Am J Transl Res 2023;15(2):1052-1062
www.ajtr.org /ISSN:1943-8141/AJTR0149267

Original Article

Comprehensive analysis of immune-related genes for classification and immune microenvironment of asthma

Bin Feng[1*], Tong Zhou[2*], Zhiyi Guo[2*], Jieyu Jin[1], Sheng Zhang[1], Jun Qiu[1], Jun Cao[1], Jia Li[3], Xia Peng[3], Juan Wang[3], Yanru Xing[4], Renxin Ji[5], Longwei Qiao[6], Yuting Liang[1]

[1]Center for Clinical Laboratory, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China; [2]Medical College of Soochow University, Suzhou 215006, Jiangsu, China; [3]Department of Laboratory Medicine, Shanghai General Hospital, Shanghai JiaoTong University School of Medicine, Shanghai 201209, China; [4]Basecare Medical Device Co., Ltd., Suzhou 215006, China; [5]The School of International Medical Technology of Shanghai Sanda University, Shanghai 201209, China; [6]Center for Reproduction and Genetics, School of Gusu, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Nanjing Medical University, Suzhou 215006, Jiangsu, China. [*]Equal contributors.

Received January 20, 2023; Accepted February 14, 2023; Epub February 15, 2023; Published February 28, 2023

Abstract: Objectives: To determine the effects of immune-related genes (IRGs) and immune landscape of induced sputum, and develop novel, non-invasive diagnostic molecular therapeutic targets for asthma. Methods: GSE76262 datasets were used to identify differentially expressed IRGs in asthma. Key IRGs were detected using a protein-protein interaction network. Receiver operating characteristic (ROC) curves were analyzed to investigate the diagnostic value of key IRGs. Gene set enrichment analysis (GSEA) was performed with WebGestalt. Single-sample gene set enrichment analysis and CIBERSORT were used to investigate the immune landscape of induced sputum. Results: A total of 75 potential IRGs were associated with asthma, most of which were involved in the NF-kappa B signaling pathway. ROC analysis showed AUC values for the hub pathway ranging from 0.676-0.767, with moderate diagnostic value for asthma. We also identified IRGs-related cytokines (TNF-α, IL-1β, IL-8 and IL-6) in 76 asthma and 91 control serum samples to further explore diagnostic efficacy, showing a cumulative AUC of 0.998 for these four related cytokines. Analysis of immune cell infiltration levels showed that follicular helper T cells, activated dendritic cells, activated mast cells and eosinophils were significantly higher and macrophages M0 and macrophages M2 were significantly reduced in sputum from patients with asthma. Conclusions: IRGs-related cytokines and immune infiltration may contribute to the diagnosis and immune classification of asthma.

Keywords: Immune-related genes, asthma, induced sputum, bioinformatics

Introduction

Asthma, a chronic inflammatory disease of the airways that affects both children and adults, is characterized by variable airflow limitation, heterogeneous airway inflammation and remodeling, and clinical symptoms [1, 2]. Asthma diagnosis relies on characteristic symptoms and variable expiratory airflow limitation [3]. Despite this, significant misdiagnosis still occurs due to the heterogeneity of this clinical syndrome [4]. Misdiagnosis of other diseases as asthma may lead to unnecessary side effects and costs due to improper use of asthma treatments [5]. Failure to diagnose asthma can also lead to poor disease prognosis and high mortality [6, 7]. Therefore, development of novel asthma biomarkers would improve diagnostic accuracy, prognosis and effective use of healthcare resources.

The immune microenvironment and immune-related genes (IRGs) have been implicated in asthma progression. In allergic asthma, allergens can stimulate allergic-specific T helper 2 (Th2) cells to produce type 2 cytokines, including IL-4, IL-5 and IL-13, which can cause the accumulation of a large number of eosinophils in the airway wall, excessive production of mucus and synthesis of immunoglobulin E (IgE) by allergen specific B cells, which can be detected in serum [8]. Moreover, airway remodeling is

closely related to chronic airway inflammation, characterized by immune cell infiltration and activation, such as mast cells, eosinophils, dendritic cells (DCs), and innate lymphoid cells (ILCs) [8, 9]. Although recent studies have also shown that autophagy, carbocisteine and long noncoding RNAs play important roles in asthma development by promoting airway remodeling and decreasing the lung function [10-12], many other key genes remain to be identified to further elucidate the pathophysiology of asthma. Therapeutically targeting the immune-inflammatory responses has significantly changed the treatment of patients with uncontrolled asthma [13]. However, the molecular characteristics describing IRGs interactions and immune cell infiltration in asthma have yet to be fully explained.

