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Exploring the Regulatory Mechanism of CXCL16 Molecule-Related Antigen Presentation Using lncRNA-mRNA Co-Expression Network Analysis

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Aim: To investigate the regulatory mechanism of CXCL16 molecule-related *Aspergillus fumigatus* (*A.f.*) extract-induced antigen presentation in a mouse asthma model based on the long non-coding RNA (lncRNA) and mRNA expression profile.

Methods: *CXCL16* knockout mice and wild-type mice were administered with *A.f.* extract by intratracheal instillations to induce asthma airway inflammation. High throughput chip sequencing was used to screen for lncRNA and mRNA expression profile differences in lung tissue between the groups. A lncRNA-mRNA co-expression network was constructed through gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Wild-type mice received intraperitoneal injections with CXCL16 neutralizing antibodies, and the bioinformatics and inflammation results were validated using RT-PCR and ELISA.

Results: Compared with wild-type mice, CXCL16 knockout mice showed 120 lncRNA and 388 mRNA upregulated in lung tissue, while 1984 lncRNA and 301 mRNA were downregulated. The constructed lncRNA-mRNA co-expression network included 244 differentially expressed lncRNAs and 49 differentially expressed mRNAs. Among them, the core network's expression of the hub gene Idh1 and the top four lncRNAs was validated in the CXCL16 neutralizing antibody asthma model.

Conclusion: A comprehensive biological analysis of the lncRNA-mRNA co-expression network explored key genes and pathways, providing new insights for understanding their mechanisms and discovering new targets for asthma induced by *A.f.* The four differentially expressed key lncRNAs in the co-expression network (NONMMUT026034, NONMMUT028184, NONMMUT016537, and NONMMUT043155) can serve as intervention targets for CXCL16 molecular regulation of antigen presentation in mice asthma models.

Keywords: Long non-coding RNA, CXCL16, antigen presentation, co-expression network, asthma

Introduction

Asthma is a respiratory inflammatory disease with clinical symptoms including wheezing, difficulty breathing, cough, and chest tightness. The pathological and physiological changes caused by asthma are mainly characterized by airway hyperresponsiveness, excessive secretion of mucus, and chronic airway inflammation. Traditionally, asthma is include Th2 and non-Th2 asthma. Th2 asthma is a type 2 helper T cell (Th2) type airway inflammation with eosinophilia. Non-Th2 asthma mainly includes neutrophilic asthma, the main mechanism is neutrophil abnormalities and IL-7 mediated pathway activation.[1] The coexistence of eosinophilic and neutrophilic airway inflammation is considered to be mixed granulocytic asthma.[2] At present, asthma is considered to be the general name of several diseases with different mechanisms (endotype) and variable clinical manifestations (phenotype).[3,4] Classification of diseases by phenotype alone is not enough to establish sufficient or personalized treatment for patients, because several phenotypes can be superimposed, and the phenotypic characteristics are different.[5] The study of the immunological hypersensitivity endotype behind the asthmatic symptoms is helpful to the individualized treatment and prognosis research.

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Recent reports suggested that allergens such as *Aspergillus fumigatus* caused asthma and that the increase in asthma symptoms in children and adults is related to elevated levels of *Aspergillus*.[6] Moreover, intratracheal instillation of *Aspergillus fumigatus* spores into C57BL/6 mice caused asthma and was accompanied by Th2 and Th17 responses.[7,8] The microenvironment of airway inflammation in asthma comprises immune inflammatory cells, cytokines, chemokines, and other molecules.[9] In *Aspergillus fumigatus*-induced asthma, antigen-presenting cells phagocytosed antigens and process them into peptides, which are then presented to T cells to trigger inflammation, including M2 macrophage polarization, and finally lead to T helper type 2 response.[10] Cytokines and growth factors produced in inflammatory reactions were shown to promote the proliferation of airway smooth muscle cells and fibroblast by initiating transcription factors,[11,12] and extracellular matrix protein deposition promoted airway remodeling in *Aspergillus fumigatus* induced asthma.[13] However, the specific molecular mechanism of antigen presentation in the initial stage of this process is still unclear.

Long non-coding RNAs (lncRNAs) are defined as RNA with transcripts >200 nucleotides that do not encode proteins. Recent reports have shown that lncRNAs play an essential role in the progression of asthma. Increasingly, more lncRNAs are reported to regulate airway inflammation. LncRNA-AK007111 affected airway inflammation in asthma by regulating mast cell function.[14] LncRNAs PTPRE-AS1 and AK085865 regulated asthmatic airway inflammation by modulating macrophage polarization.[15,16] LncRNA lncBAZ2B was related to M2-type macrophage activation.[17] LncTRPM2-AS prevented autophagy-induced apoptosis of macrophages in asthma.[18] Furthermore, lncRNAs were reported to regulate smooth muscle proliferation and airway remodeling. LncRNAs CRNDE and Malat1 promoted the proliferation and migration of airway smooth muscle cells.[19,20] LncRNA TUG1 promoted airway remodeling and mucus production in asthmatic mice.[21] LncRNA LASI was found to be involved in an allergic asthma-associated hyperreactive response of airway epithelial cells.[22] LncRNAs have been widely studied in inflammatory response regulation, airway hyperresponsiveness, and mucus production. However, it is still unclear how lncRNAs regulate the process of antigen presentation.

Previous studies have shown that scavenger receptors (SRs) facilitate the delivery of antigens to the proteasome, leading to antigen processing and presentation, which stimulates adaptive immunity.[23] SRs such as CD36 and LOX-1 are involved in dendritic cell (DC) presentation and affect the inflammatory response.[23,24] Our recent research found that the scavenger receptor SR-PSOX/CXCL16 regulated airway inflammation by modulating dendritic cell antigen presentation (data not published). This study will focus on the mechanism of the lncRNA regulation of CXCL16 molecule-related antigen presentation.

At present, extensive data analysis and precision medicine are widely applied. This study conducted in-depth data mining based on chip sequencing research to provide helpful information for elucidating the pathophysiological mechanisms and identifying effective therapeutic targets for asthma.

Methods

Asthma Model

CXCL16 knockout mice and wild-type C57BL/6 mice 6–8 weeks were used for experiments. After airway anesthesia in mice, *Aspergillus fumigatus* extract was dripped into the airway and administered the other day for 3 weeks, after which lung tissue was collected.[6] The administration process was conducted in the barrier animal room of the Animal Experimental Center of the Beijing Institute of Heart, Lung and Blood and Vessel Disease. All animal experiments complied with the Animal Ethics Committee of the Experimental Animal Center of the Beijing Institute of Heart, Lung and Blood Vessel Disease regulations.

Total RNA Extraction and Chip Expression Profiling Detection

RNA was extracted from mouse lungs. Expression analysis was performed using sample labeling and array hybridization to purify the mRNA, which was then amplified and transcribed into fluorescent cRNA using Affinemetrix's GeneChip ® Mouse Transcriptome Assay 1.0, according to the manufacturer's instructions. The labeled cRNA was purified using a Qiagen RNeasy Mini Kit, and the hybridization solution was applied to the lncRNA chip slides. After incubating for

17 hours, the slide was placed in a hybridization instrument at 65 °C. The hybridization array was washed, fixed, and scanned.

