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Deciphering the transcriptomic landscape of systemic lupus erythematosus-associated pulmonary arterial hypertension

Yutong Li[1,2,3†], Junyan Qian[1,2,3†], Xiaoyue Deng[1,2,3†], Leyao Ma[1,2,3], Qizhi Yuan[1,2,3], Qian Wang[1,2,3], Zhuang Tian[4], Xiaofeng Zeng[1,2,3], Xinzhuang Yang[5,6*], Jiuliang Zhao[1,2,3*] and Mengtao Li[1,2,3*]

Abstract

Background Systemic lupus erythematosus (SLE) is an autoimmune disease that involves multi-organ damage. Pulmonary arterial hypertension (PAH) is one of the life-threatening complications of SLE. The underlying cause of systemic lupus erythematosus-associated pulmonary arterial hypertension has not been fully comprehended. Besides the mechanisms implicated in the development of PAH, such as damage to the endothelial cells, the aberrant activation of the immune system also plays a substantial role in the pathogenesis of SLE-PAH.

Methods In this study, peripheral blood samples from 100 patients with SLE-PAH and 95 patients of SLE without PAH (SLE-nonPAH) were obtained for RNA sequencing and comprehensive transcriptomic analysis. Pathway enrichment analysis was performed based on differentially expressed genes (DEGs) between SLE-PAH and SLE-nonPAH to elucidate the mechanisms potentially driving the development of PAH in SLE patients. Utilizing consensus non-negative matrix factorization (cNMF), we also conducted a detailed analysis to identify distinct subgroups within the SLE-PAH population. Meanwhile, the protein-protein interaction (PPI) analysis was performed and hub genes among the SLE-PAH subgroups were detected. Common transcription factors (TFs) of detected hub genes were also discovered to serve as potential therapeutic targets.

Results Inflammatory signaling pathways, notably those involving interferon and TNFα, were found to play an important role in the SLE-PAH. Utilizing cNMF method, three unique subgroups of SLE-PAH patients were delineated, each characterized by a distinct level of inflammatory activity. Specifically, the high inflammation subgroup, marked by the activity of Interleukin-6 (IL-6), exhibited a milder form of PAH. In contrast, the subgroup with moderate inflammation displayed the most pronounced PAH symptoms. Further disease enrichment analysis revealed that, beyond the dysregulated inflammatory pathways, patients with the most severe PAH exhibited distinct pathogenic

†Yutong Li, Junyan Qian and Xiaoyue Deng contributed equally to this work.

*Correspondence:
Xinzhuang Yang
xinzhuang_yang@163.com
Jiuliang Zhao
zjlpumc@163.com
Mengtao Li
mengtao.li@cstar.org.cn

Full list of author information is available at the end of the article

transcriptomic profiles that disrupted vascular smooth muscle homeostasis, a critical component of vascular health. In the most severely affected subgroup, 13 hub genes were identified. Additionally, two transcription factors commonly associated with these genes, KLF1 and GATA1, were discovered, which may serve as potential therapeutic targets.

Conclusion Our investigation establishes inflammation as a key driver in the development of SLE-PAH. Patients who presented with concurrent dysregulations in inflammatory responses along with PAH-specific pathogenic markers exhibited a marked increase in the severity of their PAH. The insights gleaned from our transcriptomic analysis reveal the intricate interplay between inflammatory mechanisms and the molecular substrates of PAH. This nuanced understanding paves the way for more targeted and effective therapeutic approaches for SLE-PAH.

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that can demonstrate severe multiorgan damages. Pulmonary arterial hypertension (PAH) is a severe complication of SLE that can affect about 3% SLE, with an unfavorable prognosis that often serves as a major cause of mortality among SLE patients [1–3]. PAH can also significantly impair the patient's physical capacity and diminish their quality of life [4–7].

PAH is a subtype of pulmonary hypertension, often designated as type I PH [8]. The underlying mechanism of SLE-PAH has not yet been thoroughly understood. SLE-PAH's pathophysiology differs dramatically from other non-connective tissue disease-related PAH (CTD-PAH). The unique pathophysiology of SLE-PAH is linked to aberrant immune system activity and several investigations have found an association between immune complexes and SLE-PAH [9–11]. Positivity of anti-ribonucleoprotein antibody has been reported to increase the risk of PAH in SLE patients [9]. The deposition of antinuclear antibodies, immunoglobulins, and complement in pulmonary vessels, a finding reminiscent of the immune complex deposition in lupus nephritis, has been reported in several studies [10–13]. A novel HLA-DQA1*03:02 mutation in the major histocompatibility complex (MHC) region has been associated with poor prognosis in SLE-PAH. This finding suggests a role for aberrant immune cell function in SLE-PAH pathogenesis [14]. The mutation of HLA-DQA1*03:02 influences the formation of a homodimer of HLA-DQ αβ heterodimers and the binding of the $CD4^+$ Tcell coreceptor molecule. The affinity of the MHC/peptide and the T cell receptor is enhanced and therefore leads to the activation of T cells. The Additionally, it has been proven that T-cell hyperactivation is a significant contributor to both inflammation and vascular remodeling in SLE-PAH [15]. Furthermore, previous research demonstrated that immunosuppressive therapy could lead to better outcomes for patients with SLE-PAH [16]. These studies collectively demonstrated that, in addition to the mechanisms specific to PAH, inflammation plays a crucial role in the development of SLE-PAH.

Previous omics research on SLE-PAH has predominantly concentrated on genomics, leaving a significant void in the examination of transcriptomes specific to this condition [14, 15, 17, 18]. As far as we are aware, our study marks the first comprehensive application of RNA sequencing to a sizable cohort of SLE-PAH patients. This has enabled us to delineate discernible subgroups within the SLE-PAH population, underpinned by their unique transcriptomic signatures. Furthermore, we conducted a thorough analysis encompassing differential gene expression, protein-protein interactions, and the identification of hub genes, alongside disease enrichment analysis, to illuminate the intricate pathogenic mechanisms at play in SLE-PAH.

Methods

Patients

The Chinese SLE Treatment and Research Group (CSTAR) is the largest national cohort that follows up on SLE patients in China [19]. This study based on the CSTAR cohort. All the enrolled patients fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria or 2019 European League Against Rheumatism /American College of Rheumatology (EULAR/ACR) classification criteria for SLE [20, 21]. The SLE-PAH patients in this study were diagnosed with right heart catheterization (RHC) and fulfilled the hemodynamic criteria: (1) mean pulmonary arterial pressure (mPAP) > 20 mmHg; (2) pulmonary vascular resistance (PVR) > 2WU (3) and PAWP ≤ 15mmHg [8]. All SLE-non-PAH patients showed neither PAH related syndromes nor echo PH signs, and all of them had peak tricuspid regurgitation velocity ≤ 2.8 m/s. Patients used steroids (prednisone > 30 mg/ day oral or pulse dose) or biological agents like rituximab were excluded. Patients with any changes in therapy with mycophenolic acid, azathioprine or methotrexate in the last 30 days were also excluded to reduce the differences caused by immunosuppressive therapy. Demographic characteristics, clinical assessments, laboratory test and medical management were recorded. The COMPERA 2.0 four-strata risk assessment system was applied to evaluate the PAH, patients within moderate or high-risk strata were designated as high-risk and the others were defined as low-risk [22]. All patients gave written informed permission. Overall, 100 SLE-PAH

and 95 SLE without PAH patients were included in this study.

