ORIGINAL RESEARCH

Immunosuppression by Inflammation-Stimulated Amplification of Myeloid-Derived Suppressor Cells and Changes in Expression of Immune Checkpoint HHLA2 in Chronic Obstructive Pulmonary Disease

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Background: The interaction between immune checkpoint and myeloid-derived suppressor cells (MDSCs) play a significant role in inflammatory diseases. But their correlation with chronic obstructive pulmonary disease (COPD) remains unclear.

Methods: The differentially expressed immune checkpoints and immunocytes in the airway tissues of COPD patients were identified by bioinformatics analysis, followed by correlation analysis and identification of immune-related differential genes for Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analysis. The results of bioinformatics analysis were verified by ELISA and Real-Time PCR and transcriptome sequencing of the peripheral blood of both COPD patients and healthy subjects.

Results: The results of the bioinformatics analysis showed that the level of MDSCs in airway tissue and peripheral blood of COPD patients was higher than that of healthy controls. The expression of CSF1 in airway tissue and peripheral blood of COPD patients increased, and CYBB was increased in airway tissue and decreased in peripheral blood of COPD patients. The expression of HHLA2 in the airway tissue decreased in COPD patients, and showed a negative correlation with MDSCs, with a correlation coefficient of −0.37. The peripheral blood flow cytometry results indicated that MDSCs and Treg cells of COPD patients were higher than those in the healthy control group. The results of peripheral blood ELISA and RT-PCR showed that the HHLA2 and CSF1 levels in COPD patients were higher than those in the healthy control group.

Conclusion: In COPD, the bone marrow is stimulated to produce MDSCs, and a large number of MDSCs migrate to airway tissue through peripheral blood and cooperate with HHLA2 to exert an immunosuppressive effect. Whether MDSCs play an immunosuppressive effect during migration needs to be further confirmed.

Keywords: chronic obstructive pulmonary disease, COPD, myeloid-derived suppressor cell, MDSC, immunosuppression, human endogenous retrovirus-H long terminal repeat-associating protein 2, HHLA2, bioinformatics analysis

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic airway inflammatory disease, which can cause extensive lung parenchymal cell damage,[1] and is characterized by chronic airflow restriction in the surrounding airway accompanied by a series of irreversible pathological changes in the lungs, which usually gradually worsens over time.[2] COPD is the third most commonly diagnosed disease with high morbidity and mortality in the world.[3,4] However, the pathogenesis of COPD is not entirely clear and reported intricate relationships among the various pathogenesis, making it difficult to

achieve effective prevention and control of COPD. Therefore, a broader and deeper study of the pathogenesis of COPD is conducive to both the prevention and control of COPD.

COPD is characterized by the simultaneous activation of innate immunity and adaptive immunity, both of which act together to drive this disease. Myeloid cells, including antigen-presenting cells (APCs) and myeloid-derived suppressor cells (MDSCs), play an essential role in regulating adaptive and innate immunity under both physiological and pathological states.[5] For instance, in tumors, inflammation, and other pathological conditions, the balance of mature differentiation of the bone marrow cells is disrupted, thereby leading to the cessation of maturation and differentiation of some myeloid cells at different stages, which plays a significant role in the negative regulation of immune response and constitute the MDSCs population.[6]

MDSCs are rarely expressed in healthy people's peripheral blood and tissues.[7] When stimulated by tumors and inflammatory states, they can expand in the bone marrow and migrate from the bone marrow to tumors or inflammatory sites for immunosuppression.[8] Enrichment of MDSCs has been observed in several tumor-related diseases and chronic inflammatory diseases and can be significantly associated with the poor prognosis of diseases.[9,10] The accumulation of MDSCs depends on two distinct correlated signals. The first set of signals can induce the expansion of immature myeloid cells by the different growth factors produced in tumor or inflammatory states. These include CSF2, CSF1, vascular endothelial growth factor (VEGF), Polyunsaturated fatty acids (PUFAs), Signal transducer and activator of transcription 3 (STAT3), Interferon Regulatory Factor 8 (IRF8), C/EBPβ, NOTCH, and other signaling pathways play a vital role in this process. The second group of signals is primarily facilitated by the production of inflammatory cytokines, including interferon γ(IFNγ), interleukin 1-β (IL-1β), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 13 (IL-13), tumor necrosis factor (TNF) and the toll-like receptors (TLR) ligand, High Mobility Group Protein 1 (HMGB1), etc. These mainly exert their effects through the activation of nuclear factor kappa-B (NF-κB), Signal transducer and activator of transcription 1 (STAT1), and Signal transducer and activator of transcription 6 (STAT6) signaling pathways.[8,11] Several previous studies have shown that the levels of both MDSCs and Treg levels in the peripheral blood of COPD patients were significantly increased and MDSCs are involved in the development of COPD.[12] However, how MDSCs can participate in the regulation of immune response in COPD remains unclear.

Immune checkpoints expressed on immune cells can contribute to immunosuppression in adaptive immunity as well. Immune checkpoints can protect the cells from immune cell interference by binding directly to the ligands expressed by themselves. As an essential part of tumor immunotherapy, studies related to the various immune checkpoints are becoming increasingly extensive. The primary immune checkpoints involved in COPD are protein programmed cell death 1 (PD-1) and programmed cell death one ligand 1 (PD-L1).[13,14] HHLA2 is a member of the B7 immunoglobulin superfamily. It has been demonstrated that HHLA2 can effectively inhibit the production of the different cytokines as well as the proliferation of CD4 and CD8 T cells in tumors[15] and play an immunosuppressive role, but there is no relevant report on COPD yet.

In this study, bioinformatics data mining was performed on the combined standardized data set of three groups of COPD samples in the GEO database to find the critical immune checkpoints. The peripheral blood of COPD patients and healthy subjects were used to verify the potential changes in immune checkpoints and MDSCs in COPD patients as well as the possible effect of their various interactions on the development of COPD. At present, there are few studies on the interaction between MDSCs and immune checkpoints in the occurrence and development of COPD. This study can provide references and new ideas for the prevention and treatment of COPD as well as the research and development of future targeted drugs.

Materials and Methods
Identification of Differentially Expressed Genes in the Public Datasets

The gene array expression series matrix files of three small airway epithelial samples were obtained from the comprehensive gene expression dataset (https://www.ncbi.nlm.nih.gov/geo/). The selection criteria for COPD datasets were used based on the workflow designed by Dai et al.[16] In total, including GSE8545 (18 COPD cases, 36 healthy subjects), GSE20257 (23 COPD cases, 112 healthy subjects), and GSE30063 (36 COPD cases, 133 healthy subjects),

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a total of 77 COPD patients and 281 healthy subjects were taken into the analysis. Given the limited sample size of the small airway epithelium in COPD patients, we used R software packages (sva package combat function and limma 3.40.6 packages) to adjust batch effects, combine and standardize the three data sets. The combat function in the sva package was applied to remove the batch effects of these three datasets.[17] (The batch correction results have been uploaded in the Supplementary File, the file named "sva_boxPlot adjust batch effects"). Meanwhile, the peripheral blood transcriptome sequencing results detected by our team were analyzed, including 3 healthy subjects and 6 COPD cases. The sequencing results have been uploaded to NCBI (number: PRJNA853498). R Packages limma[18] was used to evaluate the expression of differential genes in the various samples. To be specific, RMA and AFFY R packages were used for the background correction and quantile normalization of the expression data, and the probe set was summarized. Subsequently, the normalized expression data was entered into the lmFit, and ebayes functions and the Limma package were used to calculate the different statistics. The Benjamini and Hochberg False Discovery Rate (FDR) method was employed to adjust the P-value and obtain the q-value after the multiple tests. All the data was standardized and cross-compared. Adj. $P<0.05$, log FC$\geq$|2| when selecting the differentially expressed genes (DEGs) in COPD compared with the normal tissues.