Screening for Differentially Expressed lncRNAs and mRNAs

The initial data were analyzed using the R language and differentially expressed lncRNAs and mRNAs were identified. The Effy package performed data normalization, including converting the raw data and correcting the background. The Limma software package screened differentially expressed lncRNA and mRNA data. The threshold was | fold change | >1.5, and the adjusted p-value was <0.05. Genes that met the standard were considered differentially expressed lncRNA and mRNA. The ggplot2 and heat map packages were used to create volcano and heat maps of differentially expressed lncRNAs and mRNAs, respectively.

GO Enrichment Analysis and KEGG Pathway Enrichment Analysis of Differentially Expressed Genes

The gene ontology (GO) enrichment analysis method was used to determine the function of genes enriched in the co-expression network, including cellular components, biological processes, and molecular functions. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyzed many genetic pathways. The ClusterProfiler software package in R was used for GO enrichment and KEGG pathway analysis. GO enrichment used an adjusted p-value threshold of <0.01, and the KEGG pathway analysis used an adjusted p-value threshold of <0.05.

Construction of the Gene Co-Expression Network

According to the normalized signal intensity of differentially expressed mRNA and lncRNA, the R function cor.test (Hmisc and corrplot) was used to compute Pearson's correlation coefficient of mRNA-lncRNA pairs. The significantly correlated pairs were defined using a correlation value cutoff of 0.96. Subsequently, the co-expression network was visualized using Cytoscape software. In this study, the top five nodes were considered hub genes.

Verification of Hub Genes and Inflammation After Antibody Neutralization of CXCL16

The isotype control antibody and CXCL16 neutralizing antibody were intraperitoneally injected every four days until the end of the modeling, and then intratracheal instillation of *A.f.* was performed. The lungs were collected for hematoxylin-eosin (HE) staining, and RNA extraction, and the hub genes and the top five lncRNAs were verified by RT-PCR. The primers used for real-time PCR are in supplemental table The supernatant of the total bronchoalveolar lavage fluid was used to quantify the level of interleukin-β (IL-1-β) and interleukin-6 (IL-6) cytokines using the ELISA method.

Results

Differentially Expressed lncRNA and mRNA in CXCL16 Knockout and Wild-Type Mice

After airway infusion of *Aspergillus fumigatus* extract into wild-type mice, and *CXCL16* gene knockout mice, lung tissue lncRNA and mRNA chip sequencing analyses were performed. After normalization, 120 lncRNAs were found to be upregulated, 1984 lncRNAs were downregulated, 388 mRNA were upregulated, and 301 mRNA were downregulated. The volcano plots of differentially expressed genes are shown in Figure 1A and B, and cluster heatmaps of differentially expressed genes are shown in Figure 2A and B. The top 10 differentially expressed lncRNAs are listed in Table 1, and the top 10 expressed mRNAs are listed in Table 2.

GO and KEGG Enrichment Analyses of Differentially Expressed Genes

GO functional enrichment analysis and KEGG pathway analysis were performed to determine the potential function of differentially expressed mRNA. The analysis included three categories of results: biological process (BP), cellular components (CC), and molecular function (MF). The analysis showed that the upregulated differentially expressed

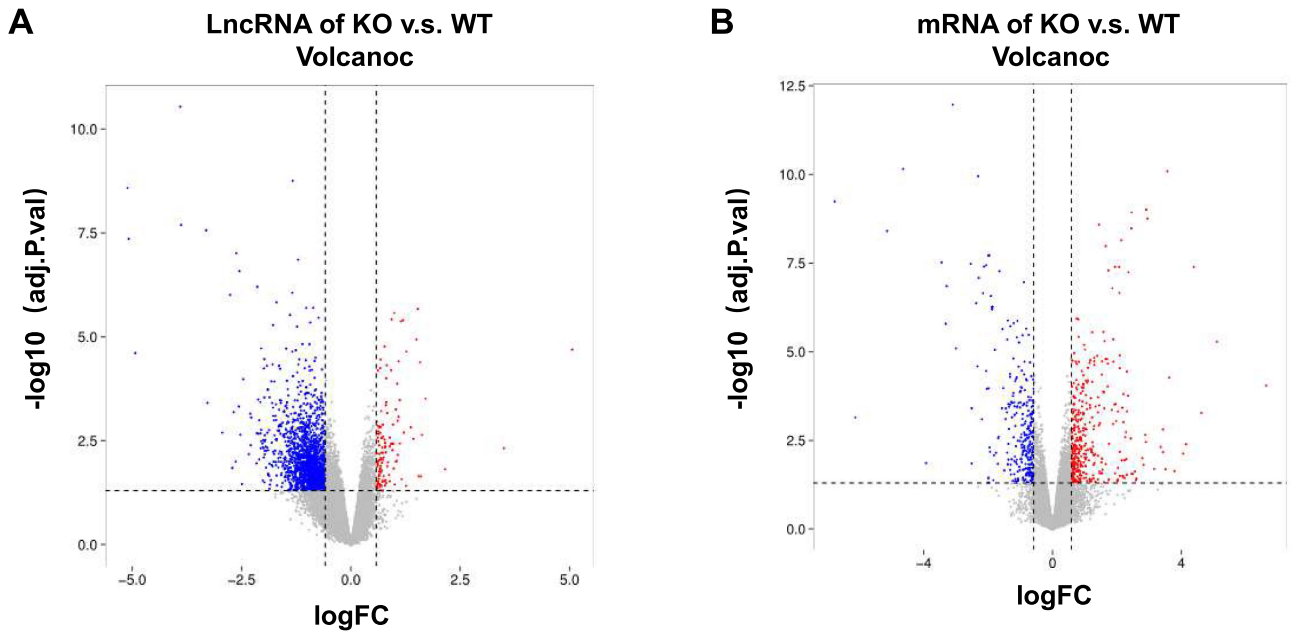

Figure 1 Volcano map of all differentially expressed lncRNA and mRNA between *CXCL16* knockout and wild-type mice. (**A**) Volcano map of differentially expressed lncRNAs. (**B**) Volcano map of differentially expressed mRNA. The blue dots represent differentially expressed genes with a logarithmic fold change of <-1, the red dots represent differentially expressed genes with a logarithmic fold change of >1, and the black dots represent genes with no significant difference in expression with an adjusted p-value of<0.05.