Sample processing and sequencing

Peripheral blood samples were collected from patients. Erythrocytes were lysed with Ammonium-Chloride-Potassium lysing buffer (Gibco). The total RNA was extracted from the peripheral blood samples via PAXgene® Blood RNA Kit (PreAnalytiX). VAHTM mRNA Capture Beads (YEASEN) were applied to isolate mRNA from the total RNA. When the mRNA quantity fell within the range of 10 to 40 nanograms (ng), proceed with normal library construction. Hieff NGS Ultima Dual-mode mRNA Library Prep Kit for Illumina (YEASEN) was utilized for library construction. The product was amplified via PCR with Thermal cyclerS1000 (Bio-Rad) to generate the library. Finally, an rRNA-depleted and strand-specific library was established and then subjected to quality control (QC) via qPCR (StepOne Plus (ABI)), ensuring a concentration above 3 nM. Libraries that passed the QC were scheduled for subsequent sequencing. The sequencing was performed by Novaseq 6000 (Illumina) with 150 bp pair-end mode.

Data processing and quality control

The RNA-seq data was output in fastq form and quality control was performed via FastQC (v 0.11.9) with default parameters and all the samples passes the QC. The sequencing data was aligned against the GRCh38 reference sequence using STAR (v 2.7.10b) [23]. The sam files were then sorted with samtools (v 1.16.1), and the bam files output from samtools were transformed to read counts via featureCounts (part of Subread). The read count data was processed in R (v 4.3.1). Low expression genes (read counts of the gene were lower than 10 in over 100 samples) were considered as noise and filtered out. Batch effects of the data were examined with principal component analysis (Supplementary Fig. 1). Transcripts per million (TPM) values were calculated from the gene counts with the following formula $TPM = \frac{(Count/Effective\,length)*10^6}{Sum\,(Count/Effective\,length)}$; ∴

Differential gene expression analysis

To detect differentially expressed gene (DEG), the R package DEseq2 was utilized with read counts of 18,630 genes as input. The differential gene expression analysis was performed between SLE-PAH and SLE-nonPAH patients to demonstrate the mechanisms underlying SLE-PAH. The genes with absolute log2 fold change over 0.5 and adjusted p value less than 0.05 were defined as differentially expressed genes.

Pathway enrichment analysis

To elucidate the pathway involved in SLE-PAH pathogenesis, various pathway enrichment analysis was performed. Gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment and Gene Set Enrichment Analysis (GSEA) was performed by R package: "clusterProfiler" (v 4.10.0). MsigDB hallmark gene sets (50 annotations) were used for GSEA [24]. The GO and KEGG enrichment was visualized in Cytoscape with Cluego [25]. The DEGs used for GSEA were sorted according to the value calculated with the following formula: $Value = -log10\begin{pmatrix}P\,value\,of\,differential\\ gene\,expression\,analysis\end{pmatrix}) /(\,sign\ (log2\ (Fold\,Change))$.

Consensus non-negative matrix factorization

In order to survey the ideal number of subgroups of SLE-PAH, consensus non-negative matrix factorization (cNMF)-based clustering was employed. CNMF-based clustering was performed with R package: NMF (v 0.26). TPM of 1,324 DEGs between SLE-PAH and SLE-nonPAH were input for cNMF-based clustering. Fifty iterations of NMF were carried out, assuming a rank ranged within 2 ~ 7, in order to determine the optimal rank and consequently the number of subgroups. The optimal rank of the clustering was determined based on high cophenetic and sillhouete. A seed of 111,111 was manually set to ensure the reproducibility of the analysis.

Principal component analysis

Principal component analysis (PCA) was performed based on the TPM values of DEGs detected previously. The PCA was based on the FactoMineR R package (v 2.9).

CIBERSORT

TPM values of our transcriptome data and the LM22 signature genes were used as input for Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) [26]. CIBERSORT (v 0.1.0) was performed at the absolute mode (sig.score as the absolute method) without quantile normalization.

Protein-protein interaction analysis and hub genes identification

Protein-protein interaction (PPI) analysis was conducted among previously identified DEGs. The network was generated on the STRING (website: https://cn.string-db.org/). The PPI analysis was then performed with the algorithm "MCODE" in Cytoscape software (v 3.10.2).

The cytoHubba software (v 0.1) in Cytoscape was used for identifying hub genes among the DEGs. The 13 algorithms provided by cytohubba were used for voting to

select hub genes. The genes that were designated as hub genes by over 8 algorithms were finally identified.

Transcription factor prediction

The common transcription factors of the hub genes were predicted on ChEA3 (website: https://maayanlab.cloud/chea3/) [27].

Disease enrichment analysis

The purpose of performing disease enrichment analysis is to identify correspondences between genes and diseases. The disease enrichment analysis was conducted with previously identified DEGs. The DisGeNET enrichment function in DOSE R package (v 3.28.2) was used for disease enrichment analysis (default parameters used) [28].

Interferon-stimulated genes (ISGs) score

The ISG score was calculated according to the following formula: $Score = \sqrt[n]{(g1 * g2 * \ldots .gn)}$, with (g1...gn) represented the read counts of interferon stimulated genes [29].

Statistical analysis

When displaying the baseline data, categorical data were displayed as percentages, while continuous data with a normal distribution were given as the mean and standardized error (SE). In order to compare continuous variables that followed a common distribution, the Student's t test was applied. For categorical variables, either the Pearson chi-squared test or Fisher's exact test was utilized. A p-value less than 0.05 was considered statistically significant. The statistical tests were carried out in R (v 4.3.1) and Graphpad Prism (v 10.1.2).

Results

Inflammation involved in the pathogenesis of SLE-PAH

We conducted a comparative analysis of gene expression between a cohort of 100 patients with SLE-PAH and 95 SLE-nonPAH patients without PAH symptom, utilizing RNA-seq data. The baseline characteristics were displayed in Supplementary Table 1. In all, there were 882 differentially expressed genes (DEGs) that decreased and 442 DEGs that increased (Fig. 1A and B). GSEA was conducted using DEGs. This study found that the SLE-PAH group exhibited a significantly elevated level of inflammation when compared to the SLE-nonPAH group. Figure 1C demonstrates that the upregulated DEGs have a high enrichment in the TNFα signaling pathway through the NFκB, interferon gamma, interferon alpha, and inflammatory response gene sets. For the purpose of enhancing comprehension of the fundamental mechanisms, a Gene Ontology (GO) study was performed (Fig. 1D). This analysis revealed that the gene sets associated with immune regulation and defensive response were significantly enriched, which were known to be the primary functions of TNFα and IFN. Furthermore, the enrichment of various pathways implicated in the activation of inflammation, including leukocyte activation, positive regulation of inflammatory response, and activation of immune response, was observed. This suggests that SLE-PAH patients presented higher levels of inflammation compared to SLE-nonPAH patients. Additional GSEA further confirmed the activation of TNFα signaling pathways (Fig. 1E and D). To further clarify the activation of IFN-related pathways, we examined the expression of 21 interferon-stimulated genes (ISGs) between SLE-PAH and SLE-nonPAH [30]. These genes can indirectly indicate the activation of type I IFN. The expression of the majority of these genes exhibited a substantial rise in SLE-PAH (Fig. 1F). Notably, gene sets associated with the adaptive system, particularly the function of B lymphocytes, were also identified (Fig. 1D). The enrichment of B cell activation, B cell receptor signaling pathway, and B cell regulated immunity indicating that the activation of B cells contributed to the SLE-PAH. Overall, the results indicated that inflammation contributed to the pathogenesis of SLE-PAH. Meanwhile, the activation of adaptive immune, especially that related to B cells, might also involve in the SLE-PAH.

