs in skin immunology. Hewitt RJ, et al. demonstrated that in vitro the migratory properties and the remodeling gene expression of *KRT5+* cells are modulated by extracellular matrix composition and organization, contributing to remodeling events in the fibrotic niche (42). Song et al. identified 186 preferentially expressed proteins between radiogenic fibrotic and normal skin tissues, including *KRT5* (43). In addition, *MEGF9* is associated with central neutrophils and keratinocytes in the skin (32), suggesting potential interactions between megakaryocytes and neutrophils that may play a role in priming autoimmune T cells in the pathogenesis of SSc. *MMP8*, also known as neutrophil collagenase, is an enzyme involved in the breakdown of extracellular matrix components and plays a critical role in inflammatory processes, tissue remodeling, and wound healing (44). The downregulation of *MMP8* in SSc suggests a potential impairment in tissue remodeling and repair processes, contributing to the fibrotic pathology of the disease.

In SSc, we identified 27 upregulated and 22 downregulated TFs, with a coordinated expression pattern among several key transcription factors including *MEIS1, GRHL1, TAL1, HESX1, GATA1, GFI1B, THRB, PBX1,* and *EBF1*. Among these genes, *GRHL1* has been implicated in the development and postnatal maintenance of the skin barrier (45,46). The distribution of *TAL1* peaks in megakaryocyte erythrocyte progenitor (MEP) cells showed a unique pattern, characterized by numerous peaks located in intergenic regions. Furthermore, both *GATA2* and *TAL1* peaks exhibited additional enrichment for the GATA motif, particularly in MEP (47). In a study by Wang Y, et al., *GATA1,*