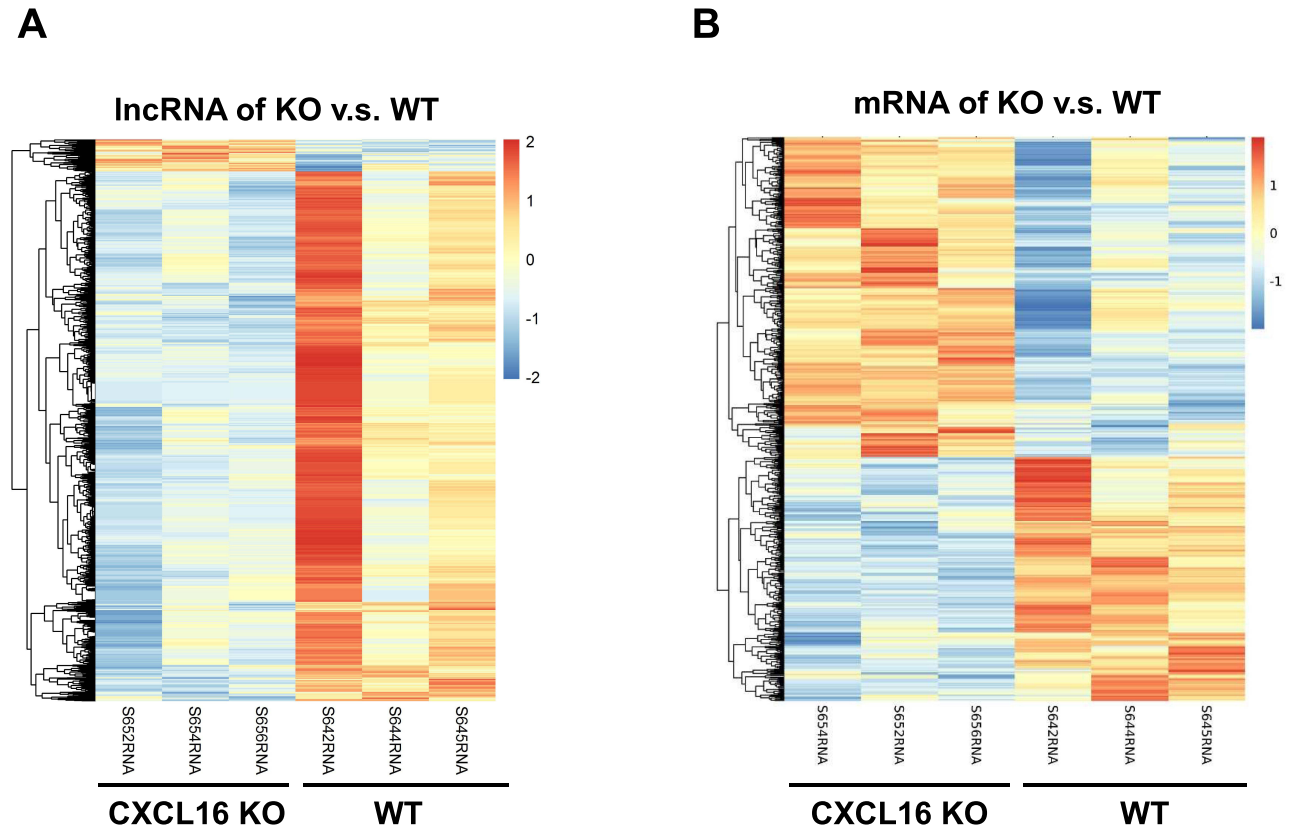

Figure 2 Cluster diagram of lncRNA and mRNA differentially expressed between *CXCL16* knockout mice and wild-type mice. (**A**) Heat map of differentially expressed lncRNAs. (**B**) Heat map of differentially expressed mRNAs. The gradient color from blue to red represents changing from downregulation to upregulation.

Table 1 The Top 10 Upregulated and Downregulated Differential Expression lncRNAs After CXCL16 Knockout

Gene_ID	Gene.Symbol	Adj.P.Val	Regulation	FC
ENSMUST00000103461	Igh-VS107	5.93E-04	Upregulated	33.359
NCBI_Gene:385277:presumedTranscript_1	Igkv4-60	1.54E-02	Upregulated	11.314
ENSMUST00000100690	Ear-ps9	2.53E-02	Upregulated	4.438
ENSMUST00000109187	Hsp25-ps1	3.68E-03	Upregulated	3.272
ENSMUST00000103477	Igh-VS107	1.05E-02	Upregulated	3.095
uc029tia.1	AB339856	3.14E-02	Upregulated	3.031
NONMMUT008692	NONMMUT008692	9.71E-04	Upregulated	2.99
ENSMUST00000100689	Ear-ps2	3.15E-02	Upregulated	2.928
ENSMUST00000132166	Gm21082	1.19E-04	Upregulated	2.888
NONMMUT054205	NONMMUT054205	4.25E-04	Upregulated	2.828
NONMMUT063072	NONMMUT063072	7.53E-07	Downregulated	−34.535
NONMMUT051994	NONMMUT051994	5.21E-06	Downregulated	−33.825
NONMMUT049977	NONMMUT049977	6.73E-04	Downregulated	−30.484
NR_037964	2610507I01Rik	5.04E-08	Downregulated	−14.929
NONMMUT032521	NONMMUT032521	3.50E-06	Downregulated	−14.723
NONMMUT063069	NONMMUT063069	4.46E-06	Downregulated	−9.918
KnowTID_00000908	KnowTID_00000908	4.08E-03	Downregulated	−9.714
NONMMUT063122	NONMMUT063122	1.01E-02	Downregulated	−7.674
KnowTID_00004196	KnowTID_00004196	6.63E-05	Downregulated	−6.774
NONMMUT050718	NONMMUT050718	2.46E-02	Downregulated	−6.543

Table 2 The Top 10 Upregulated and Downregulated Differential Expression mRNA After CXCL16 Knockout

Gene_ID	Gene.Symbol	Adj.P.Val	Regulation	FC
NM_011126	Bpifa1	1.61E-03	Upregulated	99.044
ENSMUST00000103479	Ighv3-5	2.17E-04	Upregulated	34.535
NM_008725	Nppa	4.81E-03	Upregulated	24.761
NM_001081106	Cytl1	5.00E-06	Upregulated	20.966
ENSMUST00000166380	Igkv4-53	1.41E-02	Upregulated	17.753
ENSMUST00000103500	Ighv1-12	1.75E-02	Upregulated	16.679
ENSMUST00000103459	Ighv5-17	3.19E-02	Upregulated	13.833
NM_007606	Car3	1.14E-03	Upregulated	12.295

(*Continued*)

Table 2 (Continued).

Gene_ID	Gene.Symbol	Adj.P.Val	Regulation	FC
NM_145078	2610305D13Rik	7.02E-08	Upregulated	11.876
NM_001012392	Bpifb1	2.98E-02	Upregulated	11.392
ENSMUST00000103326	Igkv1-99	3.28E-07	Downregulated	−109.137
ENSMUST00000103376	Igkv7-33	5.77E-03	Downregulated	−70.035
NM_023158	Cxcl16	9.67E-07	Downregulated	−35.261
NM_198095	Bst2	7.02E-08	Downregulated	−24.933
ENSMUST00000103327	Igkv12-98	2.40E-02	Downregulated	−15.242
NM_001167828	Trim30d	4.71E-06	Downregulated	−10.928
ENSMUST00000079237	Zfp125	9.62E-05	Downregulated	−9.987
NM_001033767	Gm4951	1.30E-05	Downregulated	−9.781
NM_023835	Trim12a	3.70E-09	Downregulated	−8.574
NM_001101475	F830016B08Rik	3.10E-04	Downregulated	−8

genes mainly participated in the top five biological processes: immunoglobulin production, immune response, positive regulation of B cell activation, defense response to bacteria, and the B cell receptor signaling pathway. The top five cellular components were enriched in extracellular space, extracellular region, extracellular exosome, immunoglobulin complex, and circulating and blood microparticles. The top five molecular functions were enriched in antigen binding, protein homodimerization activity, immunoglobulin receptor binding, oxidoreductase activity, and carbohydrate-binding (shown in Figure 3A). The downregulated differentially expressed genes involved in the top five biological processes were cellular response to interferon-beta, cellular response to interferon-gamma, response to virus, lymphocyte chemotaxis and monocyte chemotaxis. The top five cellular components were enriched in the proteinaceous extracellular matrix, external side of the plasma membrane, extracellular matrix, endoplasmic reticulum exit site, and extracellular space. The top five molecular functions were enriched in GTPase activity, chemokine receptor binding, chemokine activity, peptide antigen binding, and T cell receptor binding (shown in Figure 3B).

