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RESEARCH ARTICLE

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Identification of monocyte-associated pathways participated in the pathogenesis of pulmonary arterial hypertension based on omics-data

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Abstract

Pulmonary arterial hypertension (PAH) is one kind of chronic and uncurable diseases that can cause heart failure. Immune microenvironment plays a significant role in PAH. The aim of this study was to assess the role of immune cell infiltration in the pathogenesis of PAH. Differentially expressed genes based on microarray data were enriched in several immune-related pathways. To evaluate the immune cell infiltration, based on the microarray data sets in the GEO database, we used both ssGSEA and the CIBERSORT algorithm. Additionally, single-cell RNA sequencing (scRNA-seq) data was used to further explicit the specific role and intercellular communications. Then receiver operating characteristic curves and least absolute shrinkage and selection operator were used to discover and test the potential diagnostic biomarkers for PAH. Both the immune cell infiltration analyses based on the microarray data sets and the cell proportion in scRNA-seq data exhibited a significant downregulation in the infiltration of monocytes in PAH. Then, the intercellular communications showed that the interaction weighs of most immune cells, including monocytes changed between the control and PAH groups, and the ITGAL-ITGB2 and ICAM signaling pathways played critical roles in this process. In addition, ITGAM and ICAM2 displayed good diagnosis values in PAH.

Abbreviations: AUC, area under the ROC curve; DCs, dendritic cells; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; LASSO, least absolute shrinkage and selection operator; MCT, monocrotaline; NK cells, natural killer cells; PAH, pulmonary arterial hypertension; PCA, principal component analysis; ROC, receiver operating characteristic; scRNA-seq, single-cell RNA sequencing; SuHx, Sugen-5416 hypoxia.

Caiming Zhong, Yachen Si, and Huanhuan Yang contributed equally to this work.

This study implicated that the change of monocyte was one of the key immunologic features of PAH. Monocyte-associated ICAM-1 and ITGAL-ITGB2 signaling pathways might be involved in the pathogenesis of PAH.

KEYWORDS

ICAM-1 signaling pathway, immune infiltration, ITGAL-ITGB2 signaling pathway, monocytes, pulmonary arterial hypertension

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a chronic cardiopulmonary syndrome characterized by irreversible pulmonary vascular remodeling, bringing about elevated pulmonary artery pressure and increased pulmonary vascular resistance, and eventually leading to right heart failure or even death.[1] The incidence of PAH ranges from 5 to 10 cases in a million people per year, and its prevalence varies from 15 to 50 cases per million people.[2] The pathogenesis of PAH is very complex and needs further investigation. It has been reported that mitochondrial metabolic and dynamic dysfunction, genetic and epigenetic factors, immunity alteration, increased inflammation, sexual distinction, and right ventricular adaptation are implicated in this process.[3] Current therapeutic strategies in PAH target dysfunctional signaling pathways involved in the pulmonary vasculature to reduce right ventricular afterload. Despite reports that current therapies are of benefit to the quality of life and time to clinical worsening, these treatments do not decrease mortality of PAH.[4] Therefore, it is urgent to explore underlying pathogenesis and potential therapeutic targets of PAH further.

In recent years, the role of immunity in PAH gains increasing attention, which reveals novel insights into the underlying immunopathology. With the deepening of research, growing evidence has suggested that diverse immune cells, such as T- and B-lymphocytes, natural killer (NK) cells, monocytes, macrophages, and dendritic cells (DCs), involve in immune circuits, which connects the local inflammatory landscape in the lung and heart by interorgan communication.[5] The expansion of perivascular macrophages and the recruitment of monocytes have been proposed as critical pathogenic drivers of vascular remodeling.[6] Besides, different B- and T- lymphocytes may be critical in the pathophysiology of PAH since circulating autoantibodies and regulatory T-cells occur in animal models and patients with PAH.[7,8] Thus, understanding the process of immune infiltration in PAH is critical to devising new remedy and developing early diagnostic markers for PAH.

With the development of modern molecular biology technology (such as gene chip and high-throughput sequencing), bioinformatics analysis based on the large scale of data has gradually emerged, providing significant technical supports for the research on the pathological mechanism of complex diseases.[9] By the measurement of global gene expression levels, microarray technology can help identify differentially expressed genes (DEGs) and significant biological processes in the process of PAH.[10] In addition, on the basis of gene expression data, the CIBERSORT analysis tool can assess the proportion of 22 types of immune cell components between healthy and PAH cases.[11] However, gene microarray provides the average expression of genes in the level of the whole tissues, failing to identify the single cell type that are potentially involved in the onset and the process of diseases. While single-cell RNA sequencing (scRNA-seq) is a developing potent technique that can uncover molecular characteristics of diverse cell populations, including markers of each cell cluster, interactions between indicated cell types, and potential regulators of single cell cluster.[12] These characters mean that it is helpful to illuminate the relationship between cells and significant genes or pathways and explore the mechanism of PAH more deeply by integrating gene chip and single-cell transcriptome.[13]

In this study, we first identified the cell subpopulations closely associated with PAH by combination of gene microarray and single-cell transcriptome. Then, we focused on monocytes and obtained significant monocyte-associated candidate genes or pathways related to PAH. Furthermore, the diagnostic significance of these genes or pathways was explored.

MATERIALS AND METHODS

Raw data acquisition

For the microarray data set, we retrieved the human PAH data sets in the GEO database (https://www.ncbi.nlm.nih.gov/geo/), and selected the GSE113439,[14] GSE117261,[10] GSE15197,[15] and GSE48149[16] data sets for further analysis, which include large quantities of microarray data sets of lung from patients with PAH in

the past 10 years. We only selected the control group and PAH group in all four data sets. Notably, the platform of both GSE113439 and GSE113439 is GPL6244, while the platforms of GSE15197 and GSE48149 are GPL6480 and GPL16221, respectively. Out of the consideration that GPL6244 can identify more gene symbols than another two platforms, GSE113439 and GSE117261 were used to identify DEGs and assess the immune infiltration, and all four data sets are used to verify the diagnostic value of identified markers.

Additionally, we obtained the scRNA-seq data of lung with PAH, which included three groups (control group, monocrotaline [MCT] group, and Sugen-5416 hypoxia [SuHx] group) and six samples in each group, from an open-access online platform Mergeomics (http://mergeomics.research.idre.ucla.edu/PVDSingleCell/).[17] Although the species of samples is *Rattus norvegicus*, this data set is the only one that meet the following criterion: (1) The samples are lung tissues without cell-sorting technique; (2) The data sets are public and can be obtained. In addition, the results of this data sets were tested in the microarray data sets of human.