Analysis of GO and KEGG Pathway

Gene annotation was performed using the Database for Annotation, Visualization, and Integrated Discovery (Enrichr), which allows us to investigate the biological functions and signaling pathways in which a given gene set is involved in. Gene annotation included Gene Oncology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. GO is comprised of three independent categories: biological process (BP), molecular function (MF), and cellular component (CC). Terms with FDR < 0.05 were considered significantly enriched.

Immunocyte Enrichment and Correlation Analysis

Single-sample gene set enrichment analysis (ssGSEA) was employed to determine the immune cell types in the dataset. The immunological signature of each sample was estimated based on pre-defined immune gene sets with the ssGSEA algorithm in the R package GSVA. The immune gene sets indicated the biological functions, chromosomal localization, and physiological regulation of 28 types of immune cells. The bar plot of immune cell proportions was visualized with the ggplot2.[19–21] After determining the immune cell scores in the various samples, the two-sample t-test was applied to analyze the differences in immune cells between COPD patients and healthy control groups. Pearson correlation analysis was thereafter used to analyze the correlation of immune cells in the dataset.

COPD Patients and Healthy Individuals

A total of 22 COPD patients (cases) and 22 age-matched healthy individuals (controls) were obtained from the Traditional Chinese Medicine Hospital Affiliated to Xinjiang Medical University. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) criteria: patients with post-bronchodilator FEV1/FVC<70%, and <80% predicted FEV1. Patients were excluded if they had been diagnosed with bronchial asthma, bronchiectasis, pulmonary fibrosis, lung tumors, and tuberculosis. The 22 healthy individuals were recruited from the hospital's health checkup center. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Xinjiang Uygur Autonomous Region Hospital of Traditional Chinese Medicine (Ethical approval number: 2022XE0111-1). Written informed consent was obtained from all the participants. Venous blood was collected from all cases and controls who participated in the study.

Blood Samples

Peripheral blood samples from the healthy subjects and COPD patients were collected into the test tubes containing EDTA for cellular immunophenotype detection. 100 μL of peripheral blood from the EDTA test tube was taken, and 1 mL Trizol was added. The solution was then mixed repeatedly 20–30 times, put on ice for 15 min and stored in an ultra-low temperature refrigerator for Real-Time PCR detection. The peripheral blood was collected in the collection vessel without anticoagulant and centrifuged at 3000 RPM at 4°C for 10 min. The obtained serum samples were then immediately stored in the ultra-low temperature refrigerator for the evaluation of the soluble analytes.

Detection of the Peripheral Blood by Flow Cytometry

Peripheral blood samples from healthy individuals (14 cases) and COPD patients (14 cases) were collected for flow cytometry. The gating strategy of MDSC is referred to by Luyckx et al,[22] and the gating strategy of Treg is referred to by Sharma et al[23,24] 50 μL of peripheral blood was added into the flow tube, and 1ul of antibody was added into the negative control samples without any antibody. The following antibodies were used: FITC anti-Lin-1: CD3 (Clone UCHT1; BioLegend), CD19 (Clone 4G7; BioLegend), APC anti-HLA-DR (Clone L243; BioLegend), PE anti-CD33 (Clone WM53; BioLegend), PC7 anti-CD45 (Clone HI30; BD Biosciences); Treg used the following antibodies: PE/Cyanine7 anti-CD4 (Clone RPA-T4; BD Biosciences), FITC anti-CD25 (Clone 2A3; BD Biosciences), PE anti-CD127 (Clone HIL-7R-M21; BD Biosciences); The cells and antibodies were then incubated at room temperature for 30 min in the dark. 2 mL of red blood cell lysate (BD bioscience) was used to lysate red blood cell residues. PBS solution was added 10 min later to stop the lysis. Finally, all the dissolved residues, morphological particles, and soluble proteins were eluted by double centrifugation at 1500 RPM for 5 min. Thereafter, by using Beckman, DXFLEX flow cytometer, the stained cells were analyzed, and the results obtained were processed by Kaluza software. Approximately, up to 100,000 cells were collected in each sample and the percentage of positive cells was assessed.

Real-Time PCR

Peripheral blood from healthy individuals (22 cases) and COPD patients (22 cases) was collected for the Real-Time PCR. The total RNA was extracted by using a Trizol reagent (Invitrogen, USA). Total RNA was reverse-transcribed into cDNA using the PrimeScriptTM RT Reagent Kit (RR031A, TaKaRa, China). Thereafter, with aim of determining the target RNA expression level, Real-Time PCR was performed on ABI 7500 fast Real-Time PCR system. The results were analyzed by the 2 -ΔΔCT method. The details of primer sequence synthesis have been shown in Table 1.

Detection of the Peripheral Blood by ELISA

Peripheral blood from healthy individuals (20 cases) and COPD patients (20 cases) was collected for the ELISA. The serum was thawed at room temperature before ELISA detection, and the protein concentrations of HHLA2, CSF1, and CSF2 were detected by using commercial ELISA kits (HHLA2, JL20688, Jiang Lai biological; CSF1, JL12518, Jianglai Biology; CSF2, JL11124, Jianglai Biological). The assay was executed in strict accordance with the manufacturer's instructions.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism software (version 8.0.2). The measurement data were compared by t-test. $P<0.05$ was considered statistically significant. Pearson's correlation coefficients were computed to determine the correlation between two continuous variables. The correlation intensities were classified into five grades

Table 1 Primers Sequences of Hub Genes and Internal Reference Genes

Gene Name	Primers Sequences
HHLA2	Forward 5':GGATCCGAAGTCGTAATACACT
	Reverse 5':TAGCAGGTGTAAATTCCTTCGT
CSF1	Forward 5':GCACCCTCCTCTCACTCCCTTC
	Reverse 5':CCACCCTGCTTTCCCACAAACC
CSF2	Forward 5':TCCTGAACCTGAGTAGAGACACTGC
	Reverse 5':CGGCTCCTGGAGGTCAAACATTTC
GAPDH	Forward 5':GAGAAGGCTGGGGCTCATTTGC
	Reverse 5':TGCTGATGATCTTGAGGCTGTTGTC

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according to the absolute value of the correlation coefficient: 0.00–0.19 corresponded to very weak,0.20–0.39 corresponded to weak, 0.40–0.59 corresponded to moderate,0.60–0.79 corresponded to strong, and 0.80–1.0 to very strong. $P<0.05$ was considered significant.