The top five pathways of upregulated differentially expressed genes in the KEGG enrichment analysis included pattern recognition receptor, valine leucine and isoleucine degradation, lectins, PPAR signaling pathway, and biosynthesis of unsaturated fatty acids (shown in Figure 4A). The top five pathways of downregulated differentially expressed genes in the KEGG enrichment analysis included Epstein-Barr virus infection, human immunodeficiency virus one infection, chemokine signaling pathway, graft-versus-host disease and antigen processing and presentation (shown in Figure 4B).

Construction of the Differential lncRNA-mRNA Co-Expression Network

To clarify the function of differentially expressed lncRNAs and their role in airway inflammation induced by *Aspergillus fumigatus* extract, a gene co-expression network between lncRNA and mRNA was constructed. Then, Pearson correlation coefficients were calculated for the mRNA and lncRNA correlation analysis of all samples, and lncRNA-mRNA pairs with coefficients ≥0.96 were selected. As shown in Figure 5, we found 1674 lncRNA-mRNA interactions, including 942 lncRNAs and 325 mRNAs. Among these interactions were 707 positive lncRNA-mRNA interactions and 967 negative lncRNA-mRNA interactions.

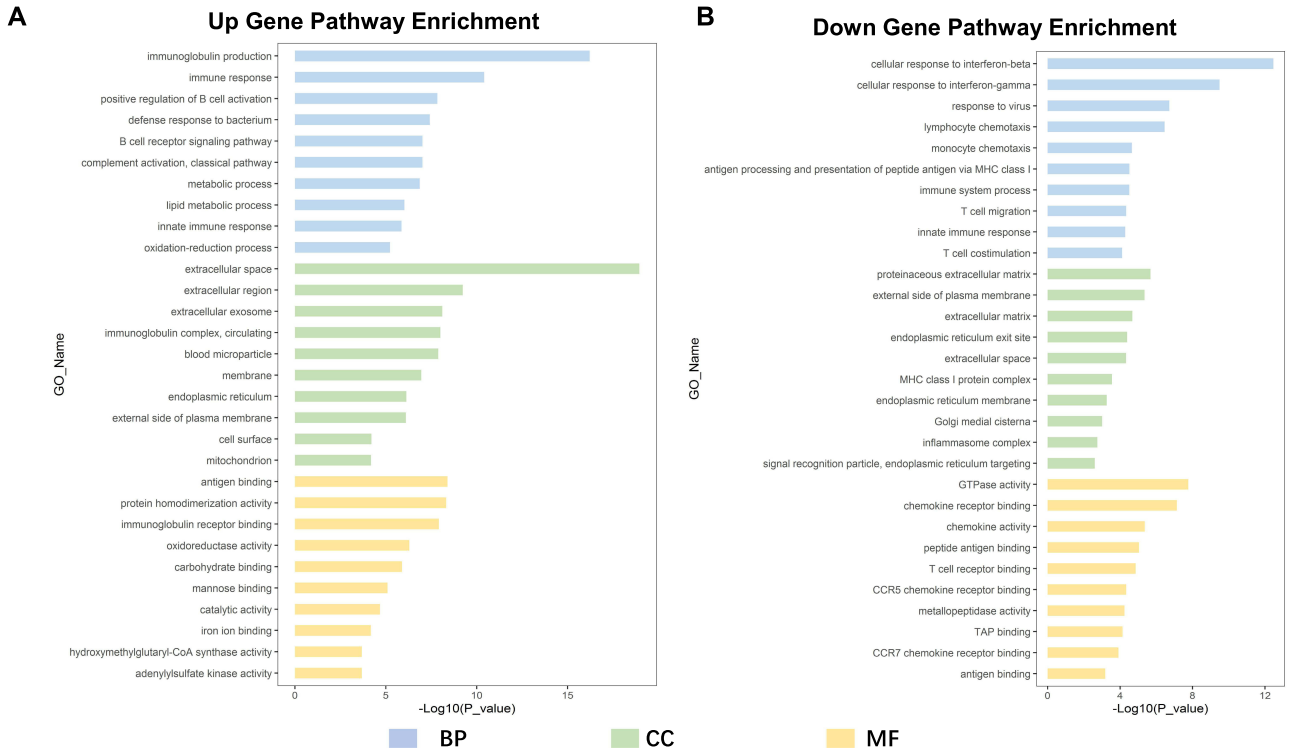

Figure 3 Gene ontology (GO) analysis of differentially expressed genes. GO analysis identified (**A**) the top 10 enriched genes related to the upregulation of gene molecular function (MF), cell composition (CC), and biological process (BP) and (**B**) the top 10 enriched genes related to the downregulation of gene molecular function (MF), cell composition (CC), and biological process (BP).

Screening and Analysis of Core Network

The involvement of upregulated differentially expressed genes in biological processes, molecular functions, and signaling pathways mainly focused on antigen binding, immunoglobulin production, immunoglobulin receptor binding, pattern recognition receptors, and PPAR signaling pathways. The differentially expressed regulated genes in the relevant pathways were deduplicated, and 119 differentially expressed genes were screened. Using these genes as target genes, lncRNA-mRNA co-expression network analysis was performed to clarify the regulatory effects of differentially expressed lncRNAs on antigen binding, pattern recognition receptor expression, and signaling pathway in airway inflammation induced by *Aspergillus fumigatus* extract (shown in Supplemental Figure 1). The co-expression network analysis included 244 differentially expressed lncRNAs and 49 differentially expressed mRNAs. The top five nodes were hub genes *Aldh9a1, CD209a, Idh1, Galm*, and *Igkv4-58*. (Table 3). A list of the top 20 co-expressed genes in the lncRNA-mRNA co-expression network is shown in Table 4.

Two core networks in the upregulation network connected three or more differentially expressed genes. The Core network 1 included 11 differentially expressed genes: aldehyde dehydrogenase 9, A1 subfamily (*Aldh9a1*), CD209 antigen, galactose mutase (*Galm*), 3-hydroxy-3-methylglutaryl CoA synthase 1 (*Hmgcs1*), hydroxysteroid (17 β) Dehydrogenase 4 (*Hsd17b4*), soluble isocitrate dehydrogenase 1 (*Idh1*), mannose receptor, C-type 1 (*Mrc1*), oxidized low-density lipoprotein receptor 1 (*Olr1*), pyruvate dehydrogenase E1 α 1 (*Pdha1*), catalase (*Cat*), and monoamine oxidase B (*Maob*); The Core network 2 included three differentially expressed genes: glutathione transferase zeta 1 (maleic acetyl acetate isomerase) (*Gstz1*), acyl-coenzyme A thioesterase 2 (*Acot2*), and haptoglobin (*Hp*) (shown in Figure 6A and B).

Four hub genes were obtained by intersecting the 14 differentially expressed genes and five hub genes in the core network graph: *Aldh9a1, CD209a, Idh1*, and *Galm* (shown in Figure 6C).