Data preprocessing of the microarray data set

Due to the batch effects, the "oligo" package (version 1.56.0) in R was used for background correction and normalization of data sets, and then principal component analysis (PCA) was conducted.[18] Additionally, we applied hierarchical cluster analysis to evaluated the bias of group by identification outlier samples according to the group by the hclust function.[19]

Identification of DEGs and functional enrichment analysis

The "limma" package (version 3.50.3) was to identify the DEGs between the control and PAH groups with the criteria of fold change >1.414 and adjusted $p < 0.05$.[20] The "clusterProfiler" package (version 4.2.2) was then used to conduct the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis with an adjusted $p < 0.05$.[21]

Immune cell infiltration analysis

The immune infiltration was estimated by ssGSEA with the "gene set variation analysis (GSVA)" package (version 1.42.0)[22] and the CIBERSORT algorithm to validate the differences in subtypes of immune cell between the control and PAH samples.[23]

Screening and verification of diagnostic biomarkers

To discover and test the potential diagnostic biomarkers for PAH, the least absolute shrinkage and selection operator (LASSO) were used to predict disease status, which was performed by the "glmnet" package (version 4.1-4).[24] Receiver operating characteristic (ROC) curves were plotted by "pROC" package (version 1.18.0) to evaluate the predictive value of the identified gene, and the area under the ROC curve (AUC) was calculated to assess the diagnostic efficacy.[25]

ScRNA-seq data analysis

The Seurat objects of the scRNA-seq data were created through the "Seurat" package (version 4.1.1).[26] Further, to elevate quality of the data, data filtering criteria were as followed: cells with >500 distinct genes and percentage of mitochondrial genes <20%. The standard Seurat clustering pipeline were performed after normalization and scaling, using the following functions in order: FindVariableFeatures with 3000 genes, ScaleData, RunPCA, FindNeighbors with the first 20 PCs and FindClusters with resolution 1, otherwise default settings.

Identification of GSVA

To illustrate the top 20 pathway involved and assess the specific regulation information, pathway analyses were predominantly performed on the 20 hallmark pathways using the GSEABase package (version 1.56.0).

Cell–cell communication analysis

Based on the expression of immune-related receptors and ligands, the scRNA-seq data of the control and PAH samples were applied to explore intercellular communications by the "CellChat" package (version 1.6.0).[27]

Statistical analysis

All significance tests in this paper, unless otherwise stated, were assessed using the two-sided Wilcoxon rank-sum test.

RESULTS

DEGs identification and KEGG pathway enrichment analysis

Microarray data of human lung tissue from PAH patients and normal controls were extracted from GSE113439 and GSE117261 data sets. Data from GSE113439 and GSE117261 data sets presented reliable quality and two distinct clusters between two groups (Figure 1a–c and Supporting Information S2: Figure 1A-C). A total of 3976 DEGs between the two groups were identified from GSE113439, including 2609 upregulated and 1367 downregulated genes (Figure 1d,e). Simultaneously, there are 245 upregulated and 214 downregulated DEGs screened from GSE117261 (Supporting Information S2: Figure 1D,E).

To further explore the underlying roles and functions of immune-related pathways in the pathogenesis of PAH, KEGG pathway analysis was carried out. Several immune-related pathways were enriched in the PAH-related DEGs from GSE113439, including NOD-like receptor signaling pathway, Th17 cell differentiation signaling pathway, TNF signaling pathway, and so on (Figure 1f). In addition, there are some pathways relevant to immunity identified from GSE117261 which consist of B cell receptor signaling pathway, Toll-like receptor signaling pathway and leukocyte transendothelial migration (Supporting Information S2: Figure 1F). The above results showed that immunity is associated with PAH.

Immune cell infiltration analysis

To characterize the landscape of immunocyte infiltration in PAH patients, The CIBERSORT algorithm was performed to assess the fraction of immunocytes. The percentages of immune cells in each sample were analyzed (Figure 2a), and ones of eight immune cell types were significantly changed in the PAH samples than normal controls from GSE113439, including CD8 T cells, monocytes, and memory B cells (Figure 2b,c). Additionally, 28 types of immune cells were included to estimate the immune microenvironment in lung tissue with ssGSEA to validate the above results. The results were presented in the form of heatmap and showed the levels of 28 immune cell types in the PAH samples and normal controls. The box plots presented differences between the two groups. Compared with the control group, 10 immune cell types (such as activated CD8 T cells, monocytes, and regulatory T cells) showed significant alterations in the PAH samples from GSE113439 data set (Figure 2d,e). Notably, data based on GSE117261 data set also revealed the similar results, suggesting that many different types of immune cells participate in the pathogenesis of PAH, including CD8 T cells and monocytes (Supporting Information S2: Figure 2).

Identification of distinct cells types

We collected scRNA-seq data of published rat PAH models: MCT induction and SuHx induction to further clarify the underlying immune features of PAH. The data set included three groups (control group, MCT group, and SuHx group), and each group included six samples. A total of 33,392 cells are included. Considering the high percentage of mitochondrial genes of the quality control in the previous study (the percentage of mitochondrial genes <0.5), we filtered the cells again, and 13,947 cells are finally included in the following analysis. We identified 21 distinct cell types expressing established markers for epithelial, stromal, lymphoid, and myeloid cell populations and others, including B cells, T cells, NK cells, DCs, macrophages, monocytes, mast cells, ciliated cells, clara cells, and fibroblasts (Figure 3a–c and Supporting Information S2: Figure 3A). Compared with the control group, percentages of several immune cells were changed in PAH groups. Notably, a decrease in the normalized cell fractions of monocytes in both MCT group and SuHx group was observed (Figure 3d,e and Supporting Information S2: Figure 3B,C). The above results revealed that monocytes might be critical in the pathogenesis of PAH, which was also reflected in the results based on microarray data analysis.

Characteristics of monocyte gene expression profile

The variance analysis revealed the top 10 significantly DEG across the samples, including Cd74, Cxcl2, Il1b, and Ccl5, which were associated with immune process (Supporting Information S2: Figure 3E). To further explore the role of monocytes in PAH, we compared the gene expression profiles of monocytes between the control and PAH samples. More than 60 DEGs were identified in monocytes, including Ccrl2, Cd274, and Ccl3 (Supporting Information S2: Figure 3D), which played critical roles in immunity, and all of which increased in PAH group. Chemokine receptor-like 2 (CCRL2) can bind with chemokine-like receptor 1 (CMKLR1) and promote the recruitment of CMKLR1-expressing immune cells, such as monocytes, DC, and NK cells.[28] The protein expression of CMKLR1 in lung tissues was elevated in a rat model of PAH.[29,30] Cd274, also known as programmed cell