Results

Increased Levels of MDSCs and Treg Cells in the Peripheral Blood of COPD Patients

The levels of immunocytes (MDSCs and Treg cells), which exhibit immunosuppression effects, in the peripheral blood of COPD patients and healthy subjects were detected by using flow cytometry. The results demonstrated that the proportion of MDSCs and regulatory T cells in the peripheral blood of COPD patients was significantly higher than that of the healthy control group ($P<0.01$ or $P<0.0001$) (Figure 1). These observations suggested that elevated levels of MDSCs and Treg cells in the peripheral blood of COPD patients might be involved in the occurrence and development of COPD.

## Synergistic Immunosuppression by MDSCs and Treg Cells in COPD

To further understand the changes of MDSCs and Treg cells in airway tissues and the association between the two distinct cells, we used the GSEA algorithm to analyze the differences of 28 immune cell subsets between COPD patients and the healthy control group in the combined dataset. The number of both MDSCs and regulatory T cells in the COPD group was found to be significantly higher than that in the healthy control group ($P<0.01$ or $P<0.001$) (Figure 2A). Pearson correlation analysis was used to analyze the potential correlation between immune cells, and a strong positive correlation was found between MDSCs and regulatory T cells, with a correlation coefficient of 0.93 (Figure 2B). To verify this result, whole-blood transcriptome analysis was performed on COPD patients and the healthy subjects admitted to the Traditional Chinese Medicine Hospital Affiliated to Xinjiang Medical University. The results suggested that the number of both MDSCs and regulatory T cells was markedly increased in the peripheral blood of COPD patients compared with the healthy subjects ($P<0.01$ or $P<0.05$) (Figure 2C). Moreover, there was a strong positive correlation between them, with a correlation coefficient of 0.76 (Figure 2D). This finding indicated that in COPD patients, the levels of immunosuppressive MDSCs and Treg cells were increased, and the two cells might play a synergistic immunosuppressive role.

MDSCs Amplification and Pathologically Activated Pathways Were Enriched in the Airway Tissues of COPD Patients

Thereafter, to understand the amplification and activation pathways of MDSCs in COPD, we conducted difference analysis on the gene profiles in the combined data set and finally screened out 8428 differential genes. After the intersection with 1793 immune-related genes in the database, there were only 560 differential immune genes (Figure 3A and B) identified. KEGG enrichment analysis of the differential genes using the Enrichr database demonstrated that these differential genes could participate in several important immunomodulatory pathways, including TGF-β and JAK-STAT signaling pathways, which can play an essential role in the amplification and activation of MDSCs (Figure 3C). Moreover, GO enrichment of the common differential genes included the first ten entries of biological process (BP), cell composition (CC) and molecular function (MF) (Figure 3D). It was observed that both KEGG and GO analysis enriched TGF-β signaling pathways that are critical to the immunosuppression of MDSCs and regulatory T cells, thereby suggesting that the expansion and activation of MDSCs in airway tissues of COPD patients can serve as a crucial mechanism in the pathogenesis of the disease.

Differential Analysis of Molecules Related to Amplification Activation and Immunosuppressive Function of MDSCs

To further decipher the detailed molecular regulation mechanism of MDSCs, MDSCs amplification and pathological activation signaling molecules such as IL-1β, SOCS3, CSF1, IL-4, and IRF8 in the combined data set were found to be significantly higher expression than those of healthy subjects ($P<0.05$ or $P<0.01$ or $P<0.001$ or $P<0.0001$), but that of JAK2, RB1, STAT3, and STAT1 were lower than those in the healthy subjects ($P<0.01$ or $P<0.001$ or $P<0.0001$)
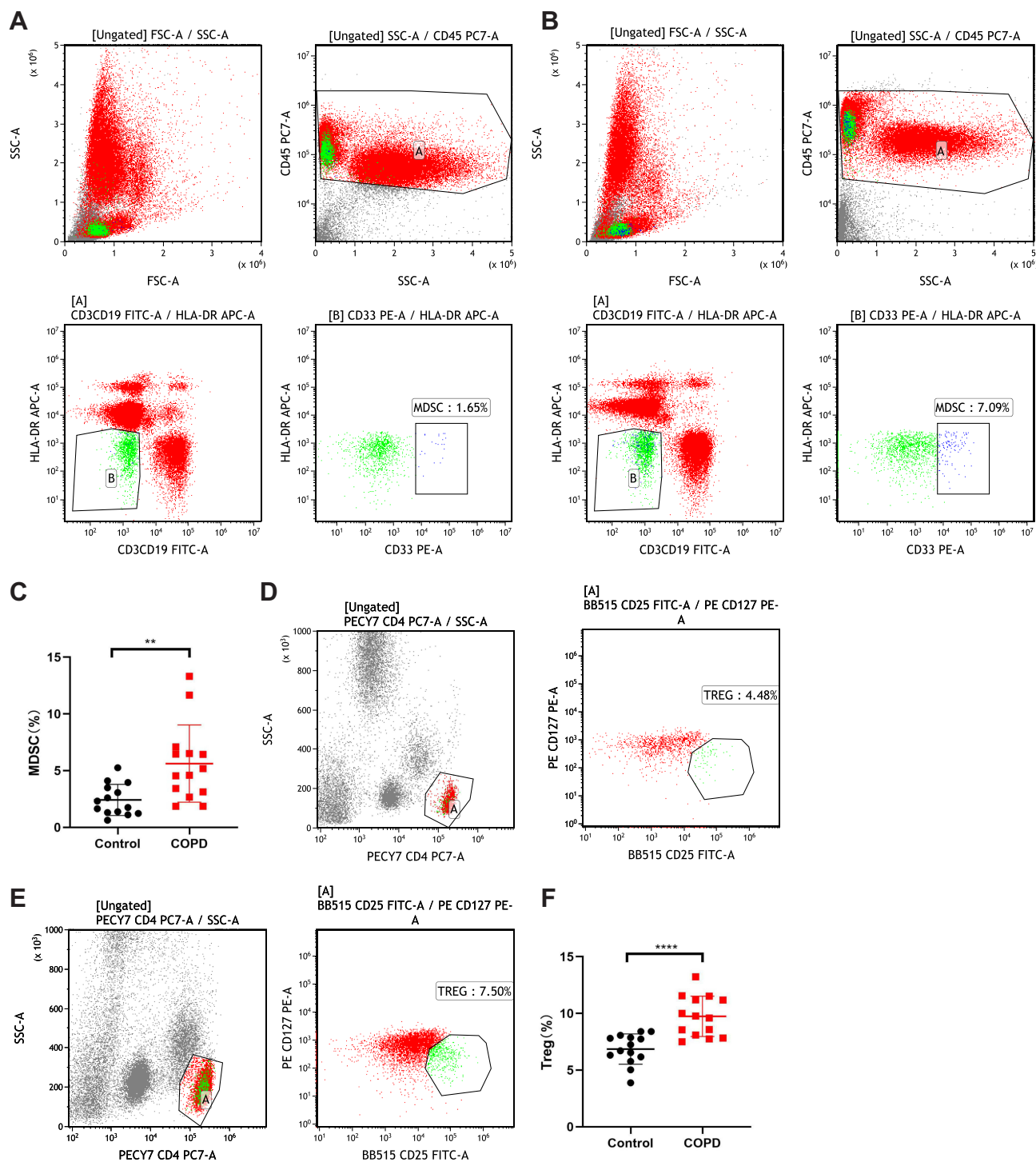
Figure 1 The differences in levels of MDSCs and Treg cells in the peripheral blood of COPD patients and healthy subjects. (**A**) Flow cytometric analysis of MDSCs in the peripheral blood of healthy subjects. (**B**) Flow cytometric analysis of MDSCs in the peripheral blood of COPD patients. (**C**) The counts of MDSCs in the peripheral blood of COPD patients and healthy subjects. (*******P*<0.01). (**D**) Flow cytometric analysis of Treg cells in the peripheral blood of healthy subjects. (**E**) Flow cytometric analysis of Treg cells in the peripheral blood of COPD patients. (**F**) The counts of Treg cells in the peripheral blood of COPD patients and healthy subjects. (*********P*<0.0001).