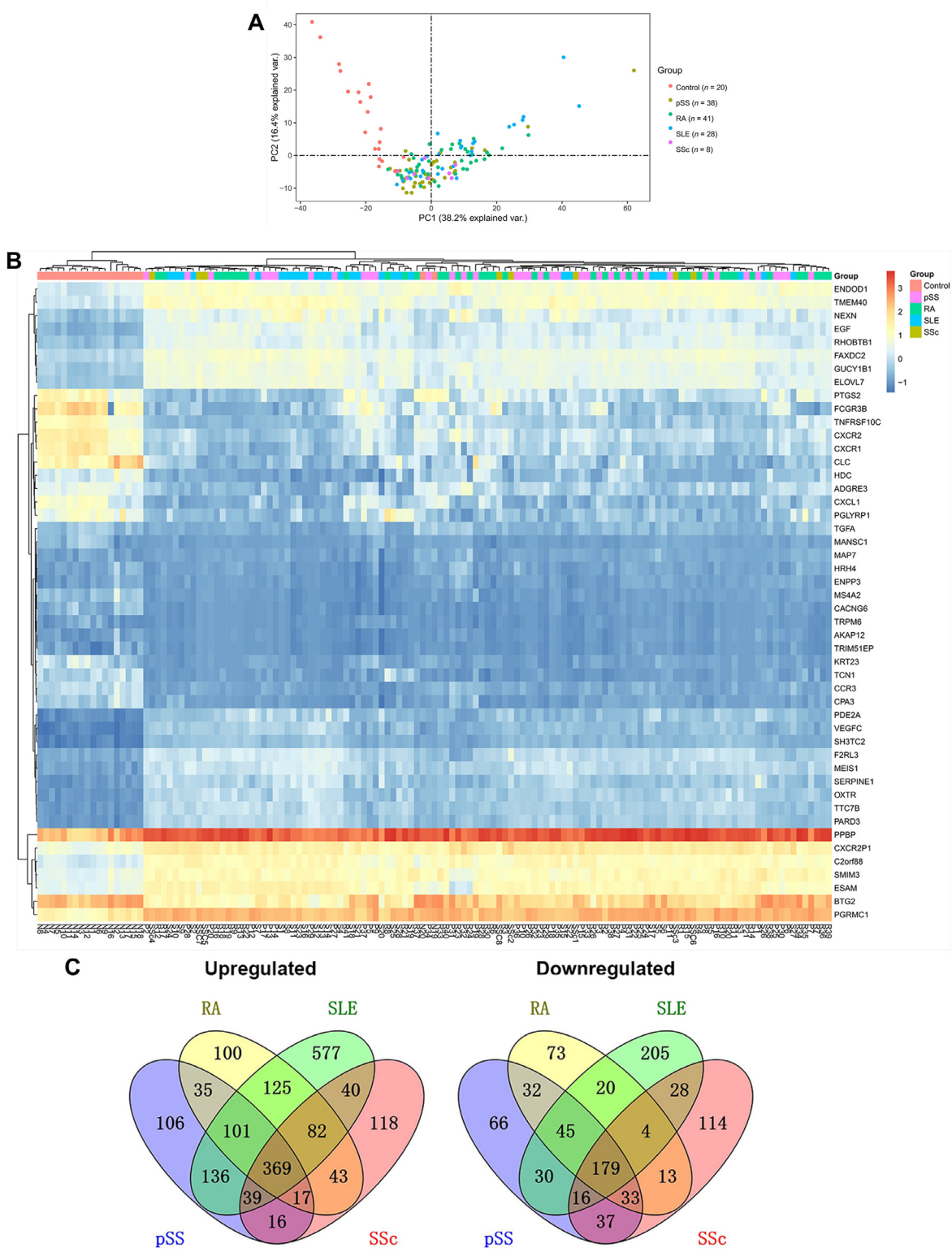

Figure 4. Shared transcriptional features in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS), and SSc. A. PCA of gene expression in PBMCs from patients with RA, SLE, pSS, and SSc, indicating overlapping transcriptional signatures among these autoimmune diseases (ADs). B. A heat map illustrates the top DEGs common to RA, SLE, pSS, and SSc. C. Venn diagrams display the overlap of DEGs, with 369 upregulated (left panel) and 179 downregulated (right panel) genes shared among the four