CNMF clustering identified distinct subgroups of SLE-PAH

Consensus non-negative matrix factorization (cNMF) was initially conducted on gene expression data from 100 SLE-PAH patients. We performed a rank survey, and as a result, three distinct clusters were identified (Fig. 2A-C, S1A). To further validate the robustness of the clustering analysis, we included 95 SLE-nonPAH patients as controls and conducted clustering analysis anew (Fig. 2D-F, S1). The analysis delineated four distinct groups, one exclusively comprising SLE-nonPAH, and two predominantly consisting of individuals with SLE-PAH. The comparison of SLE-PAH classifications in the two clustering analyses revealed a generally steady pattern, with no statistically significant differences seen (Fig. 2G). Moreover, we conducted a PCA analysis to assess the discriminative ability of different subgroups of SLE-PAH from SLE-nonPAH (Fig. 2H). The findings substantiated the subgroups identified among the SLE-PAH patient.

Subgroups of SLE-PAH patients exhibited distinct levels of inflammation

To further elucidate the differences of underlying mechanisms among three subgroups of SLE-PAH, we conducted GSEA and found that the three clusters displayed distinct levels of inflammation (Fig. 3). Based on the results of the GSEA, the three clusters were classified as high inflammation, moderate inflammation, and low

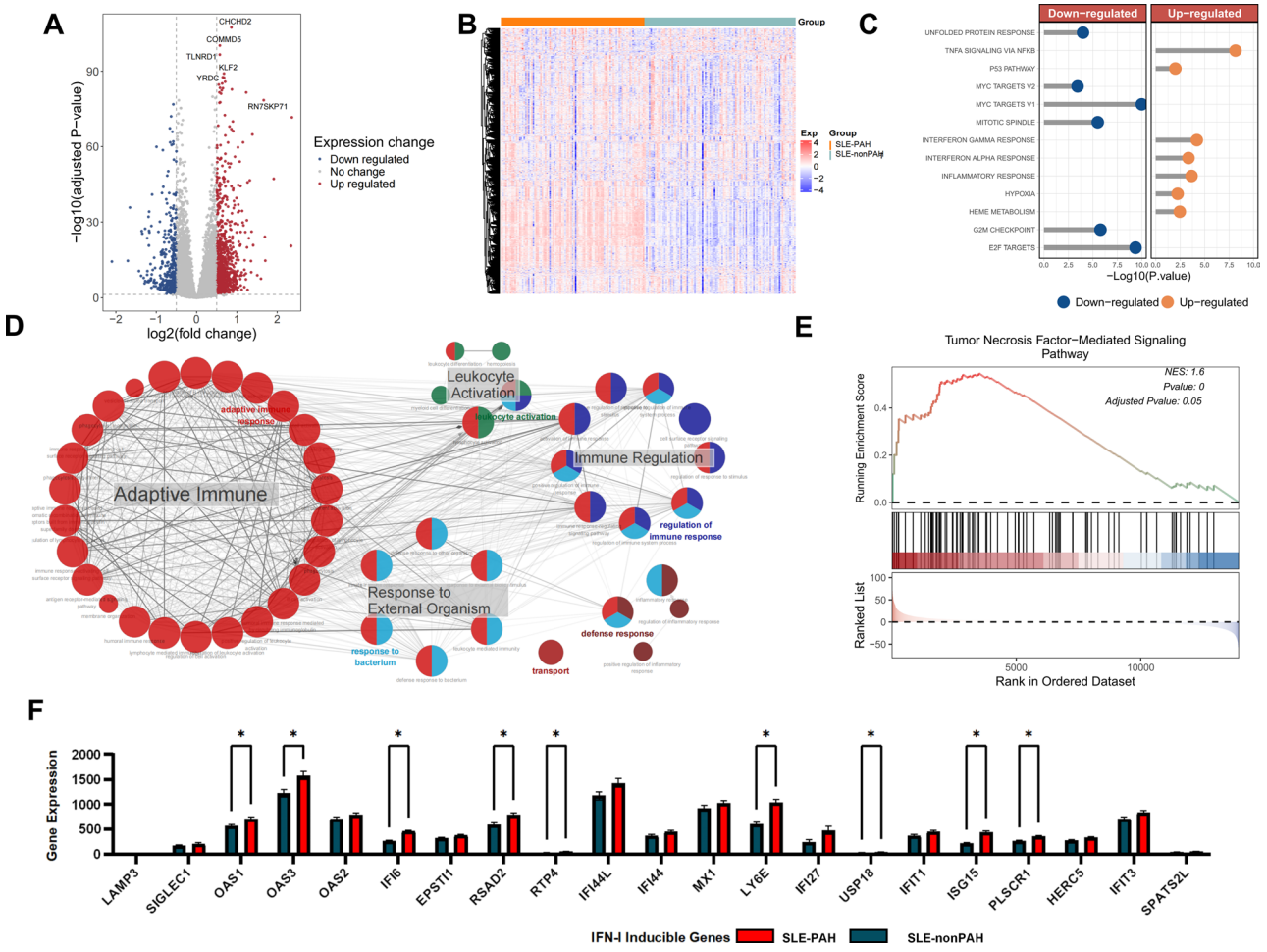

Fig. 1 Differential expression analysis between SLE-nonPAH and SLE-PAH patients. Figure 1 (**A**-**F**) Differential expression analysis between SLE-nonPAH and SLE-PAH patients. (**A**) Volcano plot of the DEGs between SLE-PAH and SLE-nonPAH, the threshold of log2 fold change is set as 0.5. The up-regulated genes are indicated in red and those down-regulated in blue. (**B**) The heatmap illustrates the DEGs between SLE-PAH and SLE-nonPAH. (**C**) The GSEA between SLE-PAH and SLE-nonPAH groups is based on MsigDB hallmark gene sets. The length of the grey lines represents the -log10 p value. (**D**) The GO analysis between SLE-PAH and SLE-nonPAH groups is depicted, with each circle representing an enriched pathway. The results are visualized using Cluego. (**E**) GSEA bases on GO gene sets. (**F**) Expression of 21 ISGs in SLE-PAH and SLE-nonPAH patients. The SLE-PAH group is labeled in red and the SLE-nonPAH group in blue (* P value < 0.05)

inflammation. The SLE-PAH subgroups characterized by high inflammation and moderate inflammation demonstrated a notable augmentation in the activation of signaling pathways associated with inflammation. The three subgroups displayed a range of distinct clinical characteristics, as illustrated in Table 1. Notable differences in the severity of PAH, evaluated via the risk stratification, were detected among these groups, with statistical significance ($P = 0.019$). Variations in BNP levels among patient subgroups were also substantial ($P = 0.007$) and indicative of the severity of cardiac involvement. Patients in the high inflammation group exhibited the mildest PAH. On the contrary, the group of patients with moderate inflammation exhibited the most severe PAH, with almost all high-risk patients belonging to this group. Meanwhile, there were no significant differences in demographic characteristics such as gender and age, as well as in immunosuppressive treatment, PAH-specific therapy, among the SLE-PAH patients in the three subgroups (Table 1).