(Figure 4A). The above-mentioned transcriptome in the whole blood of COPD patients and healthy subjects were further analyzed. Among the numerous MDSCs amplified and pathologically activated signal molecules, a total of eight distinct molecules showed differences between COPD patients and healthy subjects. These included CEBP/β, STAT6, NF-κB, JAK2, RB1, CSF1, CSF2, and IFNγ (*P*<0.05). CSF1 showed an increasing trend in both peripheral blood and airway
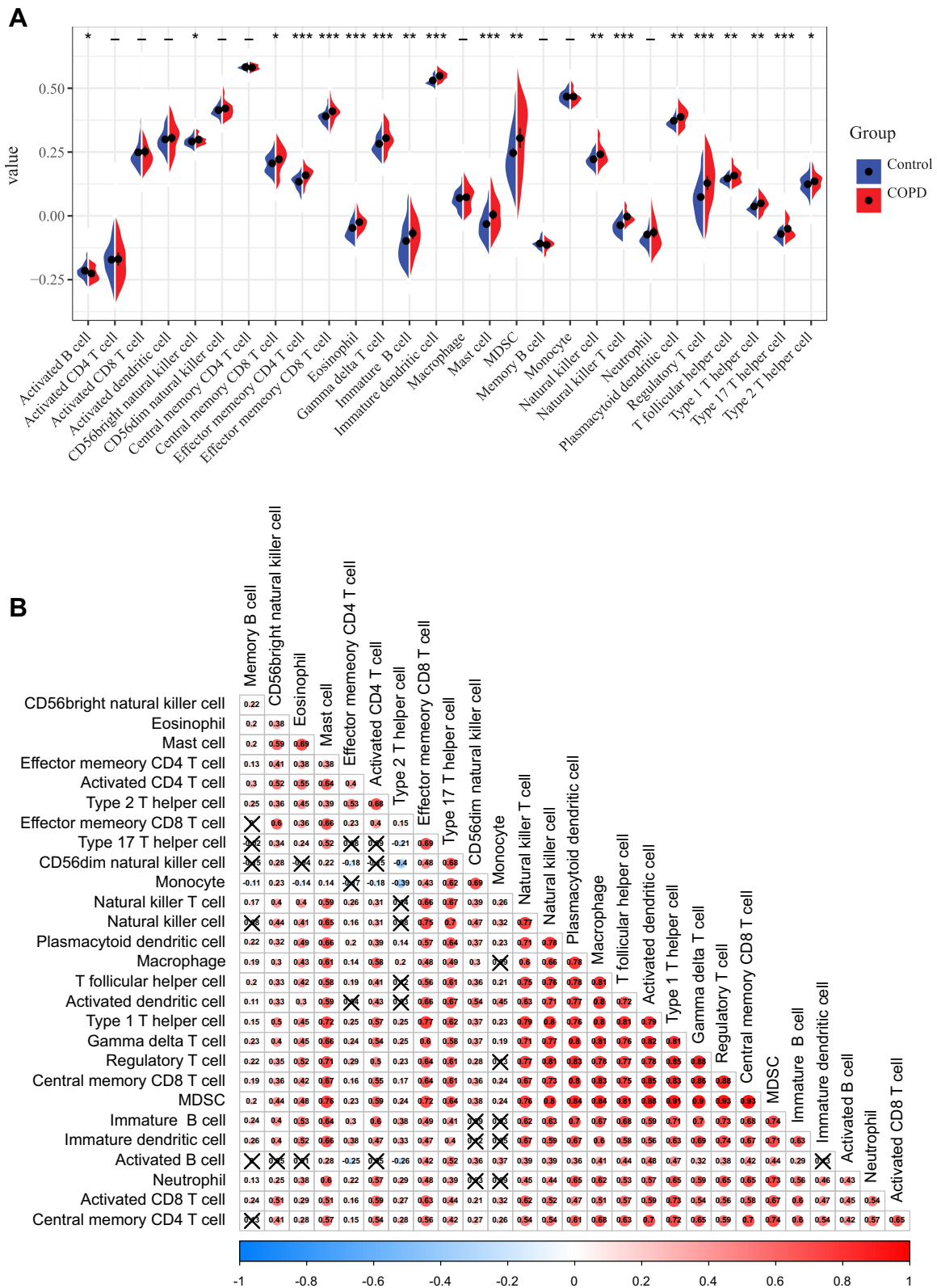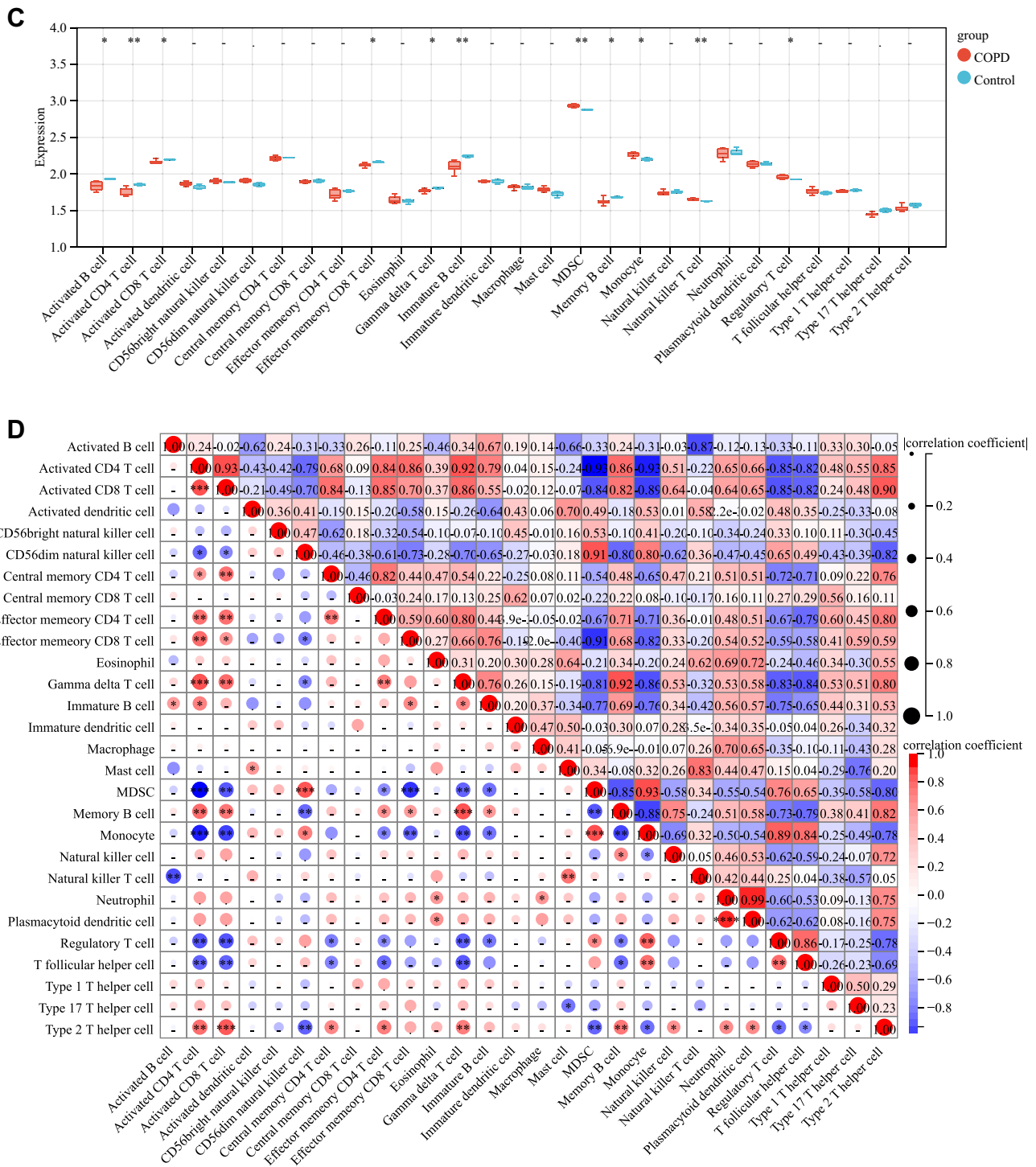
Figure 2 Continued.