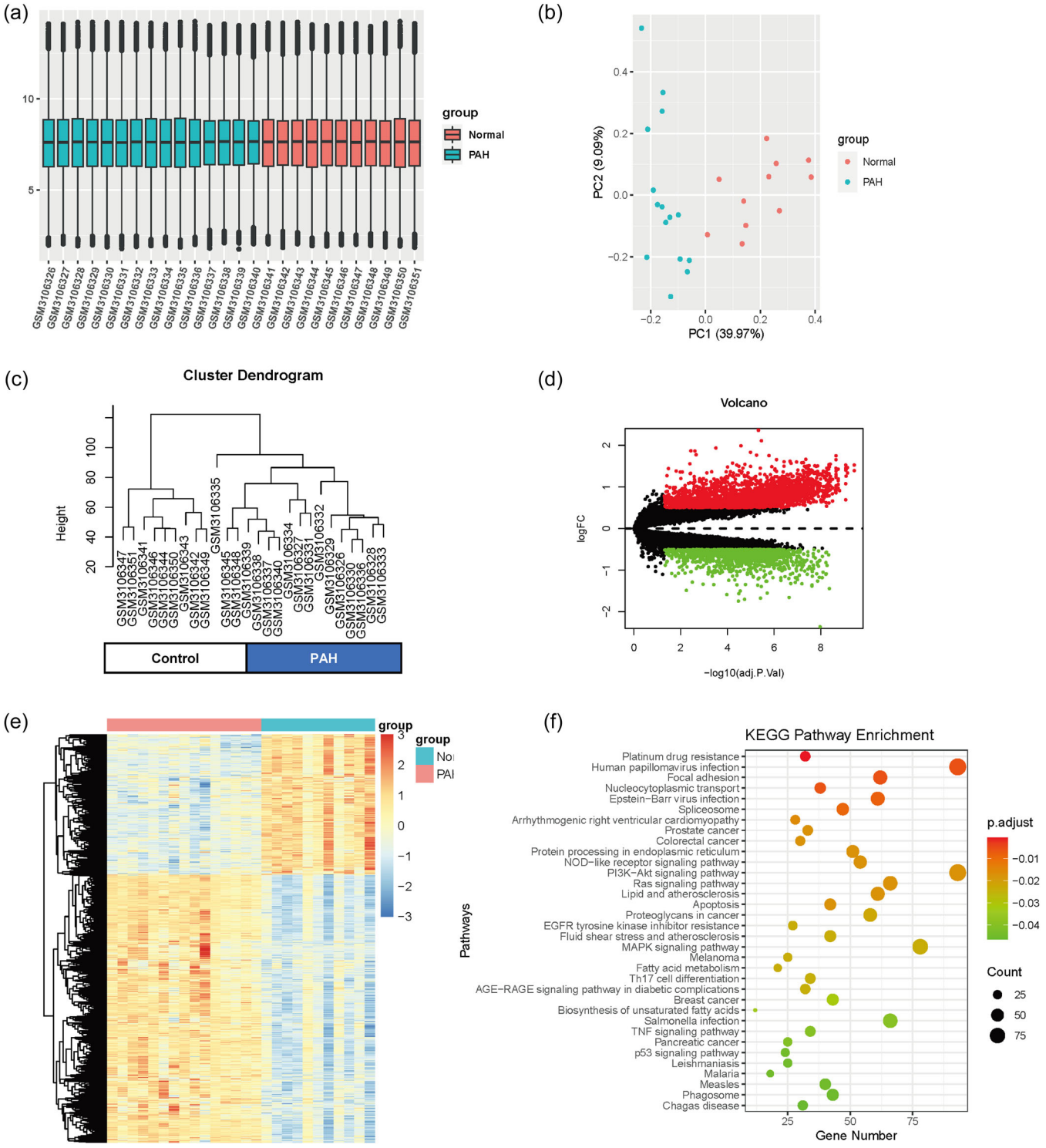

FIGURE 1 Identification of DEGs and KEGG pathway enrichment analysis based on GSE113439 data set. (a) Data after normalization of GSE113439 data set are shown. (b) Two distinct clusters are presented by principal component analysis (PCA). (c) Sample clustering was performed. (d, e) DEGs between two groups are shown in a heatmap (d) volcano plot (e). The red and green ones represent upregulated and downregulated DEGs in the volcano plot, respectively. (f) KEGG enrichment analysis of DEGs are shown. DEGs, differentially expressed genes; KEGG, kyoto encyclopedia of genes and genomes; PAH, pulmonary arterial hypertension.