ADs. D. GO enrichment analysis for the commonly upregulated (left panel) and downregulated (right panel) DEGs. E. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for the shared upregulated (left panel) and downregulated (right panel) genes.

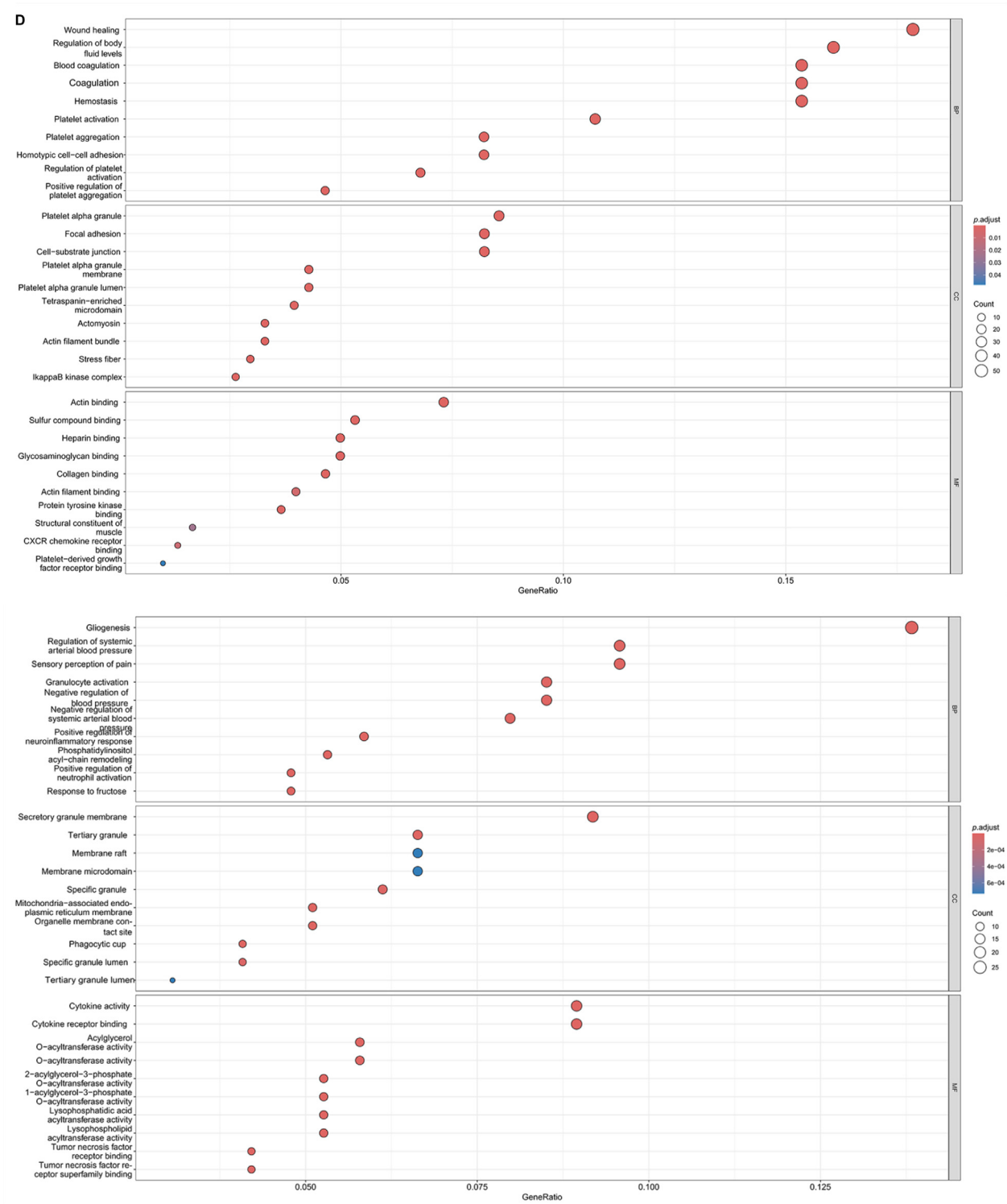

Figure 4. Continued

TAL1, and *GFI1B* were associated with specific immune cell lineages based on their expression within hematopoietic transcriptional networks (5), highlighting their importance in regulating hematopoiesis and megakaryocyte development.