Figure 2 The difference and correlation of immune infiltration of COPD patients and healthy subjects. (**A**) The differences in 28 immunocytes in the airway tissues of COPD patients and healthy subjects. (*P<005, **P<0.01, ***P<0.001). (**B**) The correlation of 28 immunocytes in the airway tissues of COPD patients and healthy subjects. (**C**) The differences in immunocytes in the peripheral blood of COPD patients and the healthy subjects as analyzed by immune infiltration scoring. (*P<0.05, **P<0.01). (**D**) The correlation of immunocytes in the peripheral blood of COPD patients and healthy subjects.

tissues of COPD patients (Figure 4C). The above results suggested that CSF1 promotes the expansion of MDSCs and can participate in the progression of COPD.

In airway tissues, TGF-β1, IL-10, and CYBB act as the key molecules of MDSCs that play immunosuppressive function, and these were found to be significantly higher in the COPD group than in the healthy subjects
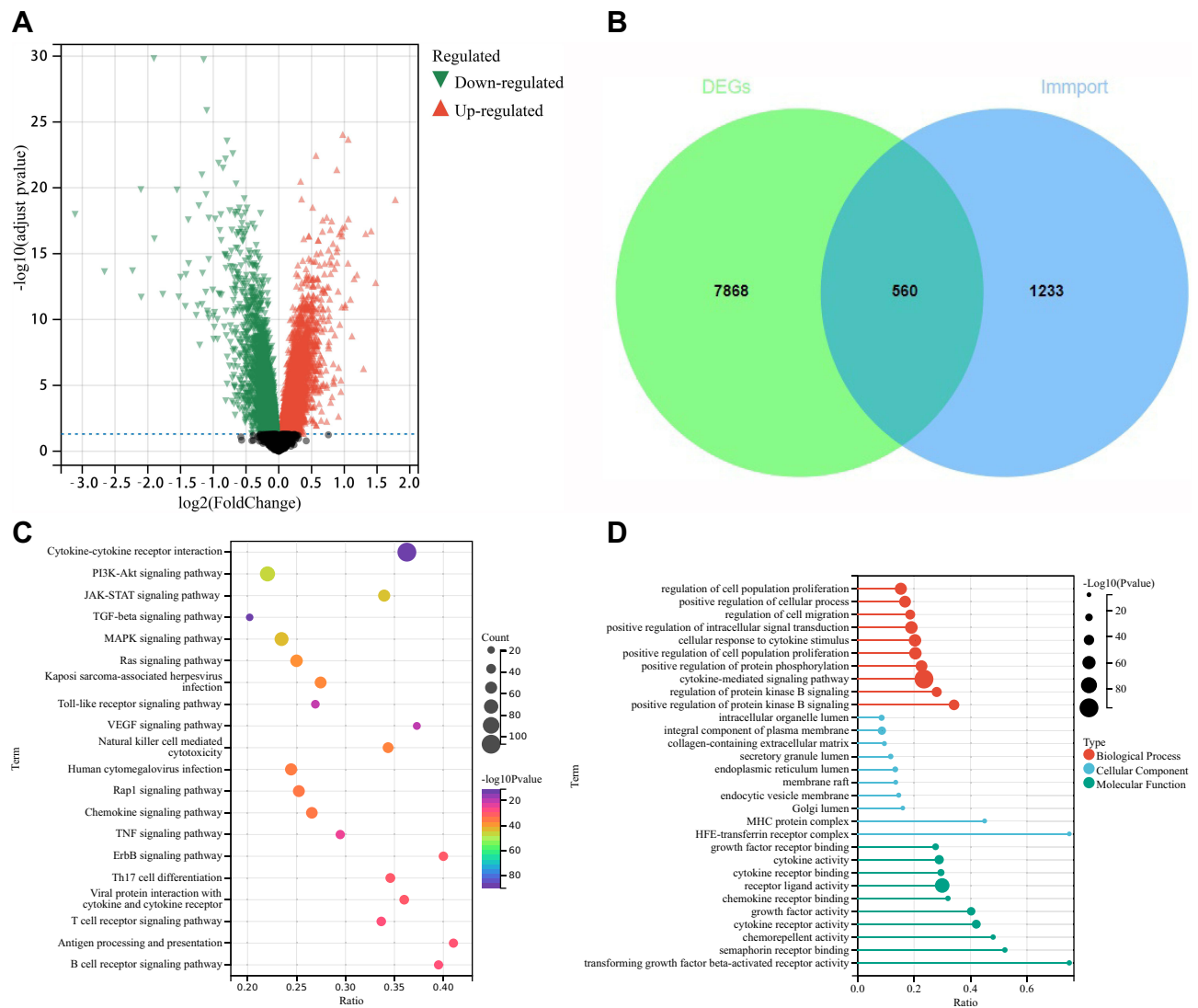
Figure 3 Screening and enrichment of the differential genes. (**A**) Screening of the differential genes in the combined dataset. (**B**) The different immune-related genes were obtained by the intersection of the screened differential genes and immune genes. (**C**) KEGG enrichment analysis was performed for differential immune genes. (**D**) GO enrichment analysis was performed on the different immune genes, including those involved in the regulation of the biological processes, cellular components, and molecular functions.