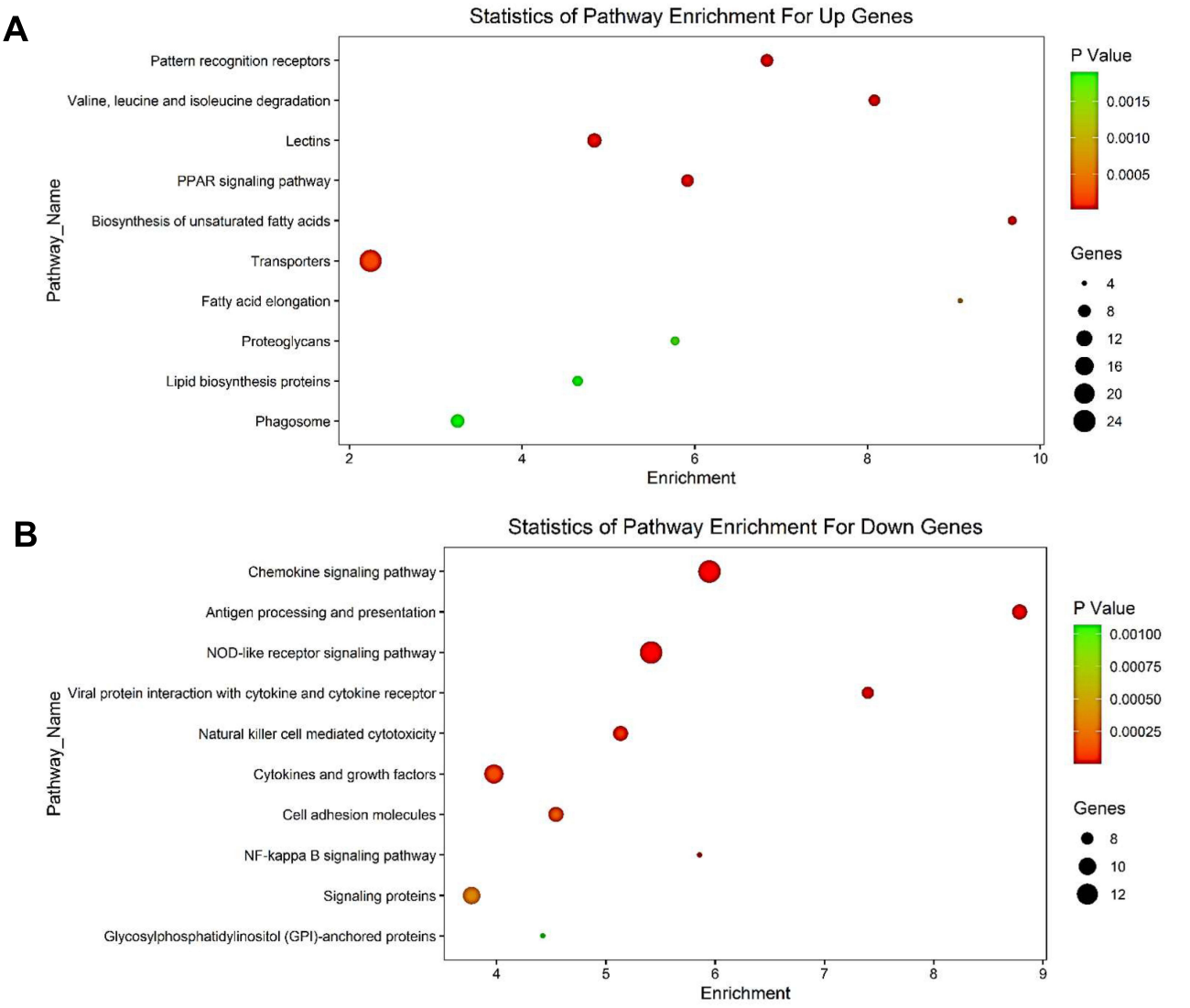

Figure 4 Pathway enrichment analysis of differentially expressed genes by the Kyoto Encyclopedia of Genes and Genomes (KEGG). (**A**) Upregulated differentially expressed genes enrichment in the KEGG pathways. (**B**) KEGG pathways for downregulated differentially expressed gene enrichment.

Verification of the Hub Gene and Inflammation After Neutralization of the CXCL16 Antibody

Wild-type mice were intraperitoneally injected with a CXCL16 neutralizing antibody or isotype control antibody, followed by airway instillation of *A.f.*, and the lung tissue was collected. After RNA extraction, RT-PCR verified the expression of the hub genes *Aldh9a1, CD209a, Idh1*, and *Galm*. As shown in Figure 7A, compared with the wild-type mice treated with isotype control antibody, the expression of *Idh1* was upregulated in the CXCL16 neutralization group, which coincided with the predicted results. The top five lncRNA related to *Idh1* in the lncRNA-mRNA co-expression network were lncRNA NONMMUT026034, NONMMUT028184, NONMMUT016537, NONMMUT043155, and NONMMUT017803. The expression of the relevant lncRNAs was verified by using RT-PCR. The expression of NONMMUT026034, NONMMUT028184, NONMMUT016537, and NONMMUT043155 decreased in the CXCL16 antibody neutralized mice compared with that in the isotype control antibody-treated mice (Figure 7B).

The airway inflammation was evaluated by HE staining. Inflammation cell infiltration of the peribronchial tissue decreased in the CXCL16 neutralizing antibody-treated mice compared with that in the isotype control antibody-treated