Herein, we explored differentially expressed IRGs in patients with asthma, to identify potential diagnostic biomarkers, by analyzing the microarray datasets for GSE76262. We also tested differential gene-related cytokines in 76 asthma and 91 control samples to further confirm its diagnostic efficacy for asthma. Moreover, the CIBERSORT and single-sample gene set enrichment analysis (ssGSEA) algorithm were utilized to investigate the infiltrative immune cells in induced sputum samples. The immune-related gene signature may both improve asthma diagnosis and further explain its pathogenic mechanism.

Materials and methods

Microarray data

Asthma clinical information and induced sputum gene expression dataset were obtained from GEO (https://www-ncbi-nlm-nih-gov.ucc.idm.oclc.org/geo/), and GSE76262 was downloaded for further analyses. Genes were selected using the R (version 4.1.1) "limma" package [14, 15] with criteria: adjusted $p < 0.05$ and $|logFC| > 0.58$. We also obtained IRGs dataset of 1,793 genes from ImmPort Portal database, which we intersected with GSE76262 to identify differentially expressed IRGs. The online tool Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to generate a Venn diagram of overlapping genes between differentially expressed genes (DEGs) and the IRGs, and a heatmap of overlapped genes was drawn using the R heatmap package.

Protein-protein interaction network and IRGs correlation analysis

Protein-protein interaction (PPI) networks of differentially expressed IRGs were predicted using STRING (http://string-db.org) and Cytoscape software (version 3.9.1) [16]. Correlation analysis of the differentially expressed IRGs was performed using Spearman correlation in the R corrplot package.

Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis using gene set enrichment analysis

Gene set enrichment analysis (GSEA) can be used to study all genes to test for statistically concordant differences between two groups. Genes with logFC were imported into the WebGestalt database for GSEA analysis [17] (http://www.webgestalt.org/) with a false discovery rate (FDR) ≤ 0.05. KEGG enrichment analyses were performed on the GSEA using WebGestalt.

Receiver operating characteristic analysis

Receiver operating characteristic (ROC) curves were used to diagnose the sensitivity and specificity of asthma, and the diagnostic efficiency of related molecules could be evaluated according to the area under the curve (AUC), reflecting the value of that molecule for asthma. Cytokine assays that have been carried out in the clinic include TNF-α, IL-1β, IL-8 and IL-6, which are closely related to asthma-related IRGs (TNFAIP3, IL1R1, IL8 and IL6R). We also assessed differential gene-related cytokines in the clinical samples to explore their diagnostic efficacy in asthma.

Clinical sample collection and cytokine test

Subjects with stable asthma were recruited from the hospital. Asthma was diagnosed using the criteria: symptoms of wheezing, cough and dyspnea; histamine that provokes a 20% fall in forced expiratory volume in the first second (FEV1) < 8 mg and/or ≥ 12% increase in FEV1 after inhalation of 200 μg salbutamol.

Healthy controls without respiratory or inflammatory illness were also recruited. The medical Ethics Committee of the First Affiliated Hospital of Soochow University approved this study (ID: 2022352). Supernatants of the non-radioactive cultures were analyzed by flow cytometry in a FACS Canto™ II (BD Biosciences, Heidelberg, Germany) using a cytometric bead array (BD Biosciences, Heidel-berg, Germany) for the presence of TNF-α, IL-1β, IL-8 and IL-6. Differences in asthma-related biomarker expressions between clinical and control samples were analyzed with Student's *t*-test.