($P<0.05$ or $P<0.001$) (Figure 4B). Transcriptional data of whole blood of COPD patients and the healthy subjects was then used to analyze the related molecules of the immunosuppressive function of MDSCs. It was observed that only TGF-β1 and CYBB were differentially expressed. The transcriptional data and biogenic data of TGF-β1 showed the same trend of change, substantially higher in the COPD group than in healthy subjects ($P<0.01$). However, CYBB showed an opposite trend from the airway database analysis results and decreased in COPD patients as compared with the healthy subjects ($P<0.05$) (Figure 4D). TGF-β1 is a non-specific immunosuppressive molecule, while CYBB is one of the specific molecules that MDSCs use to exert their immunosuppressive effects. These results suggest that MDSCs can play an immunosuppressive role by producing CYBB while increasing in the airway tissues of COPD patients, whereas such an immunosuppressive effect was not reflected in the peripheral blood.
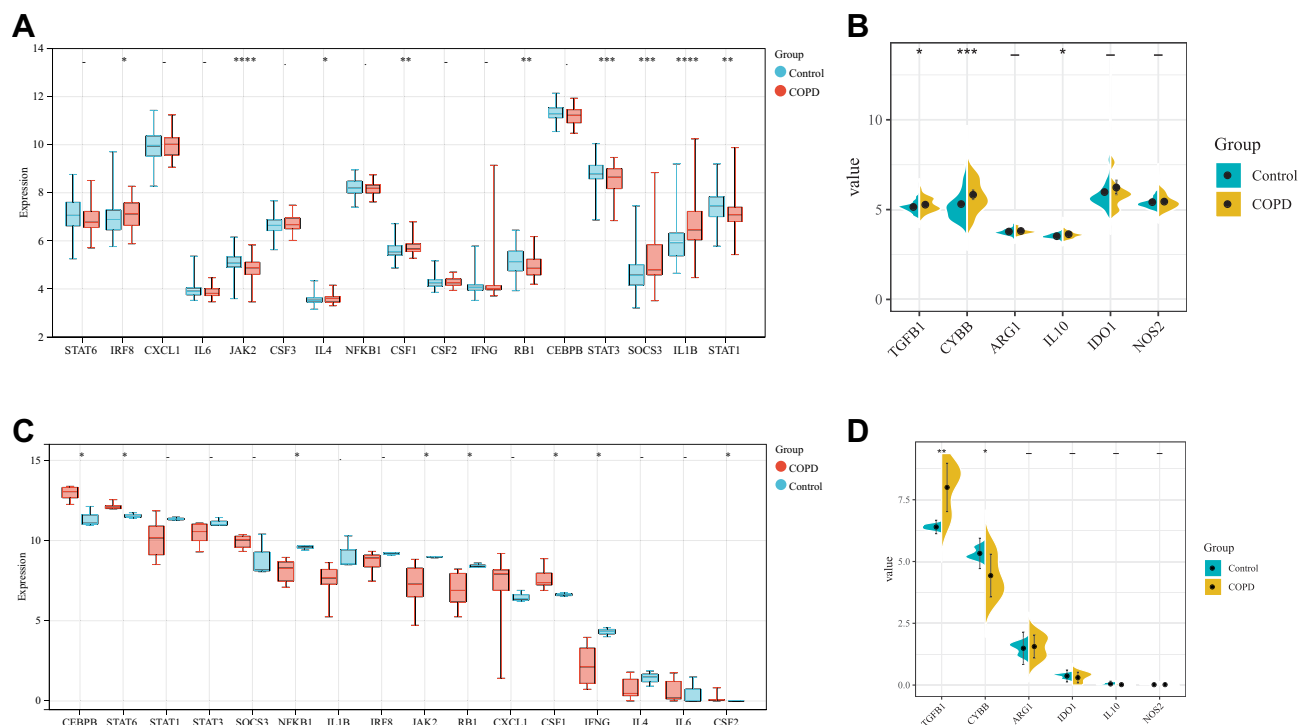
Figure 4 The differences in amplification and activation of MDSCs and immunosuppression-related molecules in COPD patients and healthy subjects. (**A**) The comparison of the expression of MDSCs amplified and pathologically activated signal molecules in airway tissues between COPD patients and healthy subjects. (*$P<0.05$,**$P<0.01$, ***$P<0.001$, ****$P<0.0001$). (**B**) The differential analysis of the immunosuppressive molecules of MDSCs in airway tissues between COPD patients and healthy subjects. (*$P<0.05$, ***$P<0.001$). (**C**) The comparison of the expression of MDSCs amplified and pathologically activated signal molecules in the peripheral blood between COPD patients and healthy subjects. (*$P<0.05$). (**D**) The differential analysis of the immunosuppressive molecules of MDSCs in the peripheral blood between COPD patients and healthy subjects. (*$P<0.05$, **$P<0.01$).

## Immunosuppression by MDSCs Might Be Associated with Immune Checkpoint HHLA2

To explore, whether immune checkpoints that serve as a vital factor in immunosuppression, can also change in COPD, we used the method of single sample *t*-test to analyze the difference of each immune checkpoint in the combined database between COPD patients and healthy subjects. The results showed that the expressions of LAG3, HHLA2, and REL were lower in COPD patients than in the healthy subjects ($P<0.01$ or $P<0.001$ or $P<0.0001$). On the contrary, the expressions of PDCD1, CD274, CD276, CTLA4, and LGALS9 were higher in COPD patients than in healthy subjects ($P<0.05$ or $P<0.01$ or $P<0.001$) (Figure 5A). Pearson's correlation analysis was employed to analyze the correlation between the immune checkpoint and immune cells. It was found that HHLA2 exhibited a significant negative correlation with immune cells in the immune checkpoint with the differential expression, and the correlation coefficient between HHLA2 and MDSCs was −0.37. The correlation coefficient with the regulatory T cell was −0.41 (Figure 5B). These results suggested that the immunosuppressive effect of MDSCs might be related to the expression of HHLA2.

Detection of Relevant Differential Genes in the Peripheral Blood by ELISA and Real-Time PCR