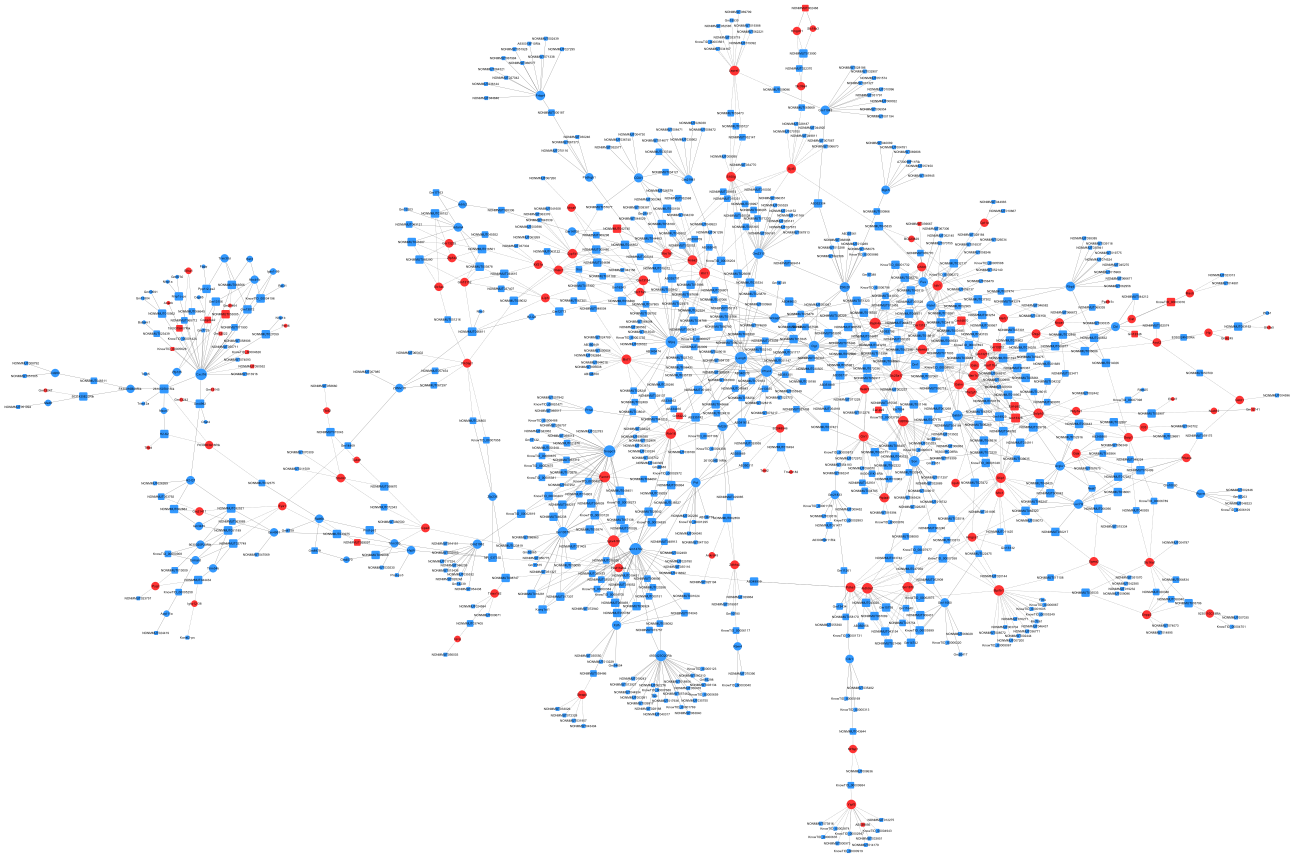

Figure 5 Co-expression network analysis of differentially expressed lncRNA and mRNA genes between *CXCL16* knockout mice and wild-type mice. The square represents lncRNA; the circles represent mRNAs; blue represents downregulation; red represents upregulation; and the line represents the co-expression relationship between lncRNAs and mRNAs.

mice (Figure 8A). The ELISA method was applied for measuring the level of the cytokines IL-1β and IL-6 in the bronchoalveolar lavage fluid. The level of IL-1β and IL-6 in the mice injected with the CXCL16 neutralizing antibody decreased compared with the isotype control antibody-treated wild-type mice (Figure 8B and C).

Discussion

Various scavenger receptors are involved in the pathogenesis of asthma. In this study, a lncRNA-mRNA co-expression network related to the scavenger receptor CXCL16 was constructed, the hub gene *Idh1* gene was screened, and four key differentially expressed lncRNAs (NONMMUT026034, NONMMUT028184, NONMMUT016537 and

Table 3 List of Top 5 hub Genes in lncRNA-mRNA Co-Expression Network After CXCL16 Knockout

GeneSymbol	Style	Biotype	Degree
Igkv4-58	Up	Protein_coding	28
Aldh9a1	Up	Protein_coding	20
Cd209a	Up	Protein_coding	18
Idh1	Up	Protein_coding	16
Galm	Up	Protein_coding	15

Table 4 List of the Top 20 Co-Expressed Genes in the lncRNA-mRNA Co Expression Network After CXCL16 Knockout

LncRNA	mRNA	Relation	P.Value	Regulation
NONMMUT066407	Olr1	−0.999815	5.15E-08	Negative
NONMMUT043152	Olr1	−0.999538	3.207E-07	Negative
NONMMUT067296	Adamts5	−0.999508	3.625E-07	Negative
NONMMUT010963	Olr1	−0.999345	6.427E-07	Negative
NONMMUT035533	Enpp3	−0.999313	7.084E-07	Negative
NONMMUT010667	Cpt1a	−0.999197	9.668E-07	Negative
AB350709	Acaa2	−0.99917	1.034E-06	Negative
AB349653	Acaa2	−0.99917	1.034E-06	Negative
NONMMUT062963	Ighv3-5	−0.999131	1.133E-06	Negative
NONMMUT023313	Maob	−0.999106	1.198E-06	Negative
NONMMUT046582	Msrb1	−0.999051	1.35E-06	Negative
NONMMUT026034	Idh1	−0.999031	1.409E-06	Negative
NONMMUT058318	Cyp1b1	−0.999003	1.49E-06	Negative
NONMMUT068941	Dhrs7	−0.998984	1.548E-06	Negative
KnowTID_00006273	Igkv4-58	−0.998918	1.755E-06	Negative
NONMMUT050709	Gstz1	−0.998891	1.844E-06	Negative
NONMMUT017803	Galm	−0.998851	1.978E-06	Negative
KnowTID_00001731	Pdha1	−0.99883	2.051E-06	Negative
NONMMUT037058	Pdha1	−0.99877	2.268E-06	Negative
NONMMUT000661	Galm	−0.998748	2.35E-06	Negative

NONMMUT043155) were identified in the co-expression network, thus providing targets for the pathogenesis and drug treatment of asthma.

More and more studies are using lncRNA-mRNA network analysis to investigate the pathogenesis of asthma and to identify potential therapeutic targets for asthma.[25] The core genes (CXCL8, FOXO3, JUN, PIK3CA, and G0S2) and their related lncRNAs (NONHSAT115963, AC019050.1, MTCYBP3, KB-67B5.12, and HNRNPA1P12) were identified in healthy individuals and asthma patients using the lncRNA-mRNA co-expression network.[26] By analyzing the data from the Gene Expression Omnibus database of asthma patients, the study determined five critical long non-coding RNAs (MALAT1, MIR17HG, CASC2, MAGI2-AS3, DAPK1-IT1) and identified eight potential new drugs (Tamoxifen, Ruxolitinib, Tretinoin, Quercetin, Dasatinib, Levocarnitine, Niflumic Acid, Glyburide).[27] Network analysis found that LncRNA PTTG3P is highly expressed in the peripheral blood of pediatric asthma patients. Knockdown of PTTG3P in vitro inhibited epithelial-mesenchymal transition (EMT), proliferation, and migration of human bronchial epithelial cells.[28] LncRNA regulates the occurrence and development of chronic airway diseases by targeting mRNA and regulating different cellular processes such as proliferation, apoptosis, inflammation, migration, and EMT, but research on antigen presentation and uptake is limited.[29,30] Our study identified 2104 lncRNAs (120 upregulated and 1984 downregulated) and 689 mRNAs (388 upregulated and 301 downregulated) in asthma lung RNA sequencing. The construction of a co-expression network, followed by GO enrichment and KEGG pathway analyses, indicated that