Immune infiltration analysis

The ssGSEA score was calculated to explore immune infiltration levels in samples from the GSE76262 datasets; this was calculated according to expression levels of immune cell-specific marker genes [18]. In the immune cell-related gene set, the enrichment scores of the genes represents the number of infiltrating immune cells [19]. The marker genes for 23 types of immune cells were descripted in the Supplementary Table 1. The ssGSEA was analyzed using the R (version 4.1.1).

The proportion of 22 immune cells in the induced sputum samples from the GSE76262 datasets were also calculated by CIBERSORT algorithm [20]. We then compared immune infiltration levels between the asthma and control samples.

Statistical analysis

Data are described as mean ± standard deviation (SD). R software (version 4.1.1) was used for all statistical analyses and the $P < 0.05$ was considered statistically significant.

Results

Identification of immune-related genes in asthma

Downloading the gene sets of induced sputum from 21 healthy controls and 118 patients with asthma revealed 848 DEGs, including 256 upregulated and 569 downregulated genes that met criteria (i.e., $|log2FC| > 0.58$, adjusted $p < 0.05$) (**Figure 1A**). A Venn diagram was constructed to show the DEGs that overlapped with IRGs (**Figure 1B**). A total of 75 asthma-related IRGs were identified for subsequent evaluation; a gene expression heatmap was shown in **Figure 1C**. The top 10 upregulated genes were: IL18R1, VEGFA, IL18RAP, IL1R2, CCL22, TNFAIP3, IL1R1, MMP12, IL2RA and CCR7. The top 10 downregulated genes were: FAM3B, TLR7, CXCR7, COLEC12, MARCO, S100A13, RBP4, TGFBR2, IREB2 and PDGFC.

PPI network of asthma-related IRGs

PPI analysis were performed to identify the interactions among asthma related IRGs. The results showed that 75 asthma-related IRGs were found to interact (**Figure 2A**), including a significant number of major genes (**Figure 2B**): CD4, TLR2, VEGFA, NFKBIA, IL2RA, IL1R1 and IL6R. There IRGs may thus be key diagnostic biomarkers of asthma.

Pathway enrichment analyses using WebGestalt

Pathway enrichment analyses of the whole gene as a rank gene list based on log2FC were performed using WebGestalt (http://www.webgestalt.org/). NF-kappa B, TNF, JAK-STAT and IL-17 signaling pathways were activated in the gene sets, while citrate cycle and oxidative phosphorylation were inhibited (**Figure 3A-D**). Notably, NF-kappa B signaling pathway was the major functional pathway involved. The main IRGs involved in the pathway were: TNFAIP3, IL1R1, PLAU, NFKBIA, NFKB1, and ICAM1.

Correlation analysis of asthma-related IRGs involved in NF-kappa B pathway

To explore the dynamic regulation mechanism of NF-kappa B pathway and IRGs characteristic of asthma progression, correlation analysis of the core genes was performed. As previous studies have shown that IL8 and IL6R play essential roles in asthma pathogenesis [21-23], we also included these genes in the correlation analysis. The results showed that 8 IRGs were positively correlated (**Figure 4A**), suggesting these genes together promote asthma development. Differentially expression and PPI analyses showed that IL1R1 is significantly increased in patients with asthma and that its dysfunction may accelerate asthma development. Our investigation also revealed that