Neutrophils, the most abundant immune cells in human blood, play a critical role in immune defense (48). Neutrophils contribute significantly to inflammation in several ADs, including SSc (49,50). In SSc, neutrophils are involved in endothelial cell apoptosis, an early event lead-

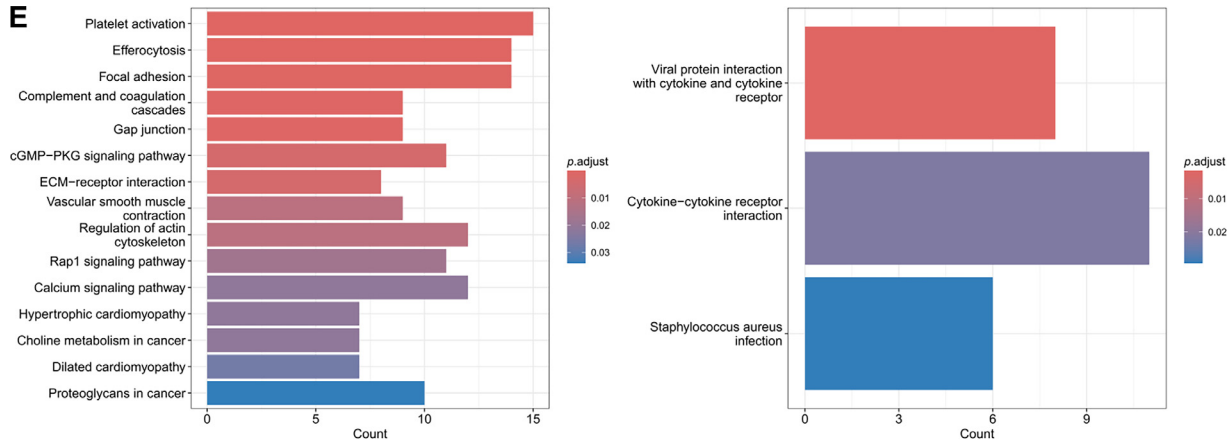

Figure 4. Continued

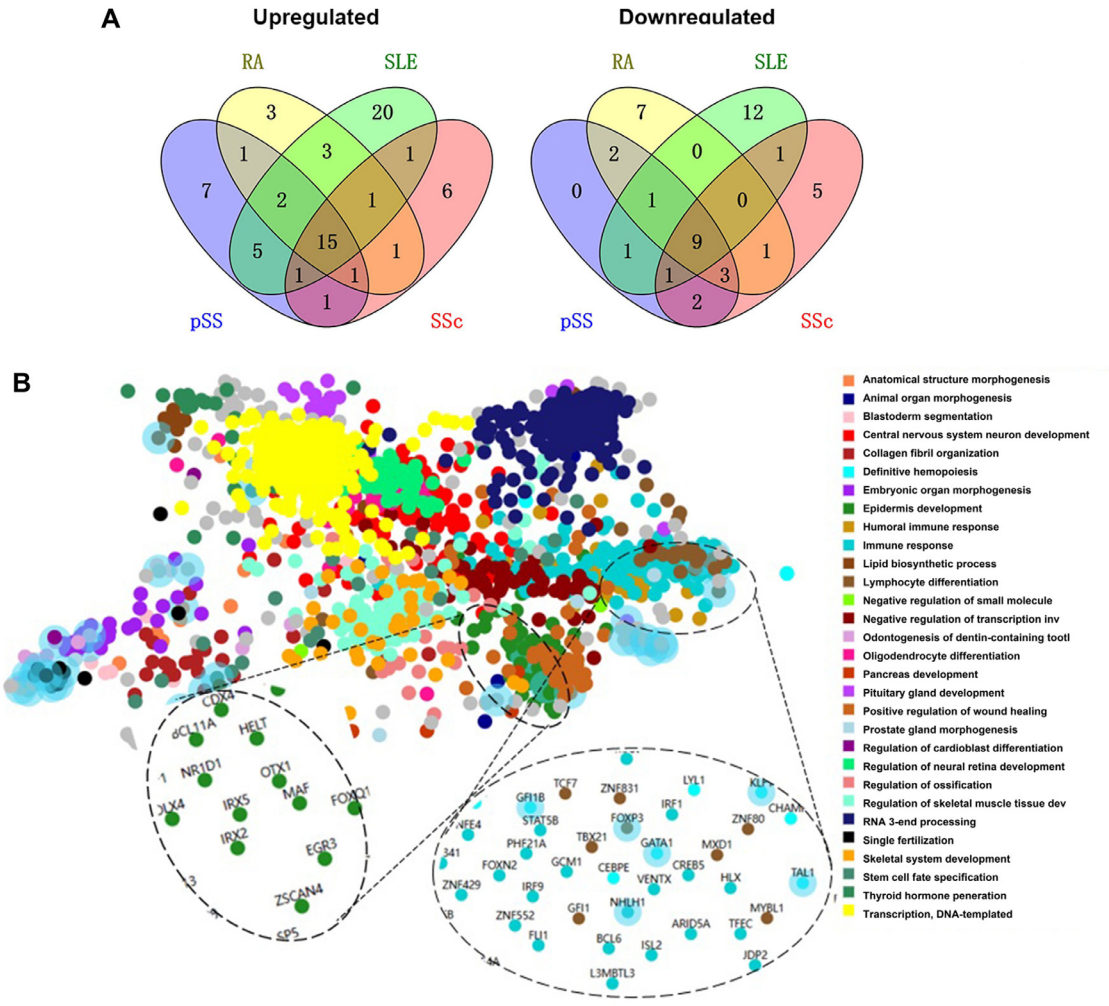

Figure 5. Core TFs indicated megakaryocyte expansion and neutrophil decrease in ADs. A. Venn diagram illustrates the 24 TFs shared across RA, SLE, pSS, and SSc, with the left panel showing upregulated TFs and the right panel showing downregulated TFs. B. TF enrichment using ChIP-X Enrichment Analysis 3 (ChEA3) identifies associations between the involved TFs. These include upregulated TFs such as *GATA1, TAL1,* and *GFI1B* and downregulated TFs such as *EGR3*. C. Cell type composition was revealed by CIBERSORTx by presenting an enrichment score of cell types in ADs compared to healthy controls (p <0.001, Kruskal-Wallis test). D. Enrichment analysis of cell type-specific signatures. E. A schematic diagram of the hematopoietic stem cell differentiation pathway regulated by specific TFs. F. Statistically significant downregulation of genes associated with neutrophils, including *CSF3R, CXCL8,* and *CXCL1*, was observed when comparing AD patients to healthy controls.