IL-6 functions in the high inflammation SLE-PAH subgroup

The results of the differential gene expression analysis between SLE-PAH and SLE-nonPAH, as well as each subgroup of SLE-PAH, were presented in Fig. 4A. A total of 4,433 differentially expressed genes (DEGs) were discovered, with 3,298 explicitly associated with the high inflammation subgroup. As previously mentioned, the subgroup characterized by mild inflammation had the least disparity when compared to SLE-nonPAH, encompassing a mere 1,031 distinct genes. GO and KEGG enrichment analysis were employed to examine the distinctive upregulated genes (1,707 out of 3,298) inside the high inflammatory subgroup, aiming to provide insights

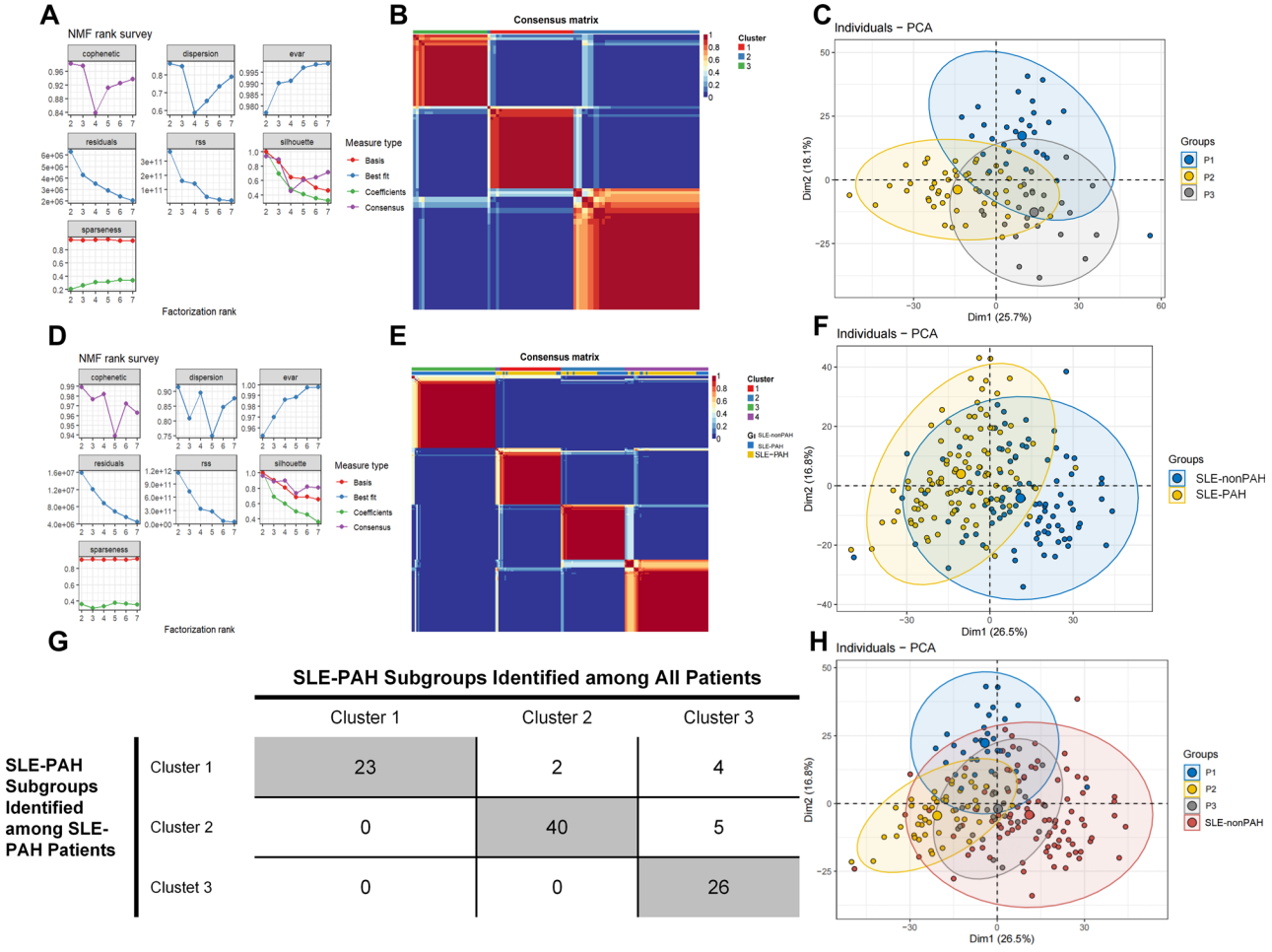

Fig. 2 CNMF clustering of SLE-PAH and SLE-nonPAH patients. Figure 2. (**A**-**C**) CNMF clustering of SLE-PAH patients. (**A**) rank survey of cNMF. An optimal rank of 3 was selected based on high cophenetic and silhouette coefficients; (**B**) Consensus matrix of the SLE-PAH patient; (**C**) PCA analysis, P1-P3 represent cluster 1–3 of SLE-PAH patients identified by cNMF. (**D**-**F**) CNMF clustering of SLE-PAH and SLE-nonPAH patients. (**D**) rank survey of cNMF. An optimal rank of 4 was selected based on high cophenetic and silhouette coefficients; (**E**) Consensus matrix of the SLE-PAH and SLE-nonPAH patients; (**F**) PCA analysis, Cluster 1–3 of SLE-PAH patients, as identified by cNMF, are represented by P1-P3. (**G**) Comparison of the classification of SLE-PAH patients from two cNMF analyses. Grey highlights indicate the quantity of SLE-PAH patients that were assigned to the same cluster. (**H**) Principal component analysis (PCA) of the three clusters of SLE-PAH patients and SLE-nonPAH patients

into its characteristics (Fig. 4B, C). The enriched GO terms primarily consisted of non-specific genes that were associated with the generation of cytokines and the activation of immune responses (Fig. 4B). Individual subgroups were assessed to determine their ISG scores, which serve as indicators of IFN levels. The subgroup exhibiting considerable inflammation displayed the highest ISG scores, as depicted in Fig. 4D [29, 30]. TNFα related pathways were also significantly up regulated in the high inflammation subgroup. Results of GSEA showed that IL6 JAK STAT3 signaling pathway was significantly up-regulated in the high inflammation subgroup, which was not observed in the other subgroups (Fig. 3). PI3K AKT mTOR signaling pathway, which is typically activated by IL-6, was also up regulated in the high inflammation subgroup (Fig. 3) [31]. The KEGG enrichment analysis revealed considerable enrichment of IL6-associated pathways, such as Th17 cell differentiation, in addition to non-specific inflammatory pathways. We used GSEA based on GO gene sets to investigate the IL6 pathway regulation in the high inflammation subgroup further. The findings showed a notable increase in IL6 production (Fig. 4E, F). In this study, the CIBERSORT algorithm was employed to assess the distribution of immune cells. The subgroup exhibiting high inflammation displayed the lowest number of regulatory T (Treg) cells, potentially attributable to the presence of IL-6 (Fig. 4G). The high inflammation subgroup consistently demonstrated the largest proportion of neutrophils, aligning with its corresponding level of inflammation.