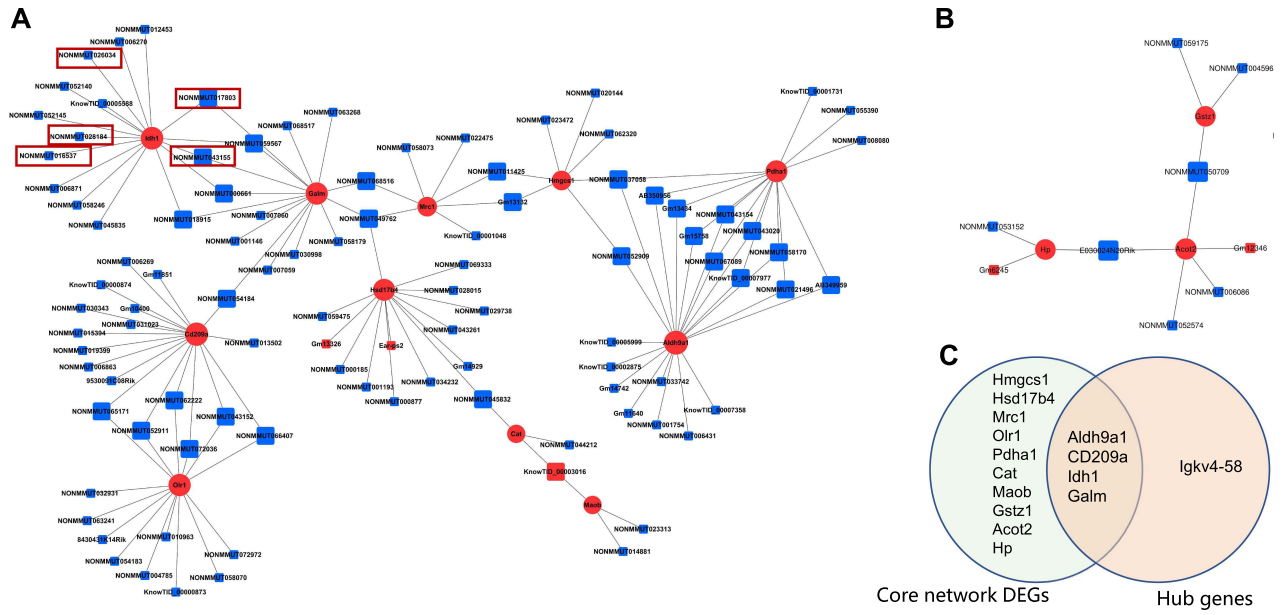

Figure 6 The core co-expression network of lncRNA-mRNA and regulatory hub genes. (**A** and **B**) Network analysis of differentially expressed genes in the core co-expression network. (**C**) Wayne diagram of upregulated core network differentially expressed genes and hub genes.

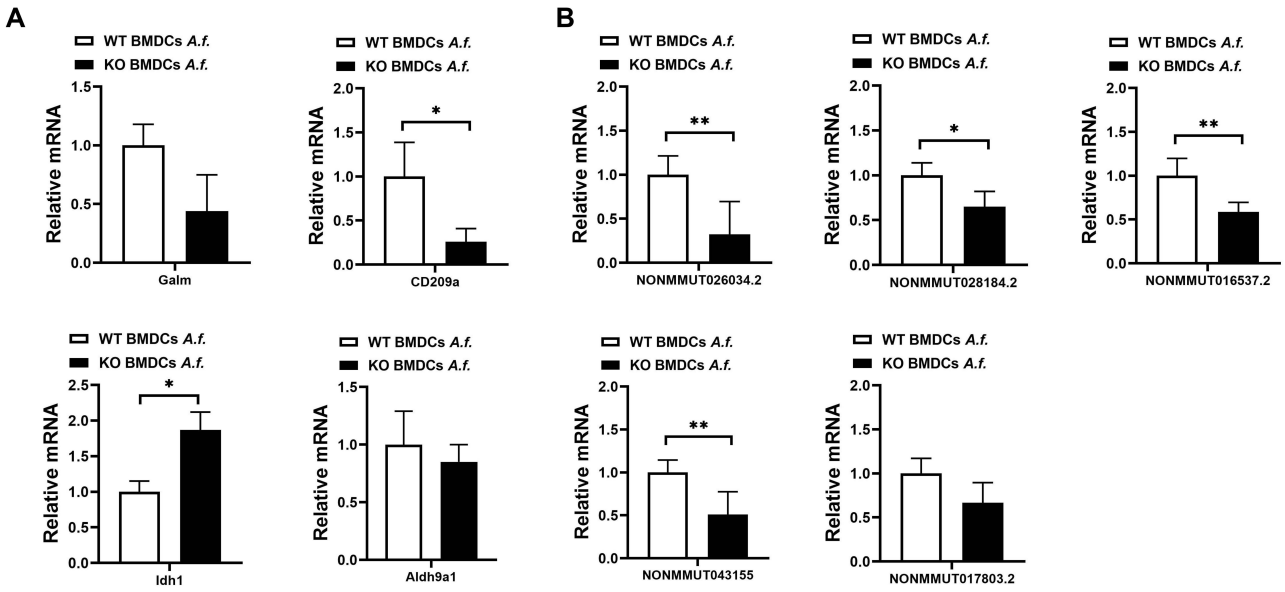

Figure 7 Validation of hub gene expression and lncRNAs after CXCL16 antibody neutralization. (**A**) RT-PCR validation of the relative expression levels of the hub genes *Aldh9a1*, *CD209a*, *Idh1*, *Galm* (n=6). (**B**) RT-PCR validation of the expression levels of lncRNA NONMMUT026034, NONMMUT028184, NONMMUT016537, NONMMUT043155 and NONMMUT017803 (n=6). * P < 0.05, ** P < 0.01 CXCL16 antibody neutralization versus isotype control-treated wild type.

upregulated differentially expressed mRNAs were mainly involved in biological processes and pathways such as immunoglobulin production, immune responses, antigen binding, pattern recognition receptors, and PPAR signaling pathways. Currently, there are still many lncRNAs that have not been annotated. Based on our analysis of the lncRNA-mRNA co-expression network, we identified the following new lncRNAs (NONMMUT026034, NONMMUT028184, NONMMUT016537, and NONMMUT043155) and mRNA (*Idh1*). We found that the mRNA level of (NONMMUT026034, NONMMUT028184, NONMMUT016537, and NONMMUT043155 is decreased, the mRNA level of *Idh1* is increased in asthma group with antibody neutralization.