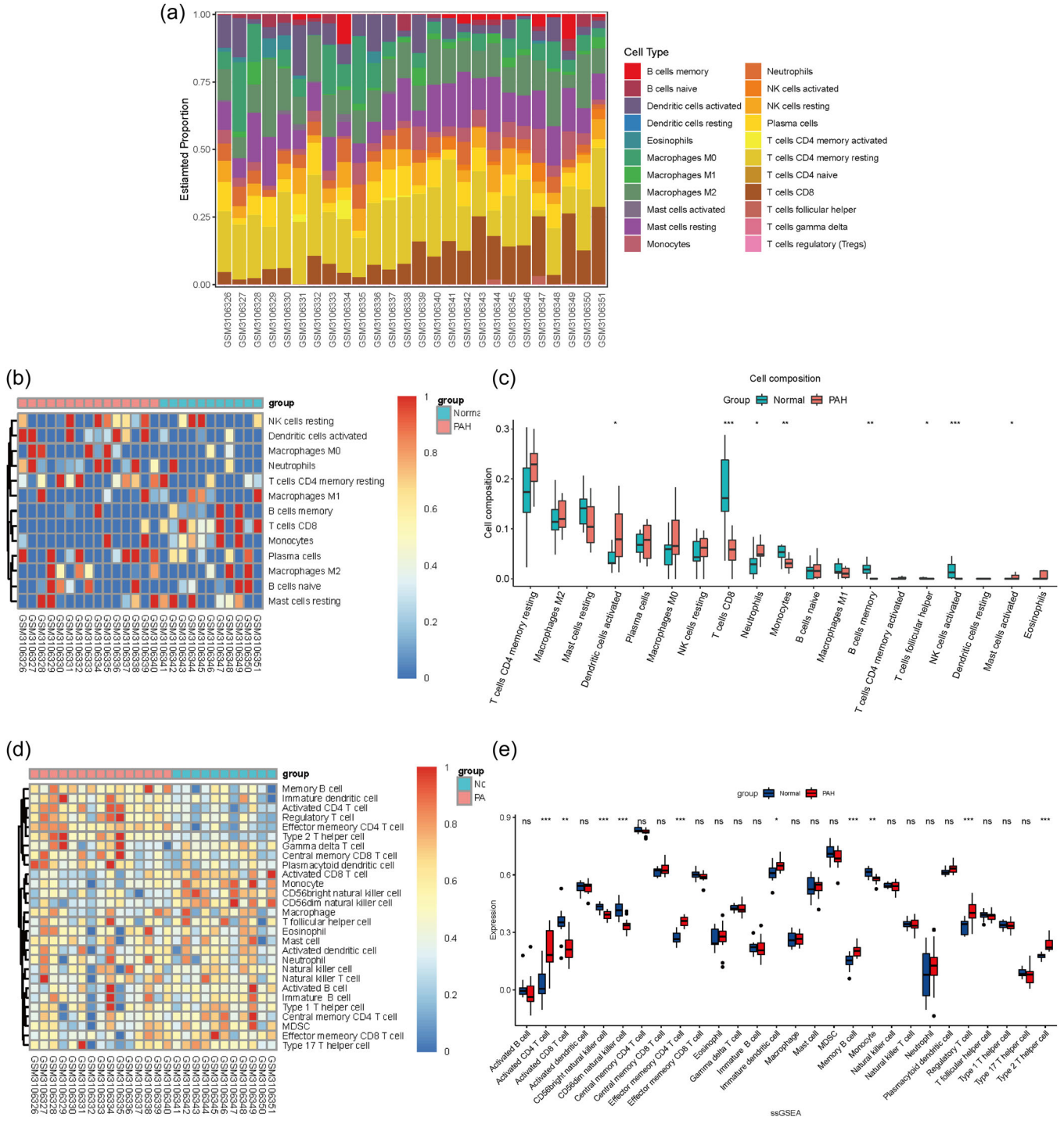

FIGURE 2 Immune cell infiltration analysis based on GSE113439 data set. (a–c) Data were analyzed by the CIBERSORT algorithm. (a) The fractions of immune cells in each sample are shown. (b) The immune infiltration levels of each sample are shown in the heatmap. (c) The fractions of 22 types of immune cells between two groups are shown. (d, e) Data were analyzed by ssGSEA. (d) Twenty-eight immune-related gene sets were enriched and shown in the heatmap. (e) The fractions of immune cells between two groups are presented. ns: no significance; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

death-ligand 1 (PD-L1), can bind with programmed death protein-1 (PD-1) to inhibit lymphocyte activation and proliferation.[31] A previous study has found that PD-1 and PD-L1 proteins were overexpressed on both T and B cells in idiopathic PAH patients, which is similar with our findings.[32] Chronic hypoxia or inflammation may upregulate the expression of PD-L1 in monocyte-derived macrophages (MoMs), and can further induce the release of cytokines, which may promote PAH.[33] Additionally, consistent with our result, C-C Motif Chemokine Ligand

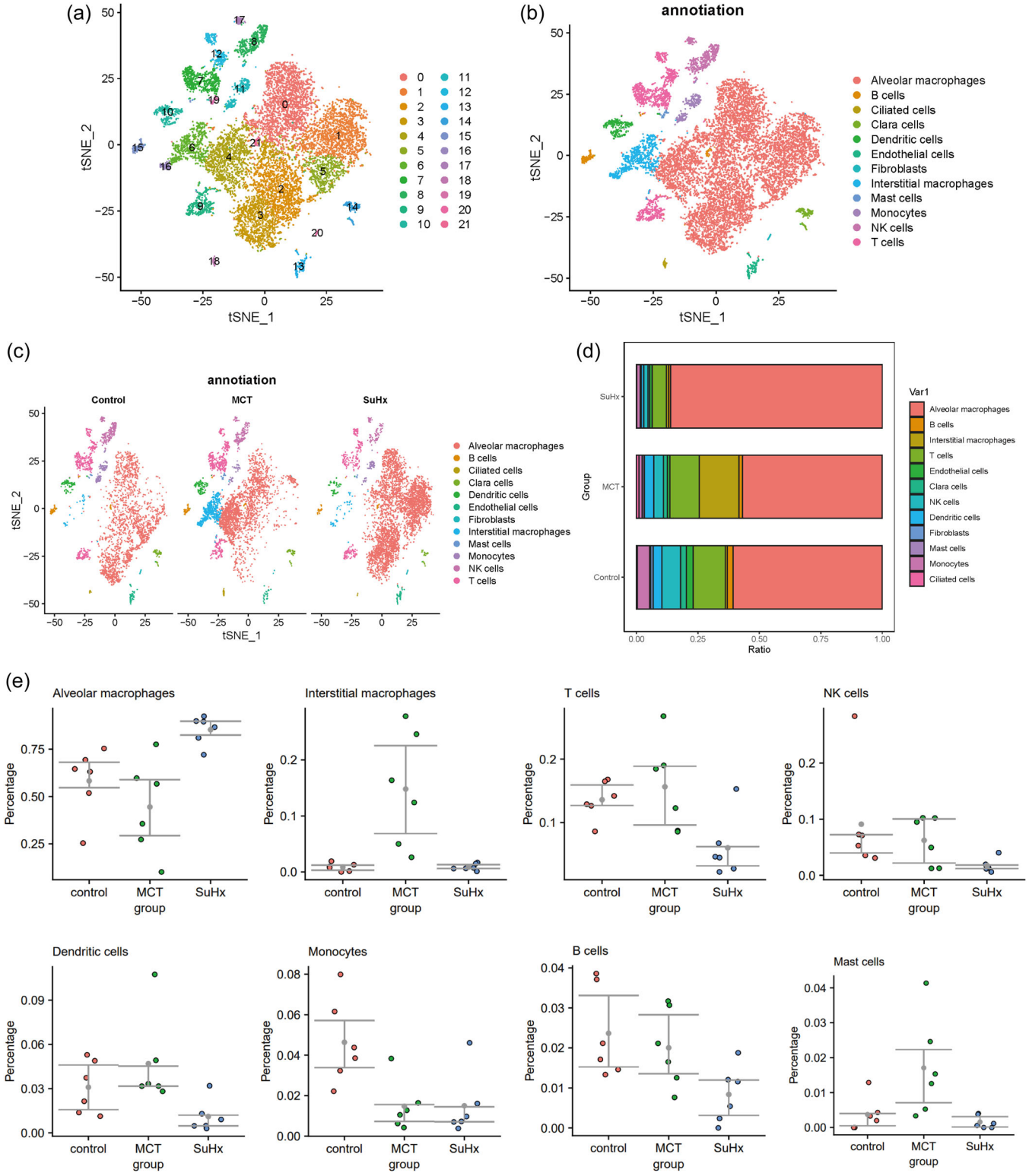

FIGURE 3 Cell composition analysis with scRNA-seq data. (a) TSNE plot displays the aggregate cells. (b) TSNE plot shows the cell types of different clusters based on identified markers. (c) The identified cell populations in different groups (six control samples, six MCT samples, and six SuHx samples) are shown in the TSNE plot. (d, e) The fractions of different cell types are shown (d), and the fractions of the immune-related cells are shown between different groups (e). MCT, monocrotaline; scRNA-seq, single-cell RNA sequencing; SuHx, Sugen-5416 hypoxia.