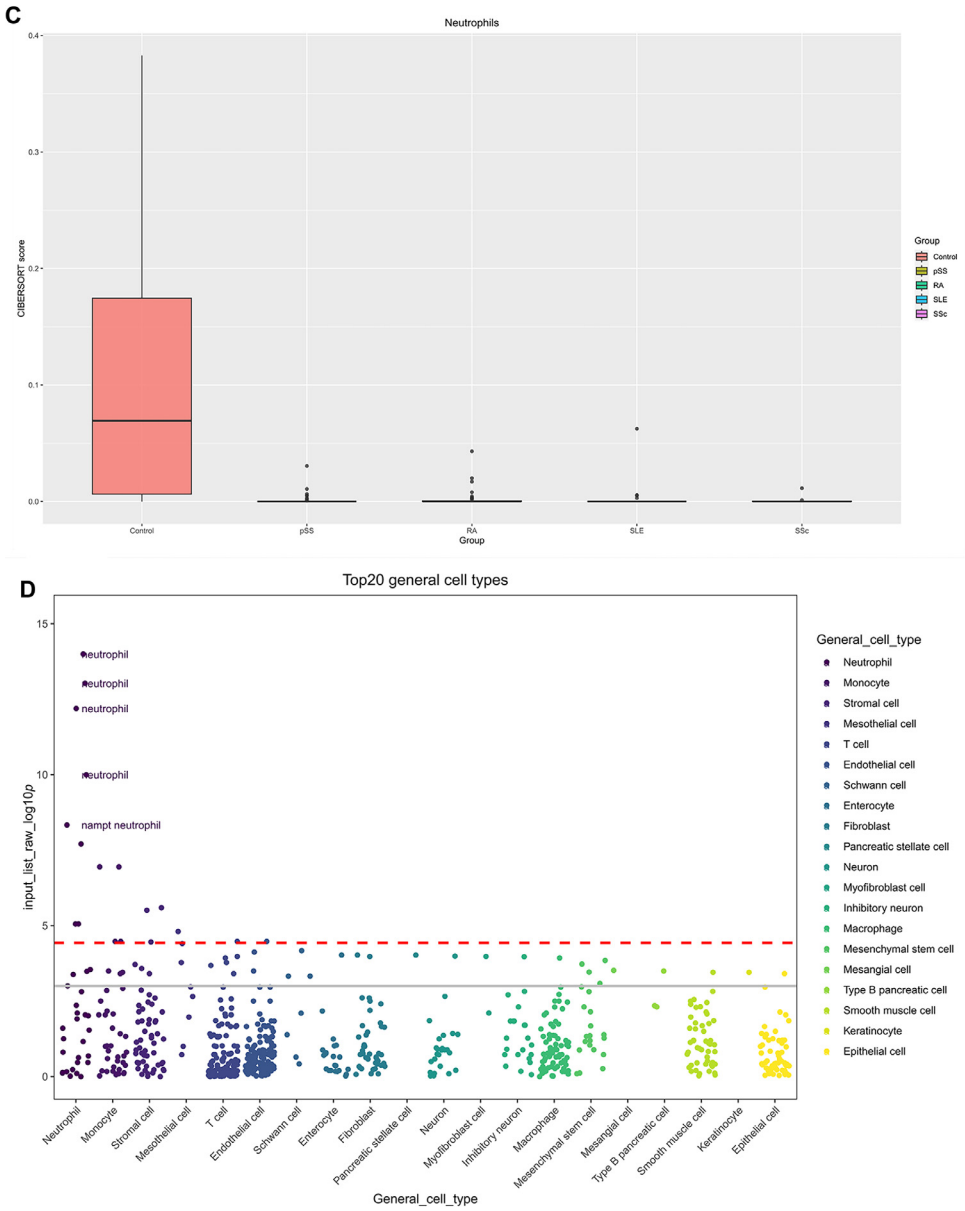

Figure 5. Continued

ing to fibrosis (51). In this study, we observed a decreased expression of neutrophils and dysregulated TFs in PBMCs from SSc patients. This finding suggests neutrophil dysfunction in SSc patients that affects cell migration (52,53), including the absence of typical chemokines (*CXCL1, CXCL8*) and receptors (*CSF3R*), that are essential for migration toward *CXCL8.* NETosis, a unique form of neutrophil death, results in the release of DNA, histones, and cytoplasmic proteins, forming a web of extracellular traps. These components can serve as autoantigens and potentially stimulate the production of anti-nuclear antibodies (ANAs) (54). ANAs are prevalent in AD, with positivity rates exceeding 90% in SSc and SLE and slightly lower rates in RA and pSS (55–58). Thus, the diminished neutrophil functions in SSc may be related to the altered immune response involving ANA production. This connection may be crucial to understanding the pathogenesis of SSc.

ADs are a complex group of conditions characterized by the immune system's misdirected attack on the body's own tissues. PCA has revealed similarities in the transcriptomes of PBMCs from patients with RA, SLE, pSS, and SSc. This suggests that these ADs may share similar pathological pathways, such as platelet and granulocyte activation, neutrophil function, and abnormal regulation of cytokine receptors, indicating a common pathophysiological basis. In ADs, PBX1 and GATA1 are consistently upregulated. PBX1 (PBX Homeobox 1) is a member of the PBX family of genes that regulate the

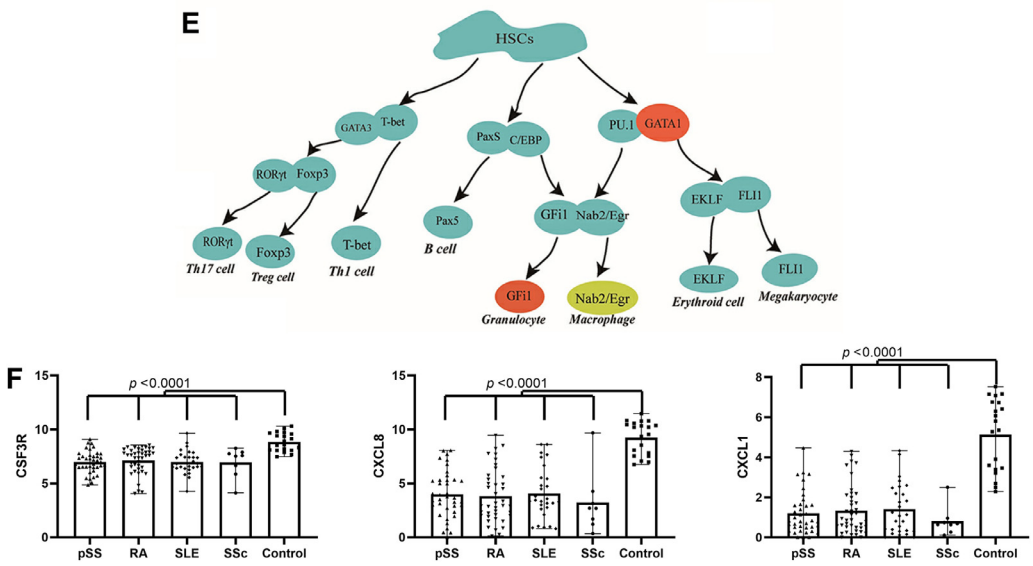

Figure 5. Continued

immune cell homeostasis (59). GATA1 (GATA-Binding Protein 1) plays a crucial role in various biological processes, particularly in the development and differentiation of hematopoietic cells, regulating the development of megakaryocytes and leukemic progression (60). Upregulation of PBX1 and GATA1 may enhance immune cell activation and inflammation, thereby exacerbating the pathological process of ADs. ADs show shared alterations in TFs, including the EGR family, which are rapid-response TFs that modulate gene expression related to endothelial function and vascular remodeling. Activated under oxidative stress, EGR family members contribute to the regulation of antioxidant enzymes and protect cells from oxidative damage (61). In this study, *EGR1, EGR2,* and *EGR3* were significantly downregulated in ADs. Immunophenotyping revealed a reduction in neutrophils within ADs, which may be related to the marked downregulation of genes such as *CSF3R, CXCL8,* and *CXCL1*. *CSF3R*, which encodes the neutrophil colony-stimulating factor 3 receptor (CSF3R), can inhibit neutrophil granulopoiesis and mobilization (62). Downregulation of CXCL8 and CXCL1 may affect neutrophil recruitment and modulation of the inflammatory response (63,64).