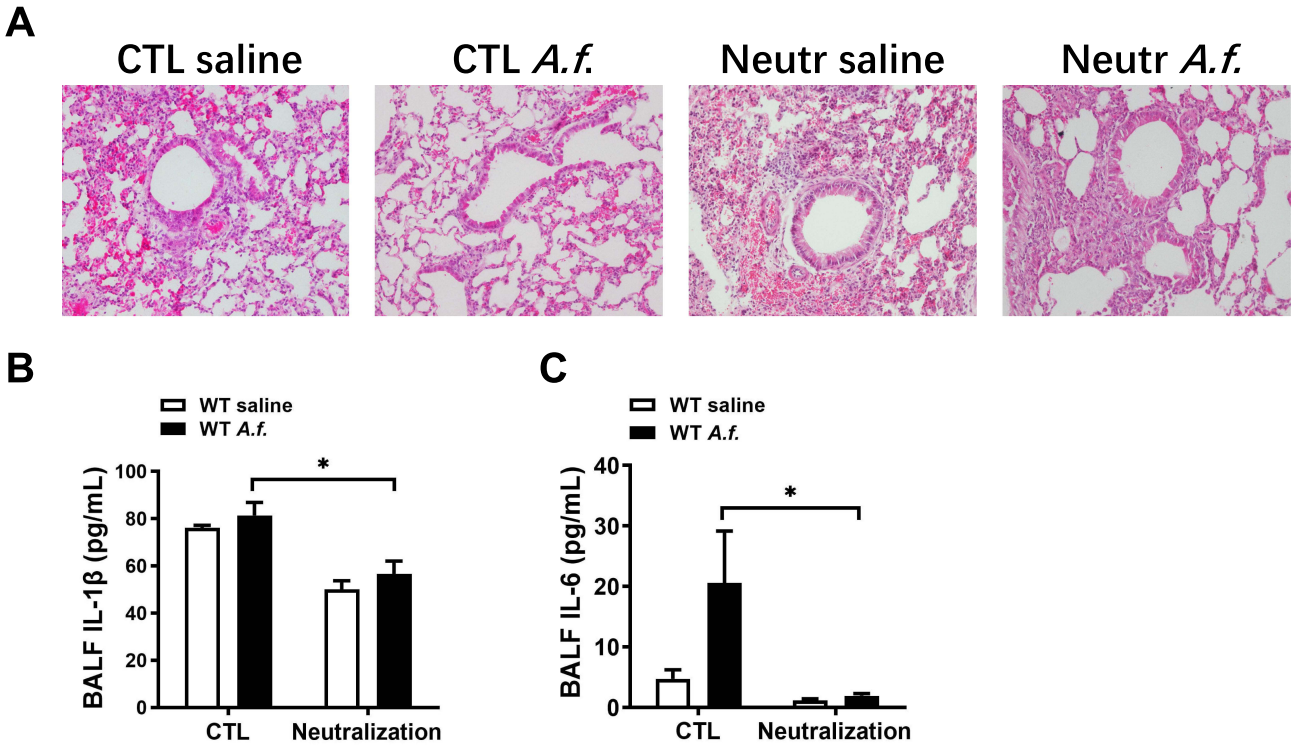

Figure 8 Validation of inflammation after CXCL16 antibody neutralization. (**A**) H&E staining of wild-type mice treated with the isotype control and CXCL16 neutralizing antibodies (×400 magnification; scale bar = 50 μm). ELISA results of (**B**) IL-1β and (**C**) IL-6 levels in bronchoalveolar lavage fluid (n=3). * P < 0.05 CXCL16 antibody neutralization versus isotype control-treated wild type mice. CTL is the isotype control antibody in mice, and Neutr is the neutralizing antibody in mice. Exploring the regulatory mechanism of CXCL16 molecule related antigen presentation using lncRNA-mRNA co-expression network analysis.

In our study, RNA sequencing results and bioinformatics analysis indicated that *Idh1*, as a hub gene, is involved in mediating the process of antigen presentation. Antigen presentation is achieved by processing antigens into peptides and exchanging antigens through MHC II molecules. Isocitrate dehydrogenase 1 is a critical enzyme in cellular metabolism, epigenetic regulation, redox regulation, and DNA repair.[31] It was found that *Idh1*^{R132H} colocalized with MHC class II.[32] *Idh1* gene mutations are associated with the non-inflammatory immune microenvironment by methylation and downregulation of antigen presentation mechanisms.[33] According to several studies, the process of antigen presentation is regulated by ferroptosis and autophagy in patients with *Idh1*^{R132H} mutation. In patients with atherosclerosis, *Idh1* mutations are associated with ferroptosis-related genes, and knockdown *Idh1* inhibited ferroptosis and foam cell formation of macrophages.[34] Patients without the *Idh1*^{R132H} mutation often showed higher xCT levels.[35] *Idh1*^{R132H} mutation and its metabolite 2-HG reduced the protein level of GPX4, which is a crucial enzyme for removing lipid reactive oxygen species and ferroptosis.[36] Patients with *Idh1* mutations had reduced levels of acyl CoA synthase long-chain family member 4 (ACSL4).[37,38] Furthermore, DCs utilize autophagy mechanisms to optimize the processing and presentation of extracellular and intracellular antigens for MHC II presentation.[39,40] Literature reports indicated that *Idh* mutations were also associated with autophagy-related genes. Overexpression of mutant *Idh1*^{R132H} strongly induced oxidative stress and autophagy marker p62 expression in wild-type cells.[41] Knockdown *Idh1* increases the ROS content of cells, induced autophagy activation, and activated MAPK signaling pathways, including ERK, JNK, and p38 signaling pathways.[42] These studies suggest that *Idh1* regulates antigen presentation of DC cells by directly or indirectly regulating autophagy or ferroptosis. Further in vivo and in vitro experiment verification is needed to determine whether *Idh1* directly or indirectly affects the process of antigen presentation.

Other research studies have found that the selected hub genes in this study exhibit polymorphisms or mutated pathogenicity. *Idh1* gene mutations have pathogenicity; its mutations and immune inflammatory response can be used for clinical diagnosis of inflammation-related diseases, such as glioma,[43,44] and can also be used for prognosis of acute precursor B lymphocyte leukemia.[45] In addition, in lung-related diseases, *Idh1* mutations promoted lung cancer

cell proliferation.[46] In the present study, we administered CXCL16-neutralizing antibodies to wild-type mice and validated the critical role of the hub gene *Idh1*, which can serve as an intervention target for antigen presentation. This analysis provides new insights into the potential mechanisms underlying the progression of asthma airway inflammation.

As physicians have recognized for many years, asthma is a heterogeneous lung disease. In recent years, many studies have strengthened the heterogeneous relationship between asthma phenotype and asthma endotype. Although the phenotype focuses on the characteristics of clinical observation, according to different pathophysiological mechanisms, immunophenotypic characteristics, and multi omic studies, such as genomics, transcriptomics, proteomics, and metabolomics, the endotype of asthma has been described in multiple dimensions. The description of these different endotypes has led to the development and testing of new asthma treatment methods for specific immune pathways, enabling doctors to provide personalized drugs for patients with severe asthma.[47] Although the previous research has made a lot of progress, more research is still needed to describe the complex internal type of asthma, identify new practical biomarkers, and develop more non IgE mediated hypersensitivity diagnostic tools[48] for better diagnosis, classification and treatment.[49] This paper is helpful to consider non-IgE-mediated endotypes behind the *Aspergillus* inflammation. The novel findings in the study is also apply to different phenotypes, such as the allergic sinusitis, may benefit from the immunological hypersensitivity endotype.

The new evidence suggests that lncRNA based phenotype-endotype provide a promising therapeutic strategy for asthma.[30] Given the lncRNA target multiple genes and complex biological processes, lncRNA based therapies are considered to have great potential for the complex endotype of asthma.[50] The therapeutic methods based on lncRNA have been limited by several challenges related to the delivery mode, stability, and tissue specificity of lncRNA, from laboratory discovery to clinical application. Since therapeutic agents can enter the lungs via the intracellular pathway, nebulization is an effective strategy to improve drug delivery and minimize side effects. The utility of lncRNA-targeted drugs for diseases related to DC antigen presentation may significantly contribute to personalized medicine. With the assistance of these emerging technologies, targeting downstream proteins (like IDH1) via lncRNA regulation holds significant potential as a crucial method for targeted therapy. It is necessary to explore more lncRNAs that exhibit disease-specific and tissue-specific characteristics.[51] Although further research is required to address the technical challenges that must be overcome for the clinical application of lncRNA, advancements in nanotechnology and RNA analogs are expected to make it possible to develop lncRNA based therapies, develop targeted drugs and more effective intervention in disease progression.

This study explored the antigen-presentation-related lncRNA-mRNA network mechanism of airway inflammation caused by *Aspergillus fumigatus* by analyzing lung tissue using high-throughput chips. In the future, we will explore the biological functions of genes at a deeper level, both in vitro and in vivo, to provide theoretical support for treating *Aspergillus fumigatus*-associated airway inflammation.

Conclusions

We constructed a co-expression network of lncRNA-mRNA related to the scavenger receptor CXCL16 screened for the hub gene *Idh1* related to antigen presentation. We identified four differentially expressed key lncRNAs (NONMMUT026034, NONMMUT028184, NONMMUT016537, and NONMMUT043155) in the co-expression network, providing support for elucidating the pathogenesis of asthma and developing precise drug treatments.

Data Sharing Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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Statement of Ethics

The study was performed in accordance with ARRIVE guidelines. Animal experiments were approved by the Experimental Animal Welfare and Ethics Committee of the Experimental Animal Center of the Beijing Institute of Heart, Lung and Blood Vessel Disease regulations.

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Disclosure

The authors report no conflicts of interest in this work.

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