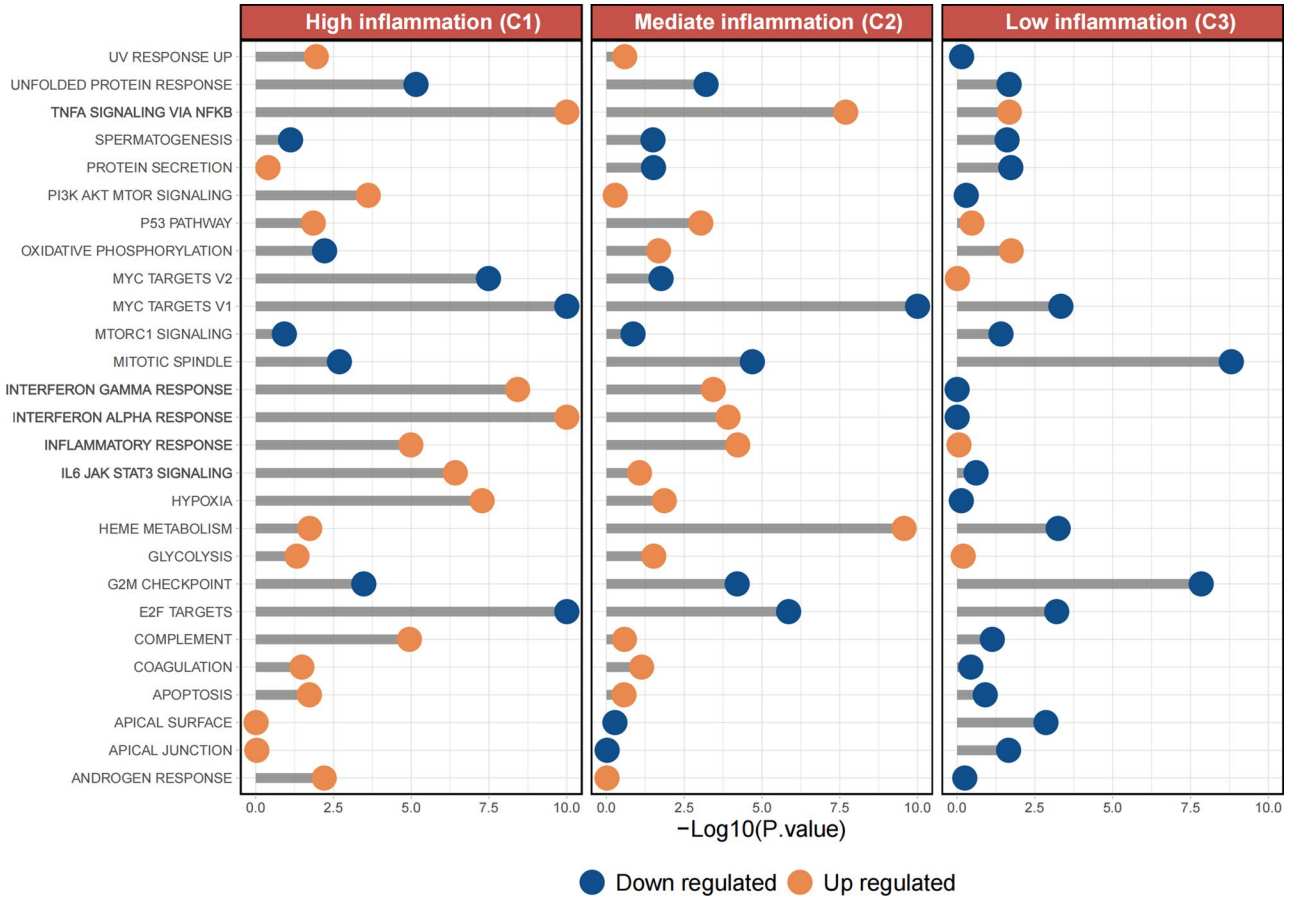

Fig. 3 GSEA of different subgroups of SLE-PAH. Figure 3. The hallmark gene sets from MSigDB are used by GSEA to identify subgroup-specific characteristics. The dots represent the direction of regulation (orange-up regulated; blue-down regulated) and the length of the grey lines represented the significance of statistical test. Only significant enriched sets (in at least one subgroup) are shown in the figure. The gene sets that reflected the level of inflammation were highlighted with bold font

The moderate inflammation SLE-PAH subgroup demonstrated characteristics of IPAH

The subgroup characterized by moderate level of inflammation exhibited the most pronounced severity ofmoderate inflammation PAH. In order to deepen our understanding of the underlying mechanisms, we conducted GO enrichment analysis on the unique sets of genes displaying differential expression among moderate inflammation patients with SLE-nonPAH patients (Fig. 5A). In conjunction with previously reported gene sets associated with immune response, unique sets implicated in the maintenance of blood cell homeostasis were also shown to be prominent. A KEGG enrichment analysis was conducted using the identical genes, and the outcomes are depicted in Fig. 5B. Pathways associated with ion transporters and ion homeostasis, which are commonly involved in idiopathic pulmonary arterial hypertension (IPAH), showed a substantial enrichment [32–34]. Disease enrichment analysis was then performed based on those unique DEGs. The predominant diseases identified were not those associated with the immune system, but rather diseases linked to the stability of blood vessels (central retinal vesselvascular tortuosity), the transmission of nerve impulses (cecocentral scotoma), and the stimulation of smooth muscle (ventricular preexcitation and preexcitation syndrome) (Fig. 5C). Vascular contraction and remodeling are the main pathogenic mechanisms of PAH, and abnormal activation of the sympathetic nervous system in PAH patients has also been reported [35, 36]. The findings revealed that the SLE-PAH subgroup with moderate moderate inflammation displayed characteristics reminiscent of IPAH. The hub genes of this subgroupmoderate inflammation were then identified using cytoHubba, and their shared TFs were predicted in chEA3. Overall, 13 hub genes were discovered, all of which shared two TFs: GATA1 and KLF1. Notably, GATA1 was one of the 13 hub genes (Fig. 5D, E). The expression of 13 hub genes and TFs was significantly higher in the moderate inflammation group (Fig. 5F). Overall, the SLE-PAH subgroup exhibiting moderate levels of inflammation presented features akin to IPAH, in conjunction with an increased inflammatory response.

Table 1 Clinical and demographic characteristics of SLE-PAH patients

	High inflammation	Moderate inflammation	Low inflammation	*P* Value
n	29	45	26	
Demographic Characteristics				
Female Sex, N, %	29 (100.0)	44 (97.8)	26 (100.0)	0.539
Age, mean (SD)	39.48 (8.84)	37.11 (8.63)	38.12 (8.64)	0.521
PAH Characteristics				
Risk Strastification, N, %				**0.019**
Low	29 (100.0)	37 (82.2)	25 (96.2)	
High	0 (0.0)	8 (17.8)	1 (3.8)	
mPAP, mmHg, mean (SD)	40.00 (11.36)	37.47 (13.03)	39.75 (12.61)	0.861
PVR, WU, mean (SD)	5.63 (3.68)	5.91 (3.69)	5.79 (3.22)	0.987
6MWD, m, mean (SD)	538.25 (48.13)	527.96 (53.97)	473.29 (149.63)	0.105
WHO FC				0.373
I	3 (10.3)	5 (11.1)	4 (15.4)	
II	24 (82.8)	32 (71.1)	21 (80.8)	
III	2 (6.9)	8 (17.8)	1 (3.8)	
NT proBNP, ng/L, mean (SD)	130.00 (104.70)	520.19 (1,014.48)	398.53 (1,215.10)	0.211
BNP, ng/L, mean (SD)	25.60 (17.62)	83.08 (83.88)	40.19 (36.04)	**0.004**
Treatment				
Equal Predisone Dose				0.767
No	2 (6.9)	5 (11.1)	3 (11.5)	
Low (< 0.5 mg/kg/d)	26 (89.7)	36 (80.0)	22 (84.6)	
Intermediate (0.5-1 mg/kg/d)	1 (3.4)	1 (2.2)	0 (0.0)	
High (≥ 1 mg/kg/d)	0 (0.0)	2 (4.4)	0 (0.0)	
Pulse (> 500 mg/d)	0 (0.0)	1 (2.2)	1 (3.8)	
Intensive immunosuppressive treatment, %	25 (86.2)	35 (77.8)	24 (92.3)	0.255
PAH Specific Therapy				
Bosentan, %	0 (0.0)	5 (11.1)	2 (7.7)	0.185
Anlisentan, %	16 (55.2)	23 (51.1)	10 (38.5)	0.432
Maxitantan, %	7 (24.1)	3 (6.7)	0 (0.0)	0.007
Sildenafil, %	4 (13.8)	6 (13.3)	1 (3.8)	0.398
Tadalafil, %	6 (20.7)	13 (28.9)	5 (19.2)	0.580
Leosigua, %	1 (3.4)	5 (11.1)	1 (3.8)	0.345
Treprostinil, %	0 (0.0)	0 (0.0)	1 (3.8)	0.238
Silepag, %	3 (10.3)	2 (4.4)	1 (3.8)	0.502