ADs share common gene expression patterns, but also have specific differences. SSc is characterized by skin thickening and fibrosis, which may extend to involve visceral organs. SSc is associated with unique DEGs and regulatory networks, such as *ALDH1A1, MMP8*, and *MEGF9*, which are associated with processes such as tissue remodeling, signal transduction, and cell adhesion. These specific differences likely reflect the pathophysiological distinctiveness of several diseases. In summary, ADs exhibit both commonalities and divergences in gene expression patterns, facilitating insight into their shared pathophysiological foundations and specific differences.

Conclusions

For the first time, our research demonstrated that *KRT2, KRT5, KRT74, MEGF9*, and *MMP8* are downregulated in SSc, with *KRT2* and *KRT74* showing specific downregulation in SSc compared to ADs. We identified distinct dysregulation of genes related to neutrophil function and megakaryocyte increase in SSc, while also revealing pathogenic pathways, differentially expressed TFs, and a reduction in neutrophils that were shared by SSc and other ADs. These findings enhance our understanding of the complex pathogenesis of SSc and offer potential targets for future therapeutic interventions.

Funding

This study was supported by the Guangdong Province Science and Technology Special Fund Project (210716126901097), 2023 China Ultra sonographers, Rising Stars of Science and Technology Research Program, Class A Grant, The National Natural Science Foundation of China (No. 82271853).