3 (CCL3), also called MIP-1α, can activate chemokine receptor 5 (CCR5) to mediate inflammation via inflammatory monocytes and neutrophils, which was elevated in chronic thromboembolic PAH patient plasma.[34] Besides, the expression of MIP-1α would be further increased due to promoted secretion by MoMs.[35] All these results demonstrated that with decreased proportion and changes in immune related genes, monocytes in patients with PAH showed a significant immune dysregulation, which inevitably promoted the process of PAH. Then, GSVA analysis was performed on monocytes and the results suggested that metabolism-related pathways might play important roles in monocytes in PAH (Supporting Information S2: Figure 3F).

Intercellular communications between monocytes and fibroblasts in PAH

To illustrate how the change in fraction of cells affect the pathogenesis of PAH, we analyzed intercellular communications with the CellChat package. The results showed that the intercellular communications of alveolar macrophages were most active (Figure 4a,b). Due to the complicated cell−cell communication network, we centered on single type of cells to further compare the two groups and found that not only monocytes but also other immune cells varied a lot (Supporting Information S2: Figure 4). However, monocytes and interstitial macrophages varied most in the scatter plot between the control and PAH groups (Figure 4c). Furthermore, the heatmap exhibited that monocytes interact with fibroblasts most in both groups, indicating that the interaction of monocytes and fibroblasts might be the key to the onset of PAH (Supporting Information S2: Figure 5A,B). Notably, although in PAH group the number of interactions between fibroblasts and monocytes decreased, the interaction strength increased (Figure 4d). Therefore, the incoming and outgoing signaling patterns of monocytes and fibroblasts were analyzed to further clarify the details in the interaction between monocytes and fibroblasts. In control group, no outgoing signals from monocytes to fibroblasts were detected. Interestingly, not only signals from monocytes to fibroblasts but also fibroblasts to monocytes were increased in the PAH group (Figure 4e).

ICAM and ITGAL-ITGAB2 pathways between monocytes and fibroblasts in PAH

Then, 45 signaling pathways are detected among the 12 cell populations to analyze which pathways between monocytes and fibroblasts changed in PAH (Supporting Information S2: Figure 5C). We found that the ICAM and ITGAL-ITGAB2 pathways were changed in the interaction between monocytes and fibroblasts in PAH as compared to normal controls (Supporting Information S2: Figure 5C). In addition, the cell−cell communications mediated by multiple ligand−receptors were visualized by bubble plot, which also suggested that ligand−receptors (L-R) in ICAM and ITGAL-ITGAB2 pathways changed between monocytes and fibroblasts (Supporting Information S2: Figure 5D). Among the communications between monocytes and fibroblasts, we noticed that the fibroblasts−monocytes interaction showed more changed ligand−receptors than the monocytes-fibroblasts interaction (Figure 5a and Supporting Information S2: 6A,B). Additionally, Itgb2-Icam1 (ITGAL-ITGAB2 pathway), Icam1-Itgal (ICAM pathway), and Icam1-(Itgal+Itgb2) (ICAM pathway) increased in PAH than control (Figure 5a,b). Next, we focused on ICAM and ITGAL-ITGAB2 pathways, and in PAH, the interactions between monocytes and fibroblasts presented great significance in both pathways (Figure 5c,d and Supporting Information S2: 6C,D). Furthermore, the expression of Itgal, which is the ligand or receptor in ICAM or ITGAL-ITGAB2 pathways changed significantly in monocytes (Figure 5e), which may be the potential reason of the phenomenon that in PAH group the number of interactions between fibroblasts and monocytes decreased, but the interaction strength increased (Figure 4d). Above results indicated that although the percentage of monocyte decreased, monocytes may increase the interaction strength with fibroblasts by ICAM and ITGAL-ITGAB2 pathways to influence the process of PAH.

Identification and verification of diagnostic markers in PAH

The contribution of each L-R pair in the ITGAL-ITGAB2 and ICAM pathways were calculated to identify the key L-R pairs. In the ITGAL-ITGAB2 pathway, Itgb2 was the key molecule and Itgb2-Icam2 was decreased in the PAH group compared to the control group (Figure 6a,b). Simultaneously, Icam1-(Itgam+Itgb2) and Icam2-(Itgam +Itgb2) in the ICAM pathway were both disappeared in PAH group. Compared to control group, Icam1-(Itgal +Itgb2) in ICAM pathway was obviously increased in the PAH group (Figure 6c,d). According to the above results, ROC curves were plotted based on the human microarray data (GSE113439, GSE117261, GSE15197, GSE48149), which contained 108 PAH samples and 50 normal controls (Table S1). The AUCs of ITGB2, ICAM2, ITGAL,