Furthermore, the two transcription factors, GATA1 and KLF1, could probably serve as potential candidates for potential therapeutic intervention targets.

Discussion

PAH has been reported to affect about 3% SLE patients and lead to poor prognosis [4, 5, 37–39]. However, the pathogenesis of SLE-PAH has not been fully understood yet. In this study, we analyzed the transcriptome of peripheral samples from SLE-PAH patients, and compared it to that of SLE-nonPAH patients to elucidated the mechanism underlying the onset of SLE-PAH. Using transcriptome data, we employed unsupervised clustering to categorize SLE-PAH patients into three subgroups with varying levels of PAH severity. Additionally, we clarified the mechanistic characteristics of each category. For the most severe subgroup of PAH, hub genes and their common transcription factors were discovered, which could serve as prospective therapeutic targets.

Interferon and TNFα play a significant role in SLE-PAH

The differential expression genes analysis and pathway enrichment analysis between SLE-nonPAH and SLE-PAH were conducted to elucidate the mechanism underlying SLE-PAH. The analysis demonstrated that inflammation played an important role in SLE-PAH and identified crucial cytokines that functions in the pathogenesis.

TNFα is a cytokine that can be produced by various immune cells, including macrophages, lymphocytes, natural killer cells and so on [40, 41]. TNFα is generally considered as an immune regulator that influence the development of immune cells and moderate the inflammatory processes [42]. Meanwhile, TNFα is an important regulator of apoptosis, and can exert both pro and anti-apoptosis depending on the circumstances [43].

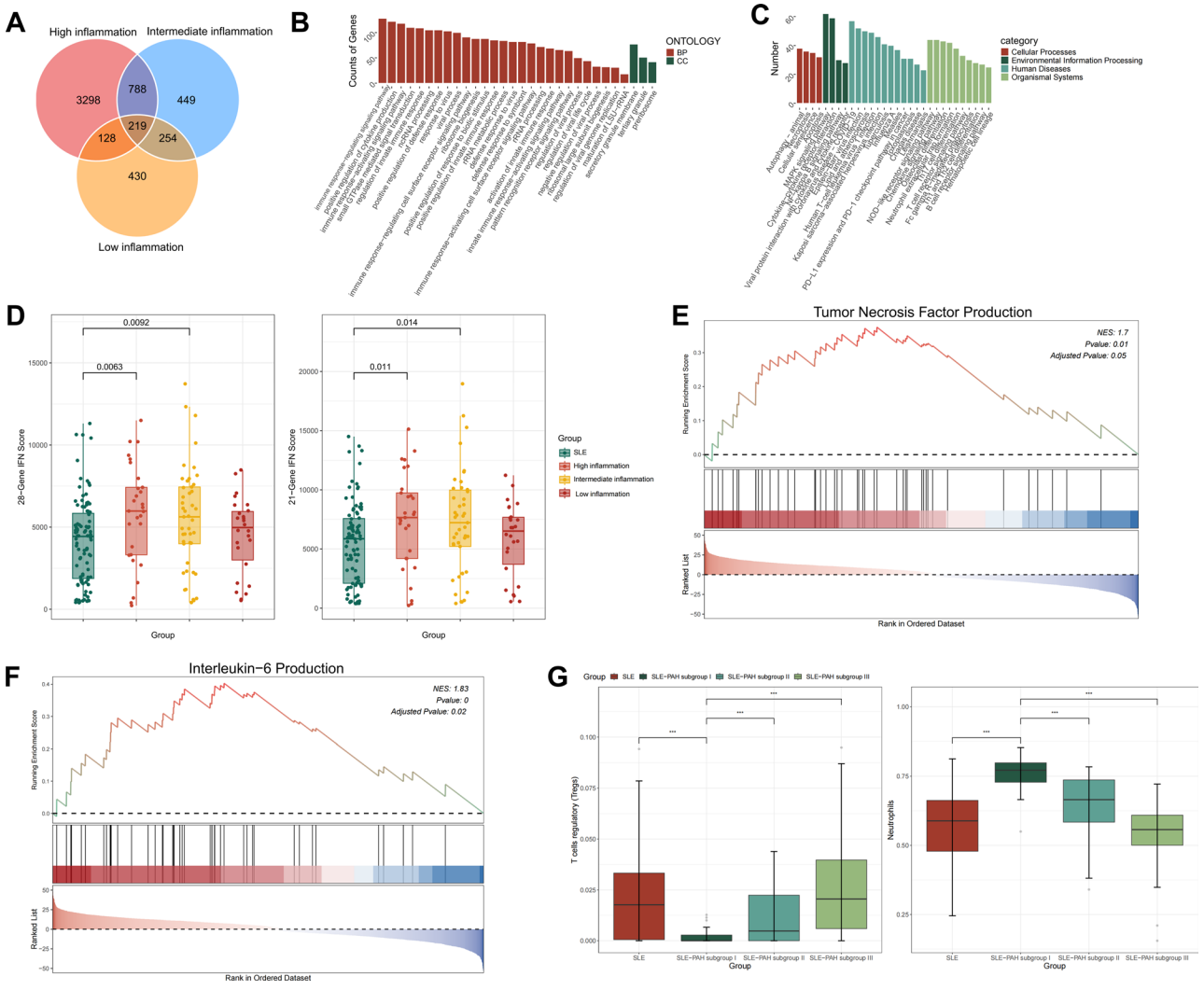

Fig. 4 Profiling the SLE-PAH subgroup with high inflammation. Figure 4 (**A**-**G**) Profiling the SLE-PAH subgroup with high inflammation. (**A**) Venn plot demonstrating the DEGs between each SLE-PAH subgroup and non-PAH SLE. (**B**) GO enrichment analysis bases on unique up-regulated genes of the subgroup characterized by high inflammation. Only the 30 gene sets with the smallest p-values are presented. (**C**) GO enrichment analysis bases on unique up-regulated genes of the subgroup characterized by high inflammation. (**D**) ISG score of each subgroup of SLE-PAH and SLE-nonPAH patients. The 28-gene score is displayed on the left and the 21-gene score is displayed on the right. (**E**-**F**) GSEA bases on GO gene sets. The descriptions of each gene sets are labeled on the top. (**G**) Prediction the proportion of immune cells via CIBERSORT