Acknowledgments

The authors thank the patients and their families for their participation in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Perelas A, Silver RM, Arrossi AV, et al. Systemic sclerosis-associated interstitial lung disease. Lancet Respir Med 2020;8:304–320. doi:10.1016/s2213-2600(19)30480-1.
2. Li J-X. Secular Trends in Systemic Sclerosis Mortality in the United States from 1981 to 2020. Int J Environ Res Public Health 2022;19:15088.
3. Poudel DR, Jayakumar D, Danve A, et al. Determinants of mortality in systemic sclerosis: a focused review. Rheumatol Int 2018;38:1847–1858.
4. Ebata S, Yoshizaki-Ogawa A, Sato S, et al. New era in systemic sclerosis treatment: recently approved therapeutics. J Clin Med 2022;11:4631.
5. Wang Y, Xie X, Zhang C, et al. Rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren's syndrome shared megakaryocyte expansion in peripheral blood. Ann Rheum Dis 2022;81:379–385.
6. Mouawad JE, Feghali-Bostwick C. The Molecular Mechanisms of Systemic Sclerosis-Associated Lung Fibrosis. Int J Mol Sci 2023;24:2963.
7. Allanore Y, Distler O, Matucci-Cerinic M, et al. Defining a unified vascular phenotype in systemic sclerosis. Arthritis Rheumatol 2018;70:162–170.
8. Sambataro D, Sambataro G, Pignataro F, et al. Patients with interstitial lung disease secondary to autoimmune diseases: how to recognize them? Diagnostics 2020;10:208.
9. Di Maggio G, Confalonieri P, Salton F, et al. Biomarkers in systemic sclerosis: an overview. Curr Issues Mol Biol 2023;45:7775–7802. doi:10.3390/cimb45100490.
10. Hughes M, Allanore Y, Chung L, et al. Raynaud phenomenon and digital ulcers in systemic sclerosis. Nat Rev Rheumatol 2020;16:208–221. doi:10.1038/s41584-020-0386-4.
11. Le JH, Cho KI. Association between endothelial function and microvascular changes in patients with secondary Raynaud's phenomenon. Clin Rheumatol 2014;33:1627–1633. doi:10.1007/s10067-014-2553-y.
12. Muruganandam M, Ariza-Hutchinson A, Patel RA, et al. Biomarkers in the pathogenesis, diagnosis, and treatment of systemic sclerosis. J Inflamm Res 2023;16:4633–4660. doi:10.2147/jir.S379815.
13. Ruaro B, Confalonieri M, Matucci-Cerinic M, et al. The treatment of lung involvement in systemic sclerosis. Pharmaceuticals (Basel) 2021;14. doi:10.3390/ph14020154.
14. Kleiveland CR. Peripheral blood mononuclear cells. The Impact of Food Bioactives on Health: *in vitro* and *ex vivo* models 2015:161–167.
15. Preglej T, Brinkmann M, Steiner G, et al. Advanced immunophenotyping: a powerful tool for immune profiling, drug screening, and a personalized treatment approach. Front Immunol 2023;14:1096096.
16. Vlachogiannis NI, Tual-Chalot S, Zormpas E, et al. Adenosine-to-inosine RNA editing contributes to type I interferon responses in systemic sclerosis. J Autoimmun 2021;125:102755.
17. Kitanaga Y, Imamura E, Nakahara Y, et al. *In vitro* pharmacological effects of peficitinib on lymphocyte activation: a potential treatment for systemic sclerosis with JAK inhibitors. Rheumatology 2020;59:1957–1968.
18. Szabo I, Badii M, Gaál IO, et al. Enhanced innate and acquired immune responses in systemic sclerosis primary peripheral blood mononuclear cells (PBMCs). Int J Mol Sci 2023;24:14438.
19. Thomas G, Frederick E, Thompson L, et al. LMWF5A suppresses cytokine release by modulating select inflammatory transcription factor activity in stimulated PBMC. J Transl Med 2020;18:1–15.
20. Ascoli C, Schott CA, Huang Y, et al. Altered transcription factor targeting is associated with differential peripheral blood mononuclear cell proportions in sarcoidosis. Front Immunol 2022;13:848759.
21. Woo M-Y, Yun SJ, Lee MJ, et al. Transcription factors regulating inflammatory cytokine production are differentially expressed in peripheral blood mononuclear cells of Behçet disease depending on disease activity. Ann Dermatol 2017;29:173–179.
22. Vitali C, Bombardieri S, Jonsson R, et al. European Study Group on Classification Criteria for Sjögren's Syndrome. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002.
23. Aringer M, Costenbader K, Daikh D, et al. 2019 European league against rheumatism/American college of rheumatology classification criteria for systemic lupus erythematosus. Arthritis Rheumatol 2019;71:1400–1412.
24. Van Den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum 2013;65:2737–2747.
25. Monaco G, Lee B, Xu W, et al. RNA-Seq signatures normalized by mRNA abundance allow absolute deconvolution of human immune cell types. Cell reports 2019;26:1627–1640.e1627.
26. Newman AM, Steen CB, Liu CL, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol 2019;37:773–782.
27. Nevinsky GA, Buneva VN, Dmitrenok PS. Multiple sclerosis: enzymatic cross site-specific recognition and hydrolysis of H3 histone by IgGs against H3, H1, H2A, H2B, H4 Histones, Myelin Basic Protein, and DNA. Biomedicines 2022;10:2663.
28. Assassi S, Swindell WR, Wu M, et al. Dissecting the heterogeneity of skin gene expression patterns in systemic sclerosis. Arthritis Rheumatol 2015;67:3016–3026.
29. Zhang T, Tseng C, Zhang Y, et al. CXCL1 mediates obesity-associated adipose stromal cell trafficking and function in the tumour microenvironment. Nat Commun 2016;7:11674.
30. Song J, Zhang H, Wang Z, et al. The role of FABP5 in radiation-induced human skin fibrosis. Radiat Res 2018;189:177–186.
31. Hewitt RJ, Puttur F, Gaboriau DC, et al. Lung extracellular matrix modulates KRT5+ basal cell activity in pulmonary fibrosis. Nat Commun 2023;14:6039.
32. Brandt-Bohne U, Keene DR, White FA, et al. MEGF9: a novel transmembrane protein with a strong and developmentally regulated expression in the nervous system. Biochem J 2007;401:447–457.
33. Sorsa T, Tervahartiala T, Leppilahti J, et al. Collagenase-2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. Pharmacol Res 2011;63:108–113. doi:10.1016/j.phrs.2010.10.005.
34. Tsioumpekou M, Krijgsman D, Leusen JH, et al. The role of cytokines in neutrophil development, tissue homing, function and plasticity in health and disease. Cells 2023;12:1981.
35. Castro-Malaspina H, Rabellino EM, Yen A, et al. Human megakaryocyte stimulation of proliferation of bone marrow fibroblasts. Blood 1981;57:781–787.
36. Ghosh K, Shome DK, Kulkarni B, et al. Fibrosis and bone marrow: understanding causation and pathobiology. J Transl Med 2023;21:703. doi:10.1186/s12967-023-04393-z.
37. Melo-Cardenas J, Migliaccio AR, Crispino JD. The role of megakaryocytes in myelofibrosis. Hematol Oncol Clin North Am 2021;35:191–203. doi:10.1016/j.hoc.2020.11.004.
38. Thachil J, Lisman T. Pulmonary megakaryocytes in coronavirus disease 2019 (COVID-19): roles in Thrombi and Fibrosis. Semin Thromb Hemost 2020;46:831–834. doi:10.1055/s-0040-1714274.
39. Freedberg IM, Tomic-Canic M, Komine M, et al. Keratins and the keratinocyte activation cycle. J Invest Dermatol 2001;116:633–640.