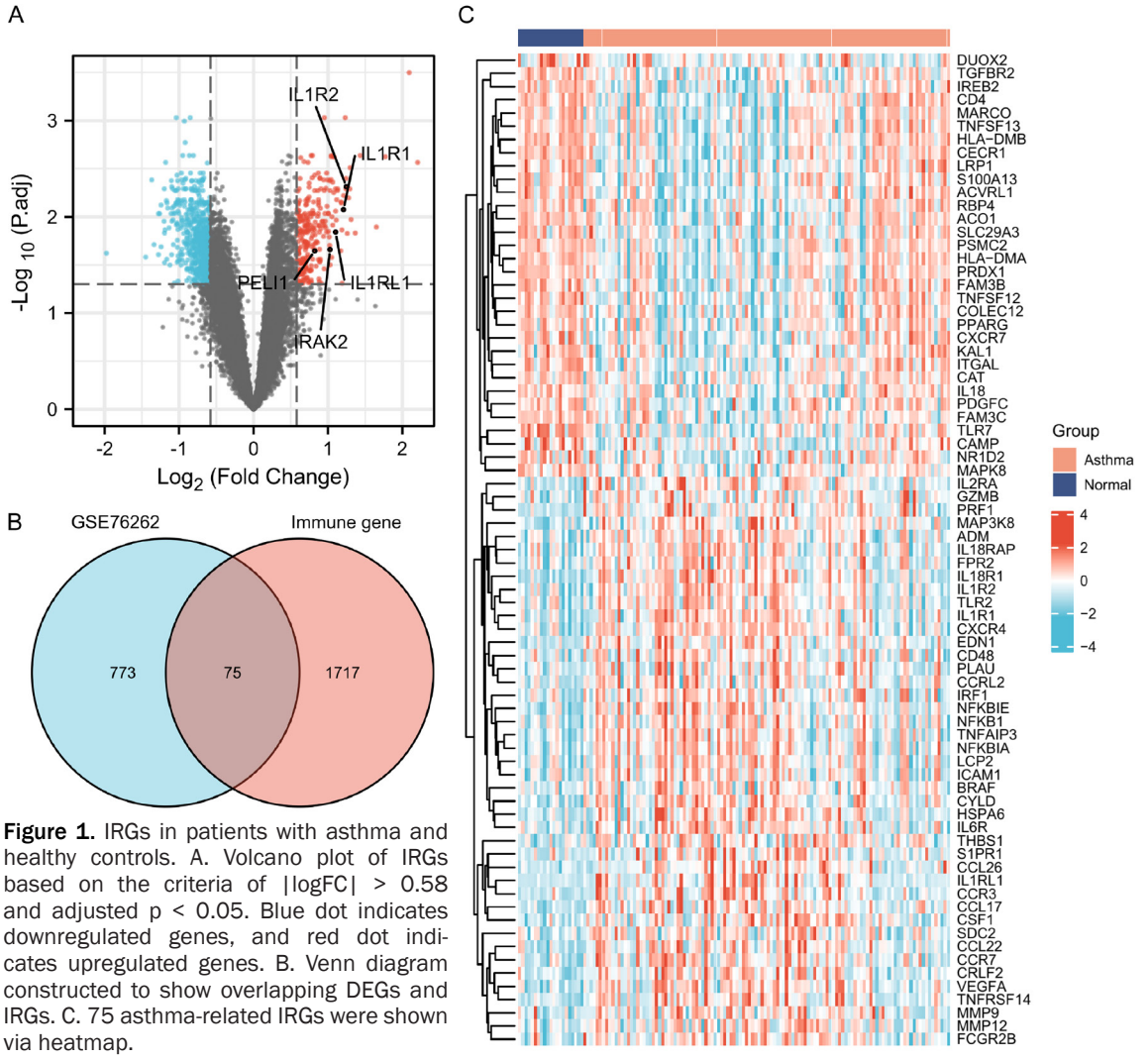

Figure 1. IRGs in patients with asthma and healthy controls. A. Volcano plot of IRGs based on the criteria of |logFC| > 0.58 and adjusted p < 0.05. Blue dot indicates downregulated genes, and red dot indicates upregulated genes. B. Venn diagram constructed to show overlapping DEGs and IRGs. C. 75 asthma-related IRGs were shown via heatmap.