TNFα has been reported to contribute to the pathogenesis of SLE. The serum level of TNFα was higher in active SLE patients [44]. The genomic polymorphisms of TNFα promotor have also been proven to correlate with the susceptibility to SLE [45]. The correlation of TNFα with SLE might attribute to both abnormal activation of immune cells and production of autoantibodies resulted from increased apoptosis [46, 47]. TNFα has also been reported to contribute to PAH via Caspase-4/11-mediated apoptosis of pulmonary artery endothelial cells [48]. In this study, TNFα related pathways and gene sets were enriched in SLE-PAH via GSEA, indicating that the function of TNFα was activated in SLE-PAH in comparation to SLE. The gene sets related to infection might also resulted from the activation of TNFα signaling. In addition to induce apoptosis of endothelial cells, the immune cell abnormal activation induced by TNFα may also contribute to SLE-PAH. TNFα is also one of the most important activator of NFκB signaling pathway [49]. The NFκB signaling could activate Blc-xL and Bcl-2 to improve the survival of B cells [50]. Meanwhile, NFκB also participates in the negative selection of self-reactive B cells [51]. The GSEA analysis between SLE-PAH and SLE identified significant activation of TNFα and downstream NFκB signaling, and the GO enrichment clarified the involvement of B cell activation in SLE-PAH. The crosstalk between NFκB and B cell function could partially elucidate the mechanisms of SLE-PAH.

IFN family is another cluster of cytokines that functions in defensing viral infection and regulating inflammation

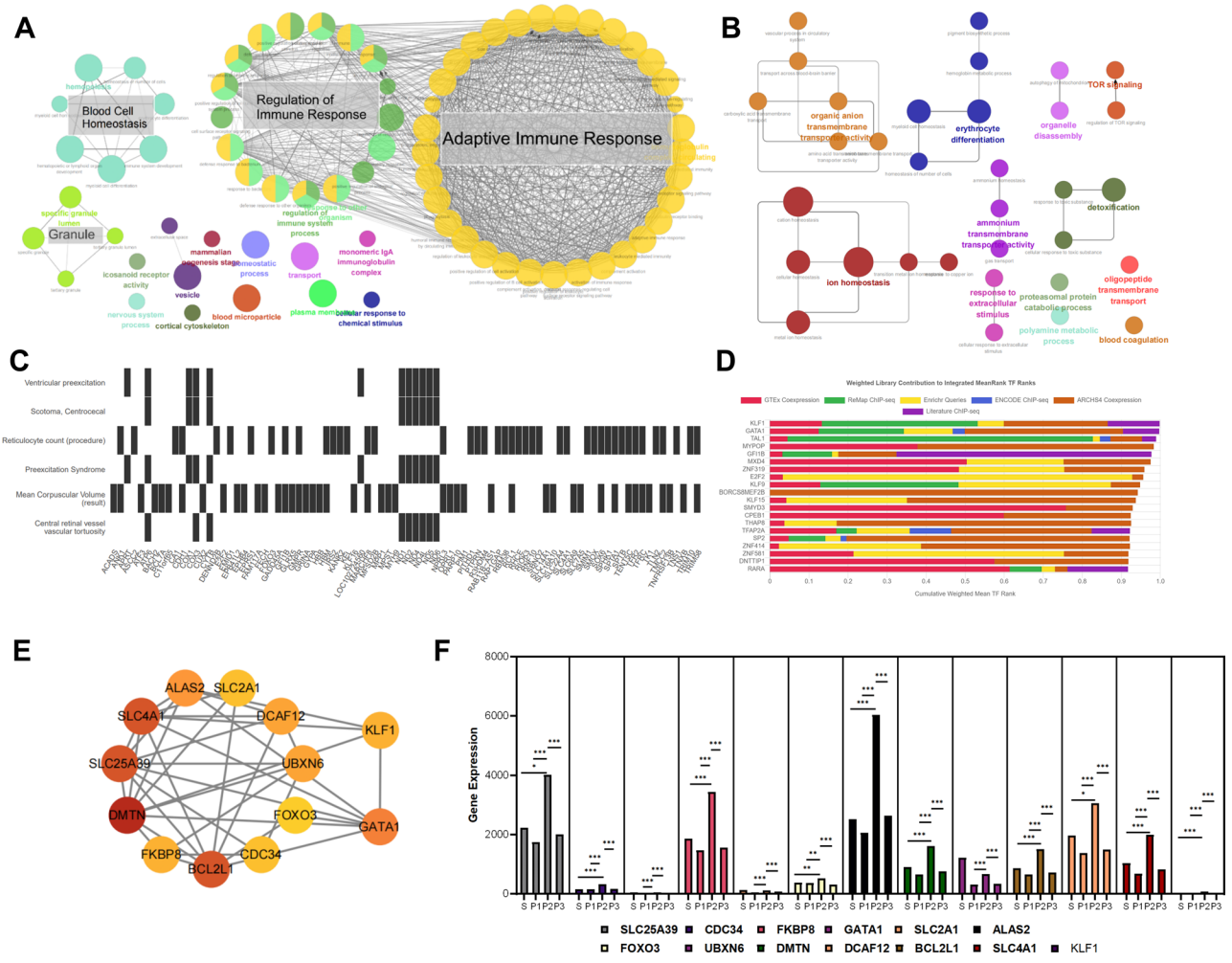

Fig. 5 Profiling the SLE-PAH subgroup with moderate inflammation. Figuer 5 Profiling the SLE-PAH subgroup with moderate inflammation (**A**-**F**). (**A**) GO enrichment analysis bases on unique differential genes of the subgroup with moderate inflammation. The results were visualized via Cluego. (**B**) KEGG enrichment analysis bases on unique differential genes of the subgroup with moderate inflammation. The results were visualized via Cluego. (**C**) Disease enrichment analysis bases on unique differential genes of the subgroup with moderate inflammation. Each gray block represents the enrichment of genes on the x-axis in the diseases on the y-axis. (**D**) Prediction of TFs on the chEA3 website. The bar chart represents the contributions of evidence from different database sources to the prediction ranking of TFs. GATA1 and KLF1 are the only two transcription factors that are shared among the 13 hub genes. (**E**) Protein-protein interaction network among the 13 hub genes and TFs. (**F**) Expression of the 13 hub genes and TFs among different subgroups of SLE-PAH and SLE. S: SLE; P1-3 SLE-PAH cluster 1–3

processes [52]. Both IFNα (type I) and IFNγ (type II) have been reported to function in SLE. Over half of the SLE patients demonstrated high expression level of type I IFN regulated genes [53]. Type II IFN signatures have also been observed to be upregulated in SLE patients, albeit less significantly [54]. Type I IFN has also been reported as crucial susceptible factor for PAH in SLE patients, but similar conclusions have not been reported in type II IFN [15]. In this study, we discovered that both IFNα and IFNγ signaling pathways were up regulated in SLE-PAH patients in comparison to those with SLE. The IFN signature was also analyzed and validated the enrichment analysis.

Overall, inflammatory levels are higher in SLE-PAH patients compared to SLE-nonPAH patients in general. New treatments for SLE-PAH may be possible by medications that target certain pathways, including TNFα and IFN, which play essential roles.