It has been demonstrated that the protein and transcription levels of HHLA2 in the peripheral blood of COPD patients were significantly increased ($P<0.0001$) (Figure 6A and D), and the protein expression level and transcription level of CSF1 and CSF2 amplified by MDSC were increased compared with those of the healthy subjects ($P<0.05$ or $P<0.01$ or $P<0.001$) (Figure 6B, C, E and F). The above results proved that MDSCs were amplified in the peripheral blood of COPD patients, but the expression trend of HHLA2 was opposite to that of airway tissue. Combined with other results of this study, it can be speculated that: on the one hand, MDSCs in the peripheral blood of COPD patients might not play an immunosuppressive role but on the other hand, the
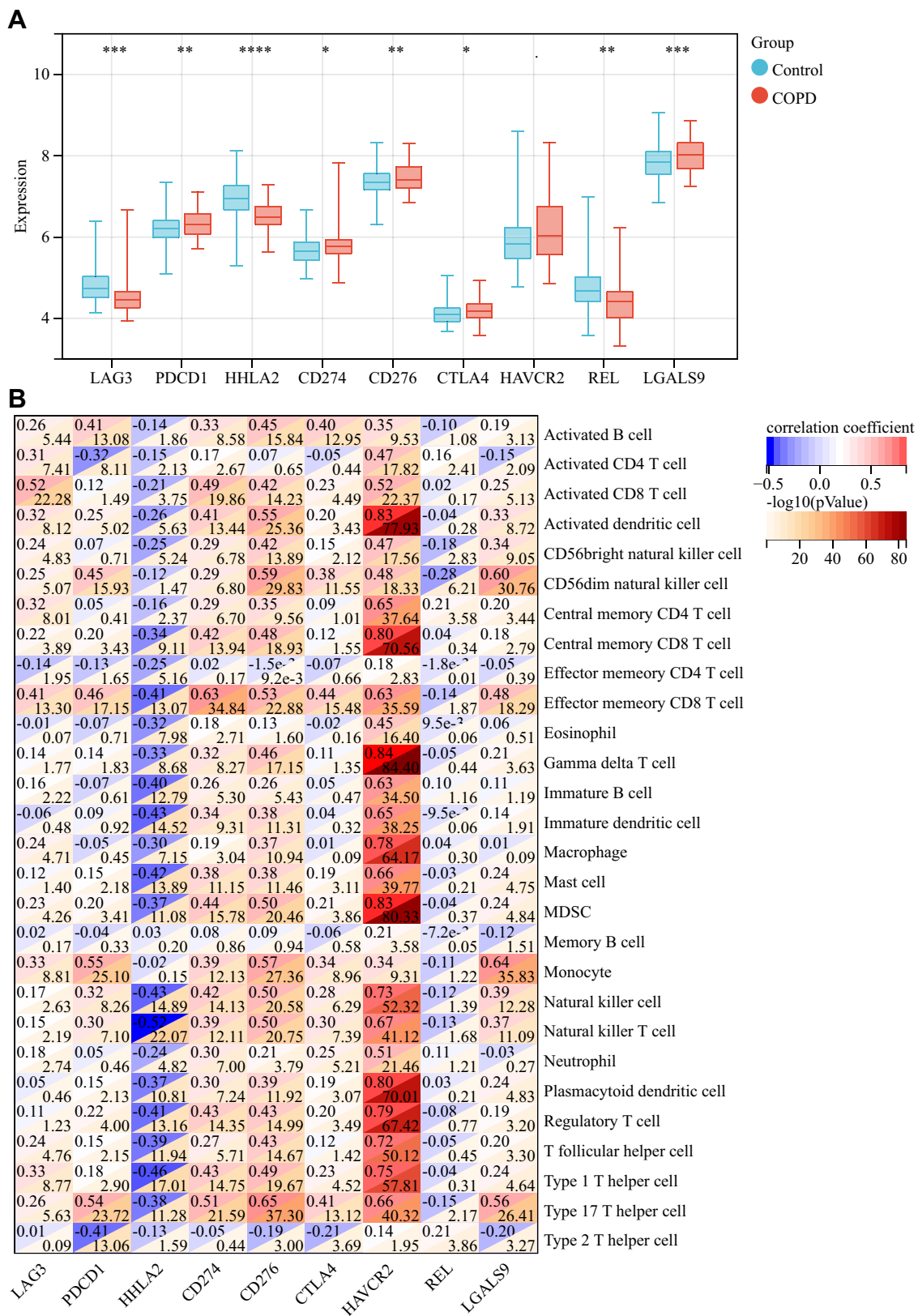
Figure 5 Analysis of the difference in immune checkpoint and its correlation with immune cells. (**A**) Analysis of the difference in immune checkpoint between COPD patients and healthy subjects. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001). (**B**) Correlation analysis between immune checkpoints and immune cells.
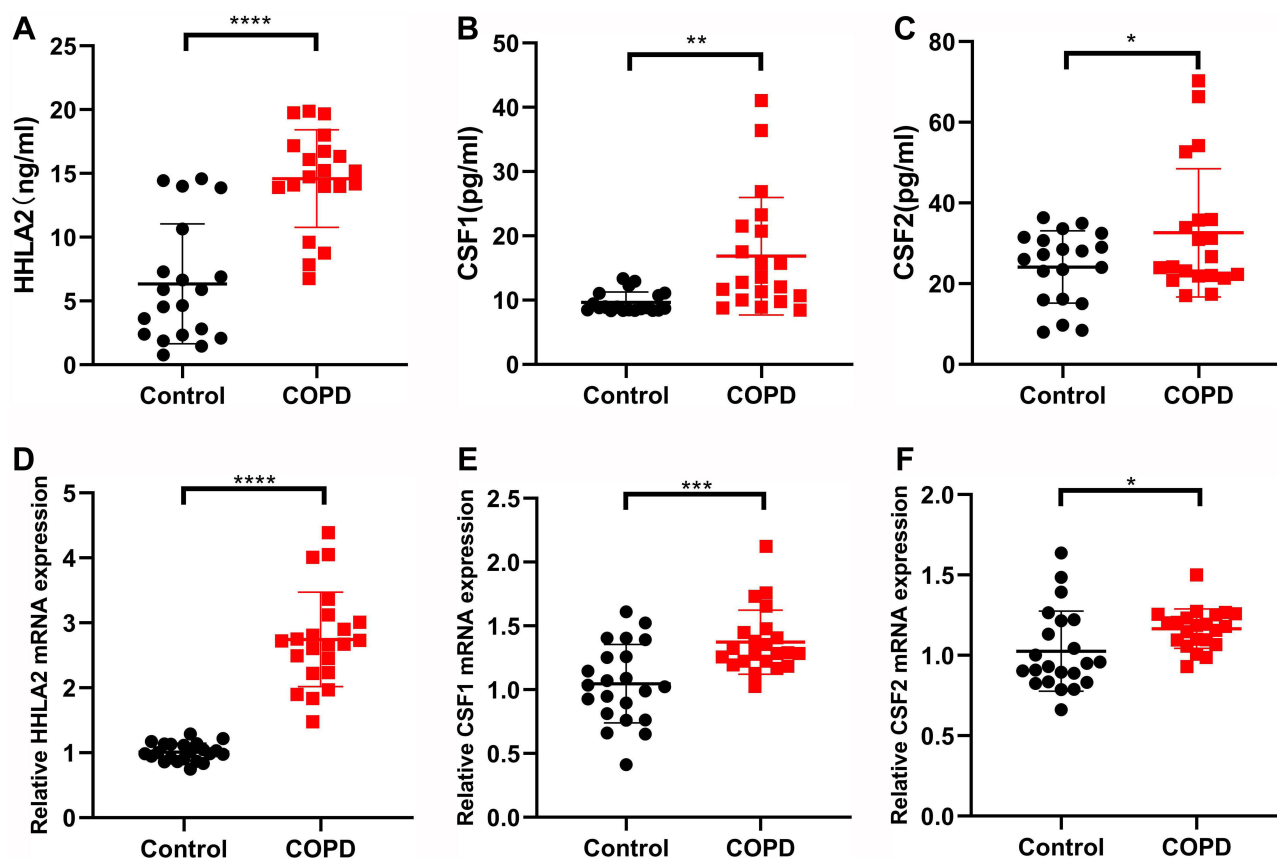
Figure 6 The detection of relevant differential protein by ELISA and mRNA by Real-Time PCR in peripheral blood (**A**) The detection of HHLA2 in the peripheral blood by ELISA. (****$P<0.0001$). (**B**) The detection of CSF1 in the peripheral blood by ELISA. (**$P<0.01$). (**C**) The detection of CSF2 in the peripheral blood by ELISA. (*$P<0.05$). (**D**) The detection of HHLA2 in the peripheral blood by Real-Time PCR. (****$P<0.0001$). (**E**) The detection of CSF1 in the peripheral blood by Real-Time PCR. (***$P<0.001$). (**F**) The detection of CSF2 in the peripheral blood by Real-Time PCR. (*$P<0.05$).

immunosuppressive effects of MDSCs in the peripheral blood of COPD were not reflected through HHLA2. This interesting hypothesis needs to be further verified in future studies.