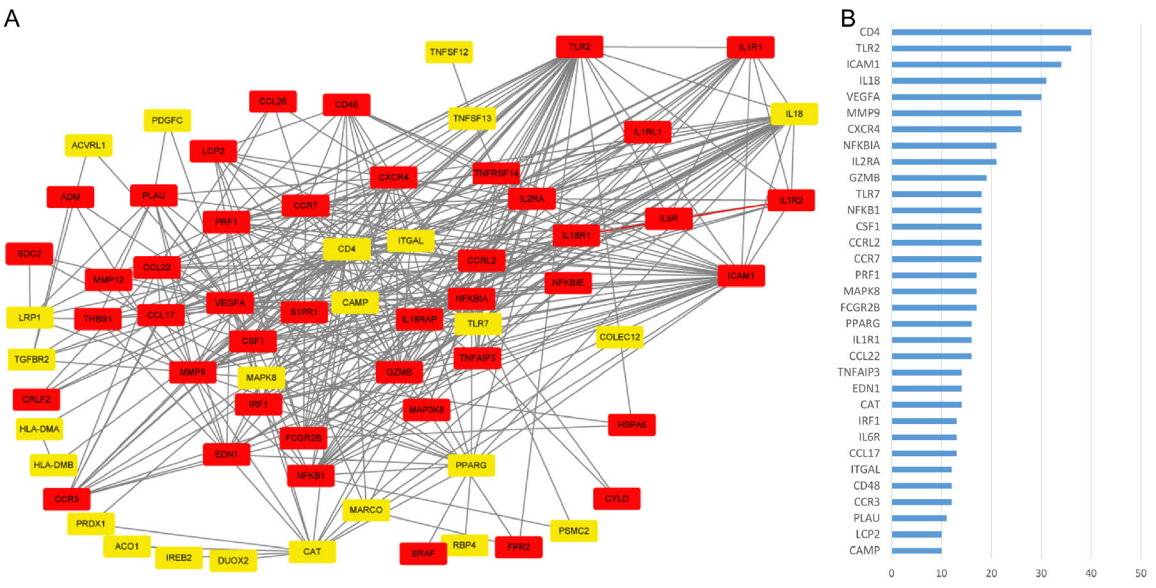

Figure 2. PPI network of differentially expressed IRGs. A. PPI network among IRGs-related DEGs. B. Number of interactions for each IRGs-related DEG.