SLE-PAH patients with high inflammation demonstrated milder PAH and activation of IL-6

In this study, cNMF unsupervised clustering was employed to partition SLE-PAH into three distinct subtypes. The findings of this study revealed noteworthy disparities in clinical manifestations and levels of inflammation among the three subtypes (Figs. 2 and 3). Among them, patients in the high inflammation subgroup exhibited the mildest PAH. Previous research published by Sun et al. in 2019 has reported two distinct subtypes of SLE-PAH, one had systemic manifestation and high

SLE activity, while the other subtype tended to have less active SLE but purer and server PAH [55]. Those patients with more active underlying SLE has been reported better to intensive immunosuppressive therapy [16, 56]. For SLE patients with mild PAH, immunosuppressive therapy along was potential to control the PAH [57]. However, the underlying mechanism has not been elucidated. According to our results, the high inflammation group has purer inflammation characteristics and less vasculopathic change. The autoimmune-mediated processes, rather than the non-inflammatory changes, dominated in the vascular remodeling of this high inflammatory subgroup, which could lead to the better prognosis and response to immunosuppressive therapy.

To profile the high inflammation subgroup in detail, further differential gene expression analysis was carried out. The comparative analysis of SLE-nonPAH and SLE-PAH revealed a notable enrichment of inflammation-related pathways within the high inflammation subgroup. The function of TNFα and IFN was activated more prominently in this subgroup. Notably, IL-6 related signaling pathways, including PI3K-AKT-mTOR and IL-6 JAK-STAT3 signaling pathway were significantly up-regulated in the high inflammation subgroup, which was not discovered in the other two subgroups. The cytokine IL-6 plays a pivotal role in the regulation of the immune system and the establishment of tolerance. The aberrant activation of interleukin-6 (IL-6) triggers the differentiation of Th17 cells while impeding the differentiation of regulatory T (Treg) cells. Consequently, this disruption of T cell equilibrium leads to the initiation of autoimmune processes and chronic inflammation [58, 59]. The production of IL-6, was proven to be up-regulated in this subgroup. Meanwhile, the proportion of Treg cells were significantly decreased. All the results supported the activation of IL-6 in the high inflammation subgroup. Level of IL-6 is higher in SLE patients and correlates with organ damages like neuropsychiatric SLE and lupus nephritis [60–62]. Nevertheless, the correlation between IL-6 and PAH was controversial. A Mendelian randomization study once proposed that increased soluble IL-6 receptor increased risk of PAH while increased IL-6 signaling reduced risk of PAH [63]. Another research suggested that targeting IL-6 JAK/STAT3 could potentially ameliorate PAH [64]. Overall, our research suggested IL-6 and its downstream pathway, especially JAK/STAT3, might serve as therapeutic targets for SLE-PAH patients with high inflammation.

SLE-PAH patients with moderate inflammation demonstrated the most severe PAH and shared characteristics of IPAH

It is worth noting that nearly all the high risk SLE-PAH patients were divided into the moderate inflammation groups. To further clarify the pathogenesis of this subgroups and discover potential therapeutic targets, gene sets enrichment and PPI analysis were carried out. The results of our study demonstrated that the moderate subgroup had distinct underlying mechanism in addition to activation of inflammation. The disease enrichment analysis identified diseases linked to the stability of blood vessels, transmission of nerve impulses, and stimulation of smooth muscle, which shared pathogenic processes with IPAH. Moreover, among the 13 hub genes, only FKBP8 and BCL2L1 are mainly considered functioning in immune processes [65–67]. SLC2A1 has been identified in rat model of PAH and induced metabolic deregulation [68]. ISG15 overexpression has been reported to induce ventricular remodeling and heart failure [69]. GATA1 is a transcription factor that was predicted to regulate all the 13 hub genes including itself. GATA1 has been reported to regulated the maturation of eosinophils, which influences the immune response and protective effects in the lung, and thus participating in the pathogenesis of PAH [70]. Meanwhile, the depletion of GATA1 has been reported to cause defect in inflammatory myeloid cells and therefore lead to diminished immune response [71]. The other TFs that shared by the 13 hub genes, KLF1, has not been reported to involved in PAH. KLF1 has been reported to regulate the expression of PD-L1 and thus influence the immune escape of non-small cell lung cancer [72]. However, the mechanisms by which KLF1 gene involvement contributes to the pathogenesis of SLE-PAH and influences prognosis remain unclear and warrant further investigation. Overall, majority of the hub genes for moderate inflammation subgroup participate in the pathogenesis of PAH. The moderate inflammation subgroup presented with both the PAH-specific pathological factors related to vascular smooth muscle homeostasis and the excessive inflammation activation associated with SLE, resulting in a double hit that leads to more severe PAH in this group.

Conclusion

In conclusion, this study leverages transcriptomic analysis to uncover the complex interplay between inflammation and the pathogenesis of SLE-PAH. The identification of distinct SLE-PAH subgroups with varying levels of inflammation provides a foundation for personalized therapeutic approaches. Notably, the discovery of potential biomarkers and therapeutic targets within these subgroups, such transcription factors GATA1 and KLF1, opens new avenues for intervention. While the study's scope is limited to peripheral blood analysis and acknowledges the need for further research, it represents a significant step toward a more nuanced understanding of SLE-PAH and the development of targeted treatment strategies.

Strengths and limitations

To our best knowledge, this is the first study that compared the transcriptome between SLE-nonPAH and SLE-PAH patients and profile the characteristics of different subgroups of SLE-PAH. This study was based on the largest and high-quality SLE cohorts in China, with patients having comprehensive clinical data and clear clinical diagnoses included. The sample size of our study is relatively large, making our results more reliable. However, this study had several limitations. Firstly, only peripheral blood samples were collected in this study, further research based on pulmonary samples is conducting in the future. Second, the differences in the peripheral blood transcriptome between SLE-nonPAH patients and SLE-PAH patients were relatively small, which might be due to the systemic inflammatory response of the SLE patients.

Supplementary Information

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Supplementary Material 1

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Author contributions

Yutong Li: Conceptualization; Methodology; Validation; Formal analysis; Visualization; Writing - review & editing; Writing - original draft; Investigation Junyan Qian: Conceptualization; Methodology; Validation; Formal analysis; Visualization; Writing - review & editing; Investigation; Funding acquisition Xiaoyue Deng: Conceptualization; Writing - original draft; Investigation; Methodology; Validation; Formal analysis; Supervision; Visualization; Writing - review & editing; Data curation Leyao Ma: Investigation; Methodology Qizhi Yuan: Investigation; Methodology Zhuang Tian: Resources Qian Wang: Resources Xiaofeng Zeng: Resources; Funding acquisition Xinzhuang Yang: Resources; Funding acquisition; Writing - review & editing; Supervision; Conceptualization; Methodology Jiuliang Zhao: Resources; Funding acquisition; Supervision; Conceptualization; Methodology Mengtao Li: Resources; Funding acquisition; Writing - review & editing; Supervision; Conceptualization; Methodology.

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Data availability

Data in this article is available on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Peking Union Medical College Hospital Institutional Review. Board and Ethical Board (Ethical number: JS-2038). The board is guided by the Helsinki Declaration and the Chinese Guiding Principles for Ethical Review of Drug Clinical Trials. Written informed consent was obtained from each enrolled patient. Clinical trial number: not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, No.1 Shuai Fu Yuan, Wang Fu Jing, Dongcheng District, Beijing 100730, China
[2]National Clinical Research Center for Dermatologic and Immunologic Diseases (NCRC-DID), Ministry of Science & Technology, Beijing, China
[3]Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Beijing, China
[4]Department of Cardiology, Peking Union Medical College, Peking Union Medical College Hospital & Chinese Academy of Medical Sciences, Beijing, China
[5]Center for Bioinformatics, National Infrastructures for Translational Medicine, Institute of Clinical Medicine & Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[6]Center for Bioinformatics, Peking Union Medical College Hospital, No.1 Shuai Fu Yuan, Wang Fu Jing, Dongcheng District, Beijing 100730, China

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