Discussion

COPD is a heterogeneous syndrome associated with an abnormal inflammatory immune response in the lung,[25] characterized by persistent respiratory symptoms and continuous airflow restriction. Studies have confirmed that changes in immune cells play an important role in COPD progression. Therefore, both inadequate and excessive immune responses are the pathological basis for the occurrence and progression of COPD.[26] In this work, we found that the proportion of MDSCs in airway tissue and peripheral blood increased in COPD patients, the expression of MDSCs amplified molecules CSF1 and CSF2 increased, and the expression of CYBB, a molecule associated with the immuno-suppressive effect of MDSCs, increased in airway tissue and decreased in peripheral blood. At the same time, we found a negative correlation between MDSCs and immune checkpoint HHLA2 in airway tissue, with decreased HHLA2 expression but increased HHLA2 expression in peripheral blood. These findings suggest that MDSC may interact with HHLA2 in COPD airway tissue and jointly exert immunosuppressive effects. But the effect in peripheral blood is not clear. MDSCs are a group of immature bone marrow-derived suppressor cells, which are involved in different immune responses in different diseases.

As one of the important immune cells, MDSCs can cooperate with Treg cells to play an immunosuppressive role.[27] MDSCs and Treg cells can promote the amplification of another cell through the production of IL-10 and TGF-β respectively, forming positive feedback regulation and driving immunosuppression.[28] The results of this study suggested that the levels of MDSCs and Treg cells in the peripheral blood of patients with COPD were significantly increased. At

the same time, the pathway of MDSCs amplification activation was significantly enriched in the airway tissue and peripheral blood of COPD patients, including CSF1 and CSF2, which promoted the expression of MDSCs amplification (Figure 6B, C, E, F). These observations suggest that COPD inflammatory states can stimulate MDSCs expansion.

As an important regulatory factor of immune cell function, the immune checkpoint plays an important role in a variety of chronic diseases.[29] Several previous studies have found strong associations between immune checkpoints and MDSCs-mediated immunosuppression, but their effects on COPD have not been clearly described. Therefore, this study focused on the correlation between MDSCs and immune checkpoints in COPD.

Immune checkpoint HHLA2, a member of the B7 family, can regulate T cell function by interacting with TMIGD2.[15,30] It possesses the function of immune monitoring and auxiliary T-cell activation and can play an important role in maintaining the stability of the immune environment. In addition, prior studies have shown that HHLA2 activates T cells by binding to its ligand TMIGD2 and promotes T cell immune response.[31] The results of this study confirmed that the HHLA2 expression in airway tissues was markedly decreased (Figure 5A). There was a significant negative correlation between HHLA2 and MDSCs (Figure 5B). These results indicate that MDSCs increase in airway tissue of COPD patients, which may play an immunosuppressive role by inhibiting T cell proliferation and activation, while HHLA2 expression decreases, which reduces the function of T cell immune response. Therefore, MDSCs and HHLA2 in airway tissue may synergistically play an immunosuppressive role in promoting COPD progression. However, in the peripheral blood, HHLA2 expression was markedly increased at both the protein and transcriptional levels (Figure 6A, 6D). In the same disease, the same molecule shows opposite expression trend in different tissues, which can be attributed to the different roles of MDSCs in different tissues.

To prove the above conjecture, we further investigated the levels of molecules related to the immunosuppressive function of MDSCs in the airway and the peripheral blood. The results showed that the levels of TGF-β1, IL-10, and CYBB, which were related to the immunosuppressive of MDSCs, were significantly increased in airway tissues, but in the peripheral blood, the expression of CYBB was decreased, and there were almost no differences in other immuno-suppressive molecules (Figure 4D). CYBB can play an important role to increase the immunosuppressive activity of MDSCs and exert an immunosuppressive effect, and its insufficient expression may potentially reduce the immunosup-pressive activity of MDSCs by affecting NADPH levels.[32,33] Based on the above results, we speculated that the airway can serve as the "main inflammatory site" of COPD, On the one hand, MDSCs are amplified and activated to produce corresponding immunosuppressive effects; On the other hand, MDSCs promote the proliferation and differentiation of Treg cells, another type of immunosuppressive cells, by producing effector molecules. However, combined with the decreased expression of CYBB in peripheral blood and the increased expression of HHLA2, this result may be due to the fact that when the inflammatory reaction occurs in the airway tissues, the growth factors were produced to stimulate the amplification of MDSCs in bone marrow, and a large number of MDSCs migrate to the airway through peripheral blood[8,34] and are activated under the stimulation of inflammatory factors to play an immunosuppressive role. Whether MDSCs play an immunosuppressive role during migration needs to be further confirmed.

In conclusion, under inflammatory conditions, amplified MDSCs, which are recruited to airway tissues and were activated by a series of inflammatory factors, can significantly inhibit the activation and function of T cells by down-regulating T cell receptors. These events can lead to the suppression of the T cell cycle and block the various immune checkpoints, thus driving and recruiting immunosuppressive Treg cells to attenuate adaptive immunity simultaneously,[35] which can in turn alleviate inflammatory responses. At the same time, because MDSCs can exert inhibitory effects on immune checkpoints, they can further inhibit HHLA2 on the surface of immune cells in COPD, which limits the excessive immune response and thus reduce airway and lung inflammation. However, the continuous increase in the number of MDSCs in COPD might lead to excessive immunosuppression, weaken the immune response, weaken pathogen-specific T-cell response, and reduce the immune function of COPD patients against different pathogens, which may contribute to the persistence of the pathogens in the body, thereby leading to chronic lung infection and frequent deterioration of the disease.[36]

There are still some limitations in our research. First, the bioinformatics results were obtained from the airway tissues of COPD patients and healthy subjects. Since human airway tissues could not be obtained, we could only conduct experimental verification on peripheral blood samples of the included individuals. Second, the number of clinical samples

included in our study are limited, and we need to confirm our conclusions in a larger COPD cohort. Third, we only verified the expression level of the differentially expressed genes in clinical samples but did not explore the potential mechanism of these genes in COPD cell models. This study observed and summarized the special change rules of MDSC and HHLA2 in COPD without discussing the specific mechanism, so we will further refine the mechanism research in future studies.

Conclusion

Our study revealed that MDSCs are elevated in airway tissue and peripheral blood in COPD patients and negatively correlated with immune checkpoint HHLA2 in airway tissue, which may jointly exert immunosuppressive effects through synergistic interaction. At present, there are few related studies in COPD, and this study may provide a new idea for the immunotherapy of COPD. Next, we will further study the related mechanism of synergistic action between MDSCs and HHLA2 through vitro experiments.

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Disclosure

The authors report no conflicts of interest in this work.

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