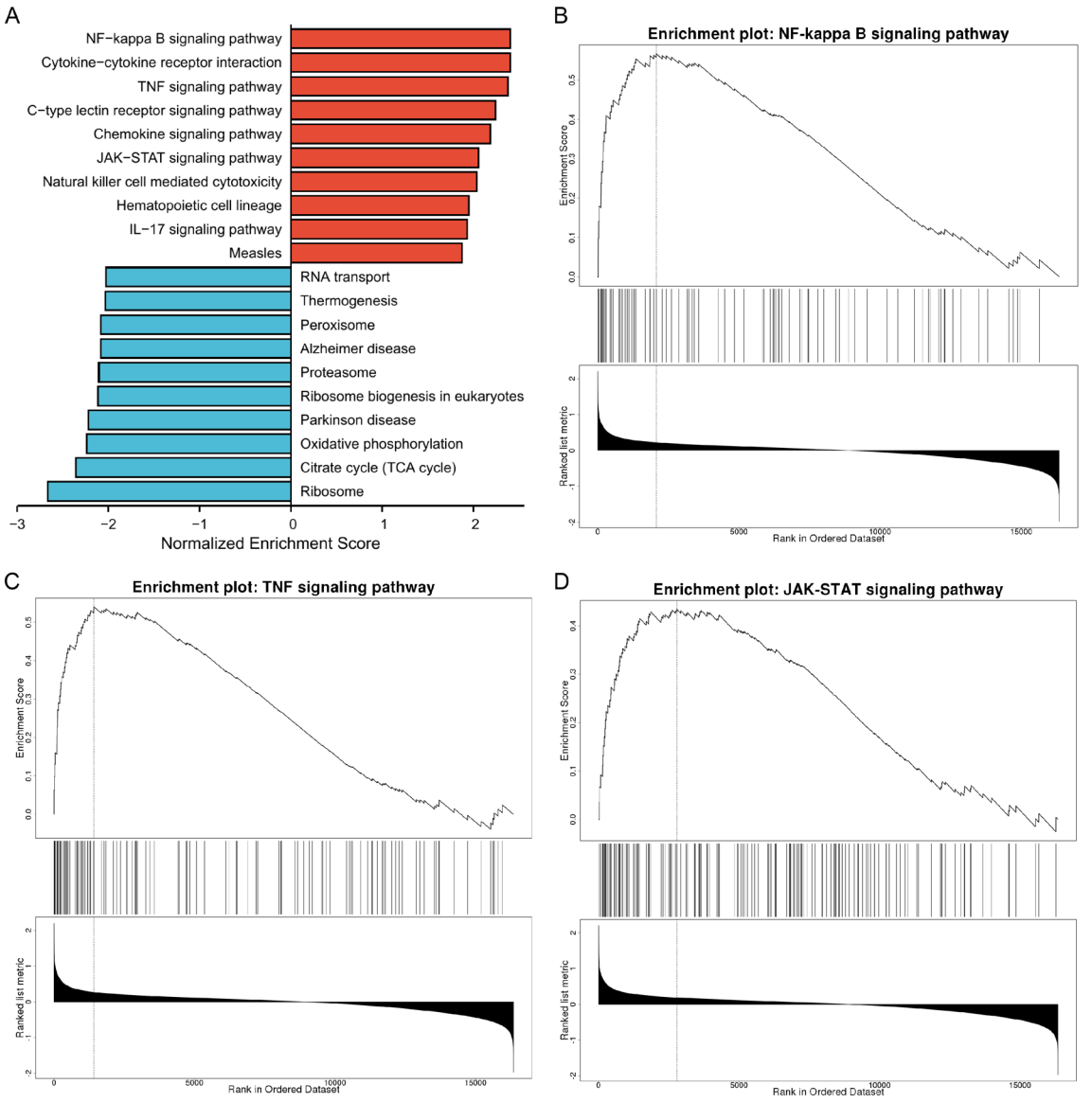

Figure 3. Gene set enrichment analysis of WebGestalt. A. Pathway enrichment analysis showed that NF-kappa B, TNF, JAK-STAT and IL-17 signaling pathway were activated in the gene sets, whereas citrate cycle and oxidative phosphorylation were inhibited. B. NF-kappa B signaling pathway was activated. C. TNF signaling pathway was activated. D. IL-17 signaling pathway was activated.

TNFAIP3, PLAU, NFKBIA, NFKB1, ICAM1, IL8 and IL6R were significantly, positively correlated with IL1R1 expression levels (**Figure 4B-H**).

Diagnostic value of asthma related IRGs

To explore the diagnostic value of 8 asthma-related IRGs above, ROC curves were drawn; the range of AUCs for these hub genes was 0.676-0.767 (**Figure 5A**). The cytokine tested clinically include TNF-α, IL-1β, IL-8 and IL-6, which are closely related to 4 asthma-related IRGs (TNFAIP3, IL1R1, IL8 and IL6R, respectively). We evaluated that the collective ROC of the combined diagnosis of these 4 genes was 0.802 (**Figure 5B**). We also tested the differential gene-related cytokines in samples from 76 patients with asthma and 91 controls to further confirm diagnostic efficacy. These results showed that TNF-α, IL-1β, IL-8 and IL-6 were significantly upregulated in the samples from patients with asthma (**Figure 5C**), with AUCs ranging from 0.518-0.997 (**Figure 5D**). The