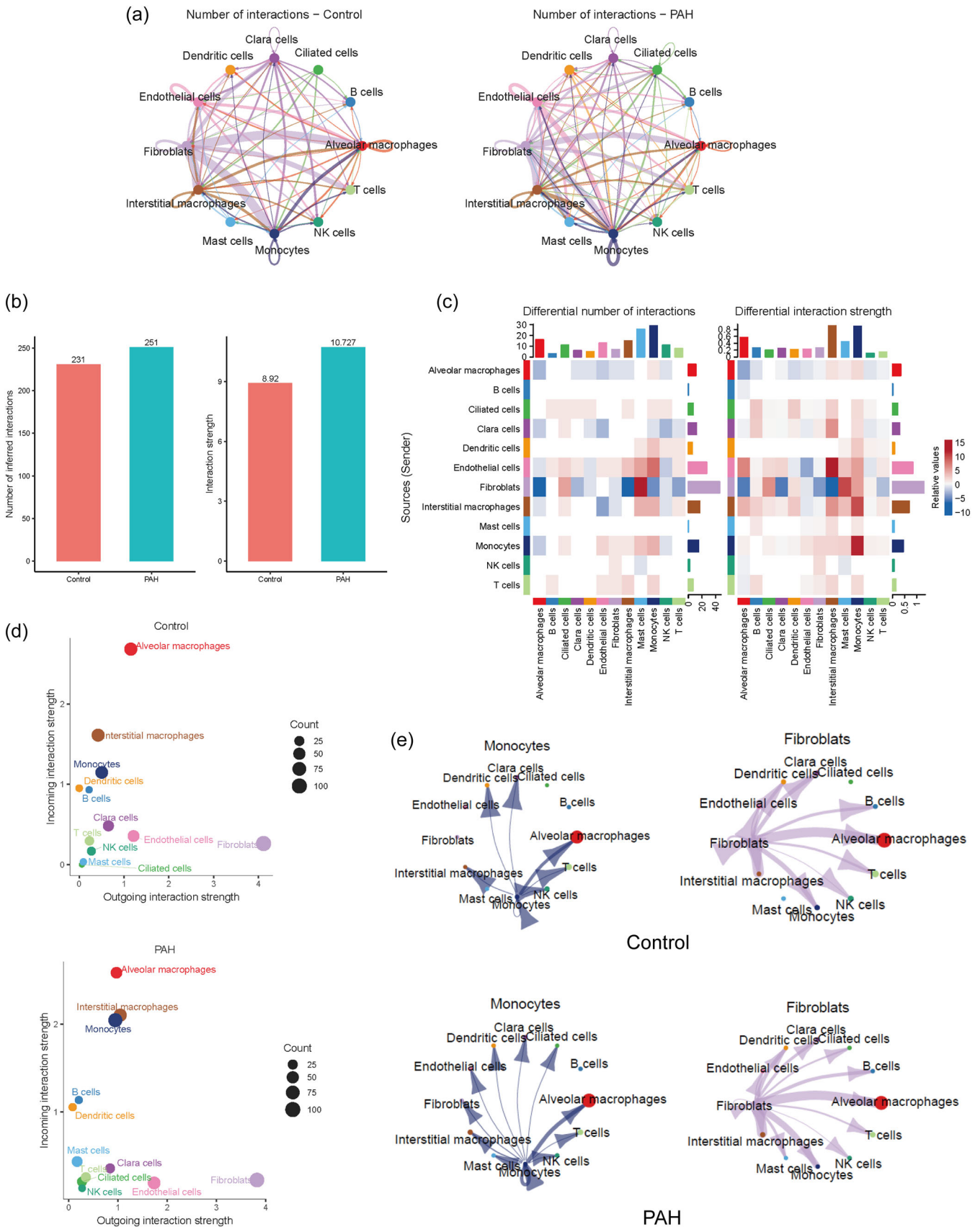

FIGURE 4 (See caption on next page).

and ITGAM were 0.642, 0.654, 0.604, and 0.759, respectively. The sensitivity and specificity of ITGB2, ICAM2, and ITGAL were 40.6% and 86.5%, 84.0% and 44.2%, and 79.2% and 42.3%, respectively. ITGAM had a sensitivity of 81.1% and a specificity of 65.4%, which displayed a good diagnosis value (Figure 6e). Furthermore, the LASSO was performed and two genes (ITGAM and ICAM2) demonstrated the high discriminatory power to diagnose PAH (Figure 6f).

DISCUSSION

PAH is a chronic and severe cardiopulmonary disorder, attributed to small pulmonary arteries proliferation and fibrosis, which causes exacerbations progressively in pulmonary vascular resistance.[2] Even though intense research efforts have been made in PAH associated field in recent years, the pathogenesis remains to be further elucidated, and the therapeutic effect is unsatisfactory. In this research, we characterized features of infiltration of immune cells and explored underlying biomarkers in PAH patients by integrating gene microarray and single-cell transcriptome based on bioinformatics analysis.

For investigating functions of immune cells in PAH, immune infiltration analysis was performed with microarray data by utilizing CIBERSORT. Our results showed that percentage of monocyte was decreased in lung tissues of PAH as compared to the control group, which was in consist with extensive profiling of cellular composition through using scRNA-seq. This is also consistent with results published by other groups.[17]

However, an increased permeability of monocytes has been found in peripheral blood of PAH in another study.[11] This might be related to monocyte recruitment and polarization. The recruitment and the polarization of monocytes are modulated by the microenvironment and facilitated by the local stimuli.[36,37] It has been demonstrated that chronic hypoxia is a critical pathogenic driver for the recruitment of monocytes to the lung. The findings suggested that monocytes have abilities to sense hypoxia, infiltrate pulmonary arteries, and promote vascular remodeling, contributing to the development of PAH.[38] In addition, it has been previously reported that blood-borne monocytes expand in lung explants from patients with PAH, which exacerbates muscularization of small pulmonary arteries and modulates local immune responses.[6] Thus, we speculated that increased monocyte infiltration in peripheral blood of PAH might be associated with mobilization of monocytes, while decreased monocyte in lung tissues might be related to transition of monocyte to macrophage or other types of cells. Data from murine models suggested that LY6C^{hi} monocytes can transform into nonresident CD11b+ infiltrating macrophages, thereby mediate pulmonary fibrosis, and result in alveolar epithelial cell-specific injury.[39–41] In another study, depletion of Ly6C^{hi} circulating monocytes by systemic administration of liposomal clodronate resulted in a reduced fibrotic response in mice, as well as a reduction in M2 macrophage numbers.[42] This is consistent with our experimental results that monocytes were reduced but macrophages (including M0, M1, and M2) tended to increase in PAH compared with control samples (Figures 2b and 3c). Moreover, in our results based on scRNA-seq, the percentage of alveolar macrophages and interstitial macrophages increased in SuHx and MCT group, respectively, which may give us a clue on the decreased percentage of monocytes (Figure 3e). These data suggest that circulating monocytes can transform into lung macrophages and play an important role in pulmonary fibrosis.

To better understand the pathogenic role of monocyte during PAH, our current study identified two significant monocyte-associated candidate signaling pathways (ICAM and ITGAL-ITGB2) related to PAH using scRNA-seq data analysis. Intercellular adhesion molecule-1 (ICAM-1) is critical in monocyte adhesion. Monocyte adhesion is a key process of monocyte trafficking across the vessel wall, which is regarded as a tightly regulated process, including the process of rolling, adhesion, and transmigration. Once monocytes attach to the endothelium, ICAM-1 enables monocytes to adhere firmly to the endothelium and migrate through the endothelial cell barrier into the site of inflammation and injury.[43] Mechanistically, ICAM-1 signaling pathway increases monocyte−endothelial cell interaction, which may contribute to the progression of secondary vascular inflammation and PAH development.[44] In addition, ICAM-1 is a potential biomarker for PAH, which is

FIGURE 4 Intercellular communications between monocytes and fibroblasts in PAH. (a, b) Overview of the intercellular communication networks in the number of interactions or interaction strength are measured by network centrality analysis and are presented by circular network plots (a) and bar plots. (c) Scatter diagrams show signals of monocytes changed most as senders and receivers in control and PAH group. (d) Heatmaps show differential number of interactions (left) and differential interaction strength among the 12 cell clusters in the overall signaling patterns between control and PAH group. (e) The signals of monocytes and fibroblasts in control and PAH group are shown. PAH, pulmonary arterial hypertension.