40. Chieosilapatham P, Kiatsurayanon C, Umehara Y, et al. Keratinocytes: innate immune cells in atopic dermatitis. Clin Exp Immunol 2021;204:296–309.
41. DePianto D, Kerns ML, Dlugosz AA, et al. Keratin 17 promotes epithelial proliferation and tumor growth by polarizing the immune response in skin. Nat Genet 2010;42:910–914.
42. Hewitt RJ, Puttur F, Gaboriau DCA, et al. Lung extracellular matrix modulates KRT5(+) basal cell activity in pulmonary fibrosis. Nat Commun 2023;14:6039. doi:10.1038/s41467-023-41621-y.
43. Song J, Zhang H, Wang Z, et al. The Role of FABP5 in Radiation-Induced Human Skin Fibrosis. Radiat Res 2018;189:177–186. doi:10.1667/rr14901.1.
44. Nwomeh BC, Liang H-X, Cohen IK, et al. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. J Surg Res 1999;81:189–195.
45. Kotarba G, Taracha-Wisniewska A, Miller M, et al. Transcription factors Krüppel-like factor 4 and paired box 5 regulate the expression of the Grainyhead-like genes. PLoS ONE 2021;16:e0257977. doi:10.1371/journal.pone.0257977.
46. Deng Z, Cangkrama M, Butt T, et al. Grainyhead-like transcription factors: guardians of the skin barrier. Vet Dermatol 2021;32:553–e152. doi:10.1111/vde.12956.
47. Subramanian S, Thoms JAI, Huang Y, et al. Genome-wide transcription factor-binding maps reveal cell-specific changes in the regulatory architecture of human HSPCs. Blood 2023;142:1448–1462. doi:10.1182/blood.2023021120.
48. Burn GL, Foti A, Marsman G, et al. The Neutrophil. Immunity 2021;54:1377–1391. doi:10.1016/j.immuni.2021.06.006.
49. Bach M, Moon J, Moore R, et al. A neutrophil activation biomarker panel in prognosis and monitoring of patients with rheumatoid arthritis. Arthritis Rheumatol 2020;72:47–56. doi:10.1002/art.41062.
50. Garcia-Romo GS, Caielli S, Vega B, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med 2011;3:73ra20. doi:10.1126/scitranslmed.3001201.
51. Barnes TC, Spiller DG, Anderson ME, et al. Endothelial activation and apoptosis mediated by neutrophil-dependent interleukin 6 trans-signalling: a novel target for systemic sclerosis? Ann Rheum Dis 2011;70:366–372. doi:10.1136/ard.2010.133587.
52. Cutolo M, Soldano S, Smith V. Pathophysiology of systemic sclerosis: current understanding and new insights. Expert Rev Clin Immunol 2019;15:753–764. doi:10.1080/1744666x.2019.1614915.
53. Zhang M, Zhang S. T Cells in Fibrosis and Fibrotic Diseases. Front Immunol 2020;11:1142. doi:10.3389/fimmu.2020.01142.
54. Sohn DH. NETosis in autoimmune diseases. J Rheum Dis 2016;23:82–87.
55. Justiz Vaillant A.A., Goyal A., Varacallo M. Systemic Lupus Erythematosus. StatPearls. Treasure Island (FL) with ineligible companies. Disclosure: amandeep Goyal declares no relevant financial relationships with ineligible companies. Disclosure: matthew Varacallo declares no relevant financial relationships with ineligible companies. 2024.
56. Miyake M, Matsushita T, Takehara K, et al. Clinical features of Japanese systemic sclerosis (SSc) patients negative for SSc-related autoantibodies: a single-center retrospective study. Int J Rheum Dis 2020;23:1219–1225.
57. Bacquet-Deschryver H, Jouen F, Quillard M, et al. Impact of three anti-TNFα biologics on existing and emergent autoimmunity in rheumatoid arthritis and spondylarthropathy patients. J Clin Immunol 2008;28:445–455.
58. Fayyaz A, Kurien BT, Scofield RH. Autoantibodies in Sjogren's syndrome. Rheum Dis Clin North Am 2016;42:419–434. doi:10.1016/j.rdc.2016.03.002.
59. Gu S, Zhang J, Han X, et al. Involvement of transcriptional factor Pbx1 in peripheral B cell homeostasis to constrain lupus autoimmunity. Arthritis Rheumatol 2023;75:1381–1394. doi:10.1002/art.42487.
60. Arkoun B, Robert E, Boudia F, et al. Stepwise GATA1 and SMC3 mutations alter megakaryocyte differentiation in a Down syndrome leukemia model. J Clin Invest 2022;132. doi:10.1172/jci156290.
61. Khachigian LM. The MEK-ERK-Egr-1 axis and its regulation in cardiovascular disease. Vascul Pharmacol 2023;153:107232. doi:10.1016/j.vph.2023.107232.
62. Liu Y, Song R, Lu Z, et al. The RNA m(6)A demethylase ALKBH5 drives emergency granulopoiesis and neutrophil mobilization by up-regulating G-CSFR expression. Cell Mol Immunol 2024;21:6–18. doi:10.1038/s41423-023-01115-9.
63. Das ST, Rajagopalan L, Guerrero-Plata A, et al. Monomeric and dimeric CXCL8 are both essential for in vivo neutrophil recruitment. PLoS ONE 2010;5:e11754. doi:10.1371/journal.pone.0011754.
64. De Filippo K, Dudeck A, Hasenberg M, et al. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. Blood 2013;121:4930–4937. doi:10.1182/blood-2013-02-486217.