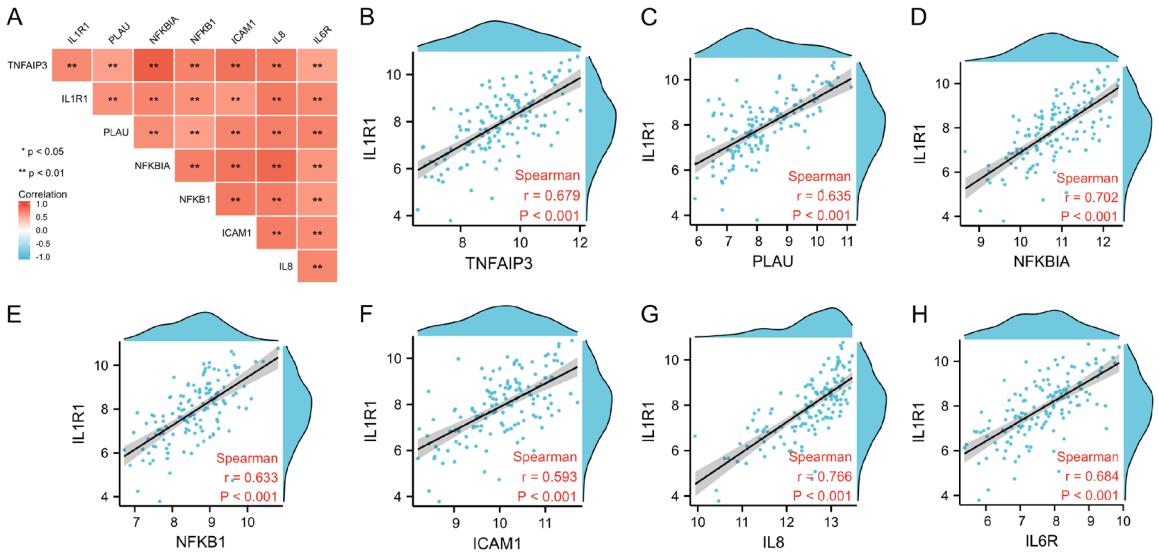

Figure 4. Spearman correlation analysis of the IRGs-related DEG. A. Correlation analysis of asthma-related IRGs involved in the NF-kappa B pathway, IL8 and IL6R. B-H. TNFAIP3, PLAU, NFKBIA, NFKB1, ICAM1, IL8 and IL6R were significantly positively correlated with IL1R1 expression levels.

cumulative AUC for these 4 related cytokines was 0.998 (**Figure 5E**).

Immune cell subtypes differences between asthma and control groups

Applying the ssGSEA algorithm identified 23 immune cell subtypes. As shown in **Figure 6A**, the infiltration levels of activated B cell, activated CD4 T cell, CD56 dim natural killer cell, eosinophil, gamma delta T cell, MDSC, macrophage, mast cell, monocyte, regulatory T cell and type 17 T helper cell were clearly higher in the asthma group. Furthermore, to validate the infiltrating immune cells in the ssGSEA algorithm, CIBERSORT analysis was performed. As shown in **Figure 6B**, CD8 T cells, follicular helper T cells, activated DCs, activated mast cells and eosinophils were significantly higher in the asthma group, whereas macrophages M0 and macrophages M2 were significantly reduced in the asthma group. We also found that IL1R1 expression was positively correlated with the infiltration levels of mast cells and eosinophil (a master immune cell subtype essential for asthma development) and negatively correlated with macrophages M2, indicating that cytokine and immune cells together promote chronic airway inflammation and participate in asthma progression (**Figure 6C-E**).

Discussion

Herein, we identified the essential genes involved in IRGs and further explored their value for asthma diagnosis and the related potential pathogenic mechanisms. These analyses were performed with 75 potential asthma-related IRGs from the intersection of the GSE76262 and ImmPort databases. Pathway enrichment analysis showed that the NF-kappa B signaling pathway was the major functional pathway involved. We then performed a comprehensive correlation analysis of asthma-related IRGs involved in the NF-kappa B pathway and other essential asthma-related IRGs (IL8, IL6R), exploring their values for diagnostic potential. The cumulative AUC for the combined diagnostic accuracy among these 4 genes (TNFAIP3, IL1R1, IL8 and IL6R) was 0.802. We also tested genes-related cytokines used for routine clinical detection samples from 76 patients with asthma and 91 healthy controls to further confirm their potential clinical diagnostic efficacy. Furthermore, we analyzed the infiltration levels of immune cell subtypes to identify whether the key IRGs work together to promote asthma progression. This study may provide new insight into the pathogenic mechanisms of asthma and may improve the asthma diagnosis procedures.