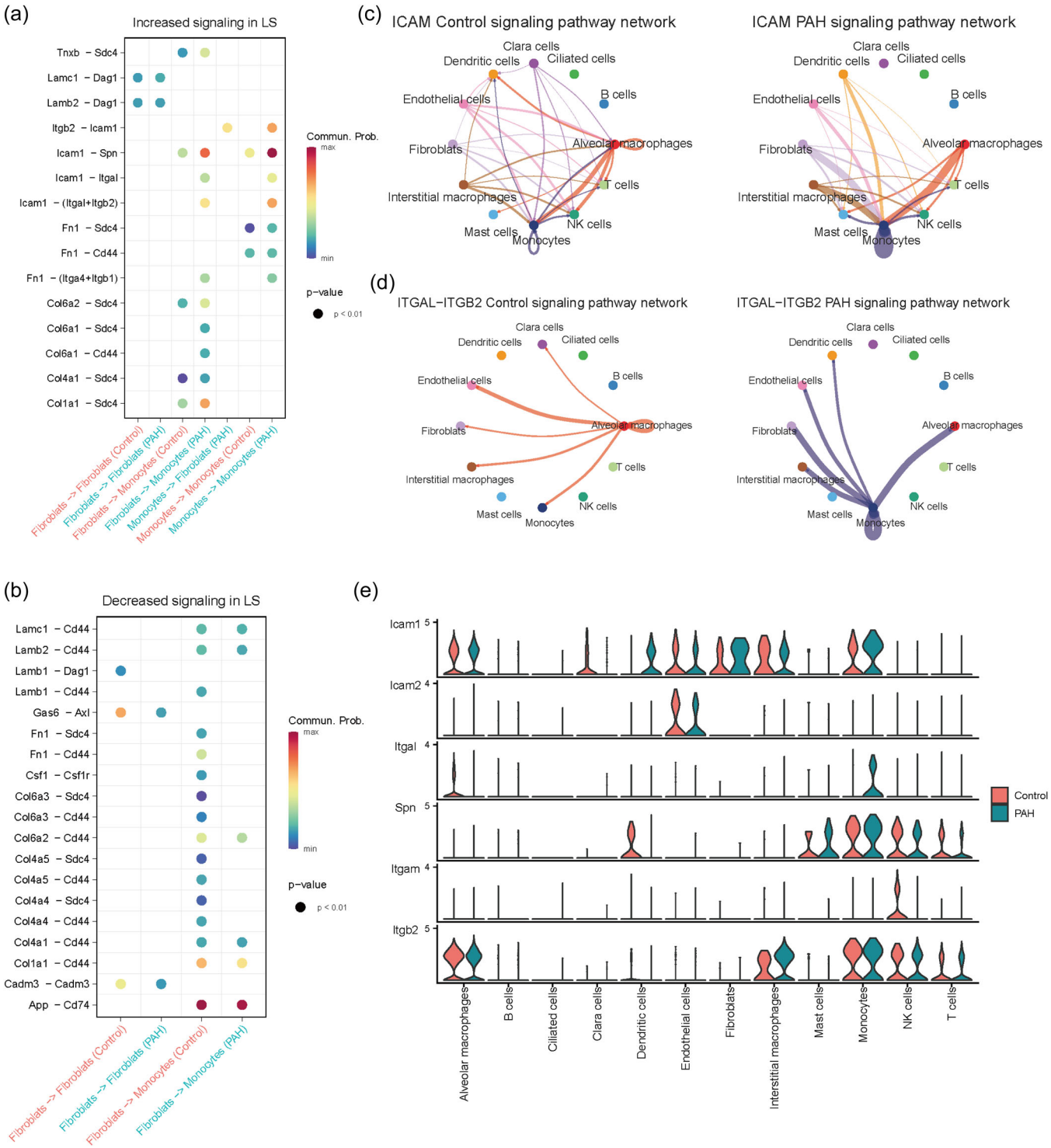

FIGURE 5 Visualization of signaling pathways or ligand−receptors mediated intracellular communications. (a, b) The increased (a) and decreased (b) ligand−receptor pairs are shown in the dot plot, which contribute to the signals between fibroblasts and monocytes. (c) Circular network plot shows the network centrality analysis of ICAM signaling pathway in control group (left) and PAH group (right). (d) Circular network plot shows the network centrality analysis of ITGAL-ITGAB2 signaling pathway in control group (left) and PAH group (right). (e) The between-group level of expression of molecules in ICAM signaling pathway and ITGAL-ITGAB2 signaling pathway are shown in violin plot. PAH, pulmonary arterial hypertension.

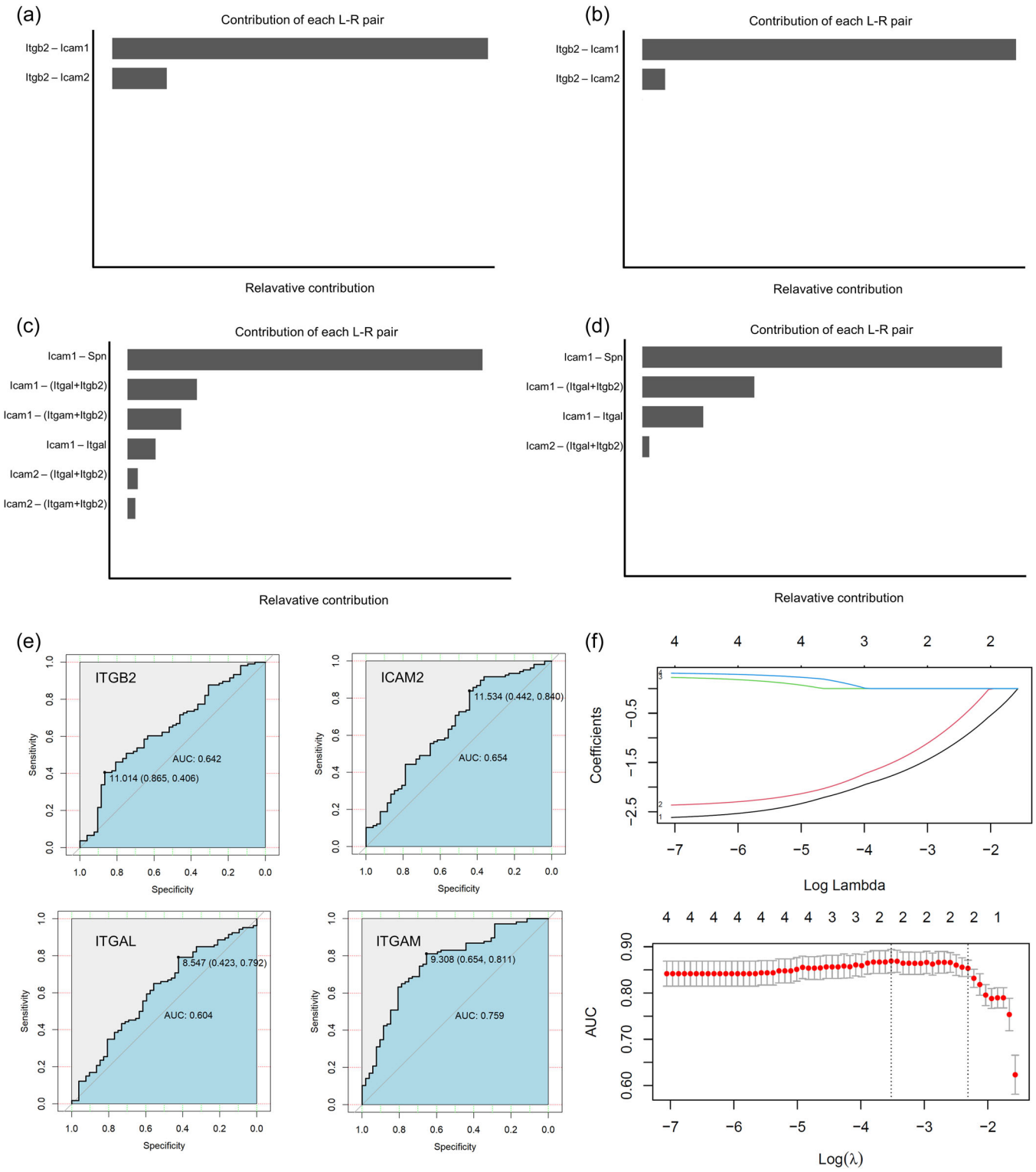

FIGURE 6 Identification of the key L-R pairs and diagnostic significance of correlated genes. (a–d) The contributor of each L-R pair in ITGAL-ITGAB2 pathway (a, b) and ICAM pathway (c, d) in control group (left) and PAH group (right). (e) ROC curves of critical genes (ITGB2, ICAM2, ITGAL, and ITGAM) in ITGAL-ITGAB2 pathway and ICAM pathway are shown. (f) Least absolute shrinkage and selection operator (LASSO) logistic regression algorithm are shown. L-R, ligand–receptors; PAH, pulmonary arterial hypertension; ROC, receiver operating characteristic.

elevated in the serum of both adults and children with PAH.[45,46] Therefore, ICAM-1 becomes a potential therapeutic target in PAH, and monitoring ICAM-1 levels might be useful in looking for the development of PAH. The combination of integrin alpha L chain (ITGAL) and beta 2 chain (ITGB2) forms the lymphocyte function-associated antigen-1 (LFA-1), which plays a critical role in the extravasation of immune cells from the bloodstream to the local tissues.[47] Through inside-out signaling, chemokine signals induce a conformational change in LFA-1 converting LFA-1 to a moderate-affinity state, which can promote cell adhesion to endothelial cells by the means of binding to ICAM-1.[48] Thus, we speculated that blockage of the interaction between LFA-1 and ICAM-1 might relieve the development of PAH.

A previous study displayed that ITGAL expression is critical for murine microglia CX3C chemokine receptor 1 (CX3CR1) expression and fractalkine (CX3CL1)-directed motility.[49] CX3CL1 and CX3CR1 are critical mediators in the vascular and tissue damage of several chronic diseases, including PAH. High levels of CX3CL1 and CX3CR1 expressed in monocytes are indispensable for survival, retention, migration, and recruitment to the site of injury. Moreover, genetic deficiency of CX3CR1 caused defective proliferation of pulmonary artery smooth muscle cell and remodeling of the lung vasculature in vitro and vivo model of PAH.[6,36] So, we guessed that inhibition of ITGAL expression might improve pulmonary vascular remodeling in PAH via suppressing CX3CL1/CX3CR1 signaling. Furthermore, the mRNA level of ITGAL is significantly upregulated in peripheral blood monocytes from PAH patients compared to the controls, indicating that ITGAL-ITGB2 signaling pathway might participate in PAH pathogenesis and that ITGAL can serve as a potential diagnostic biomarker.[50]

In addition, previous studies also have shown that MCP-1/CCR2 signaling pathway plays an important role in monocyte recruitment to the lung.[51] Recruitment of inflammatory monocytes is CCR2 dependent. Inflammatory monocytes exit from bone marrow, enter to lung, and give rise to inflammatory DCs and exudative macrophages under inflammatory conditions via the activity of CCR2.[52,53] Therefore, depletion of CCR2 may abolish inflammatory monocyte aggregation. Moreover, the progression of PAH can be facilitated by early recruitment of alternatively activated (M2) macrophages, which are thought to be polarized and activated by helper T cell type 2 cytokines (i.e., IL-4 or IL-13, IL-6) and the chemokine CCL2.[54,55] Additionally, blocking CCL2 expression attenuates disease severity.[56] Taken together, consistent with CX3CL1/CX3CR1 signaling, CCL2 may also regulate PAH progression.

The activation of pulmonary fibroblasts is one of the key components of pulmonary arterial remodeling in PAH. The primary stromal cell in the adventitia is the fibroblasts, which raises the possibility of significant fibroblast-immune cell cross-talk.[57] Fibroblasts can also release chemokines to recruit immune cells, and has some capacity for phagocytosis and antigen presentation.[58–60] Except for the interaction between fibroblasts and monocytes, that between fibroblasts and macrophages in the adventitia of blood vessels also facilitates the transmission of inflammatory signals and the progression of PAH.[37] Studies have reported that fibroblasts have abilities to recruit and activate macrophages, leading to vascular inflammation and vascular remodeling in PAH.[61] At the same time, studies have found that macrophages can receive and combine the signals sent by fibroblasts and then carry out disparate transcriptomics and metabolomics programming to keep a more stable lung microenvironment during the pathogenesis of PAH.[8,37] The crosstalk between fibroblasts and macrophages in the microenvironment of the adventitia of blood vessels are also expected to play a great therapeutic significance in improving the process of pulmonary vascular remodeling.

However, several limitations still exist in our study. First, this study focused more on immune cell infiltration in PAH and nonimmune cells that participate in the pathogenesis of PAH were not further investigated. Besides, the sample size and total number of cells involved in our study are not very large, which leads to the defect that the annotation of some cell subclusters and results of intercellular communication may not be detailed enough. Nevertheless, our study can provide foundations for further exploration of the mechanism of PAH.

CONCLUSION

The present study implicates that the change of monocyte is one of the key immunologic features of PAH. Monocyte-associated ICAM-1 and ITGAL-ITGB2 signaling pathways might be involved in the pathogenesis of PAH. Therefore, diagnostic identification coupled with therapeutic targeting of monocyte-associated genes or pathways may hold great promise in the diagnosis of PAH.

AUTHOR CONTRIBUTIONS

The project was conceived by Hao Tang, Xueli Lai, and Hui Shi. Chen Wang, Yalong Liu, and Cheng Chen searched and screened data from database. Chao Zhou and Yang Chen participated in the instruction of

analysis. Importantly, contributing equally to this study. Caiming Zhong, Yachen Si, and Huanhuan Yang analyzed the most data and completed this manuscript and share first authorship. Additionally, all authors participating this work have read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

All data in this article is from public database, and additional ethics approval is not applicable.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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