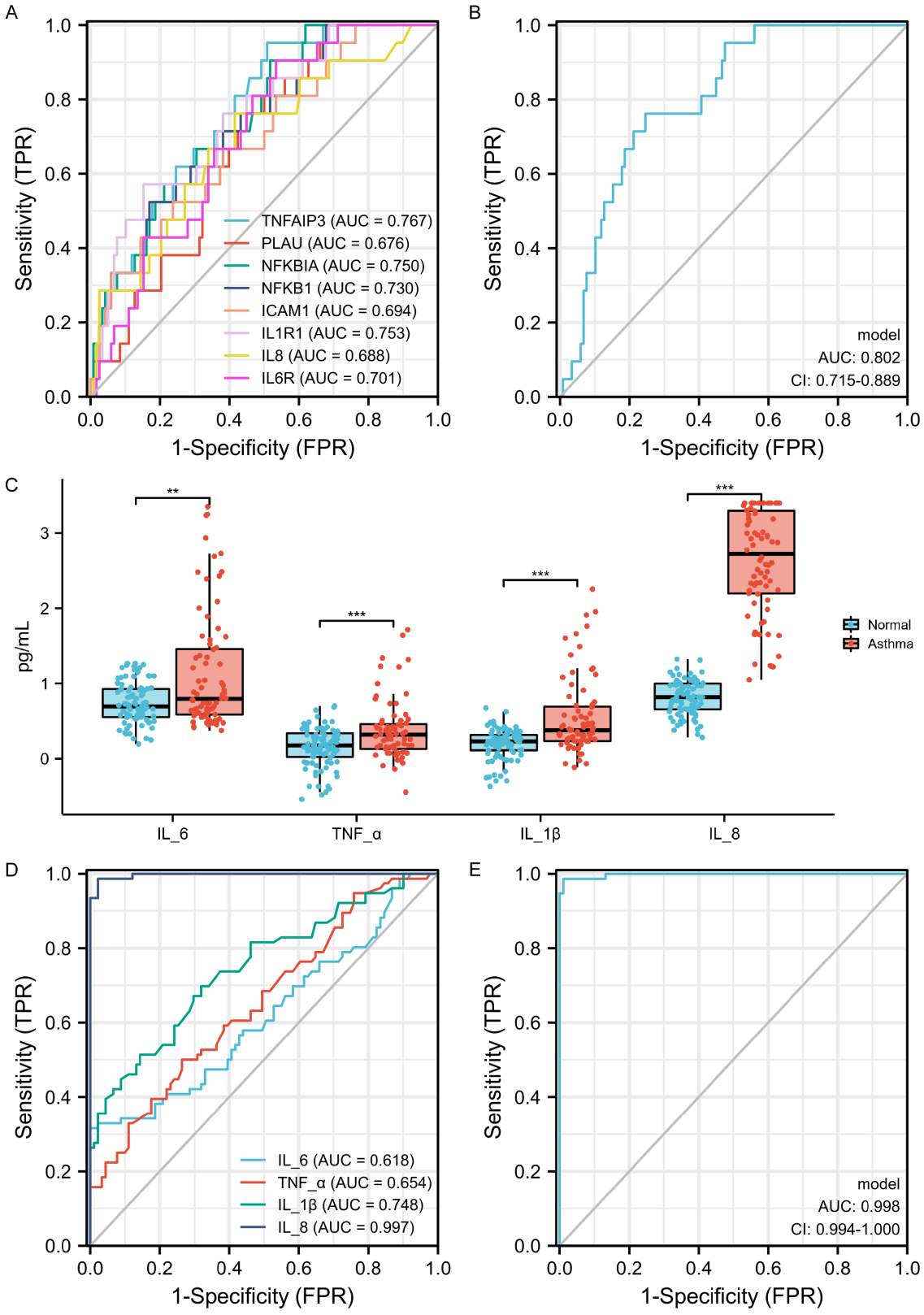

Figure 5. Diagnostic value of asthma-related IRGs. A. ROC curves were plotted, with AUCs for hub genes ranging from 0.676-0.767. B. The cumulative AUC of the combined diagnostic accuracy for 4 genes (TNFAIP3, IL1R1, IL8 and IL6R) was 0.802. C. TNF-α, IL-1β, IL-8 and IL-6 were significantly upregulated in samples from patients with asthma. D. The AUCs of these cytokine (TNF-α, IL-1β, IL-8 and IL-6) ranged from 0.518-0.997. E. Cumulative AUCs of these 4 related cytokines (TNF-α, IL-1β, IL-8 and IL-6) was 0.998.

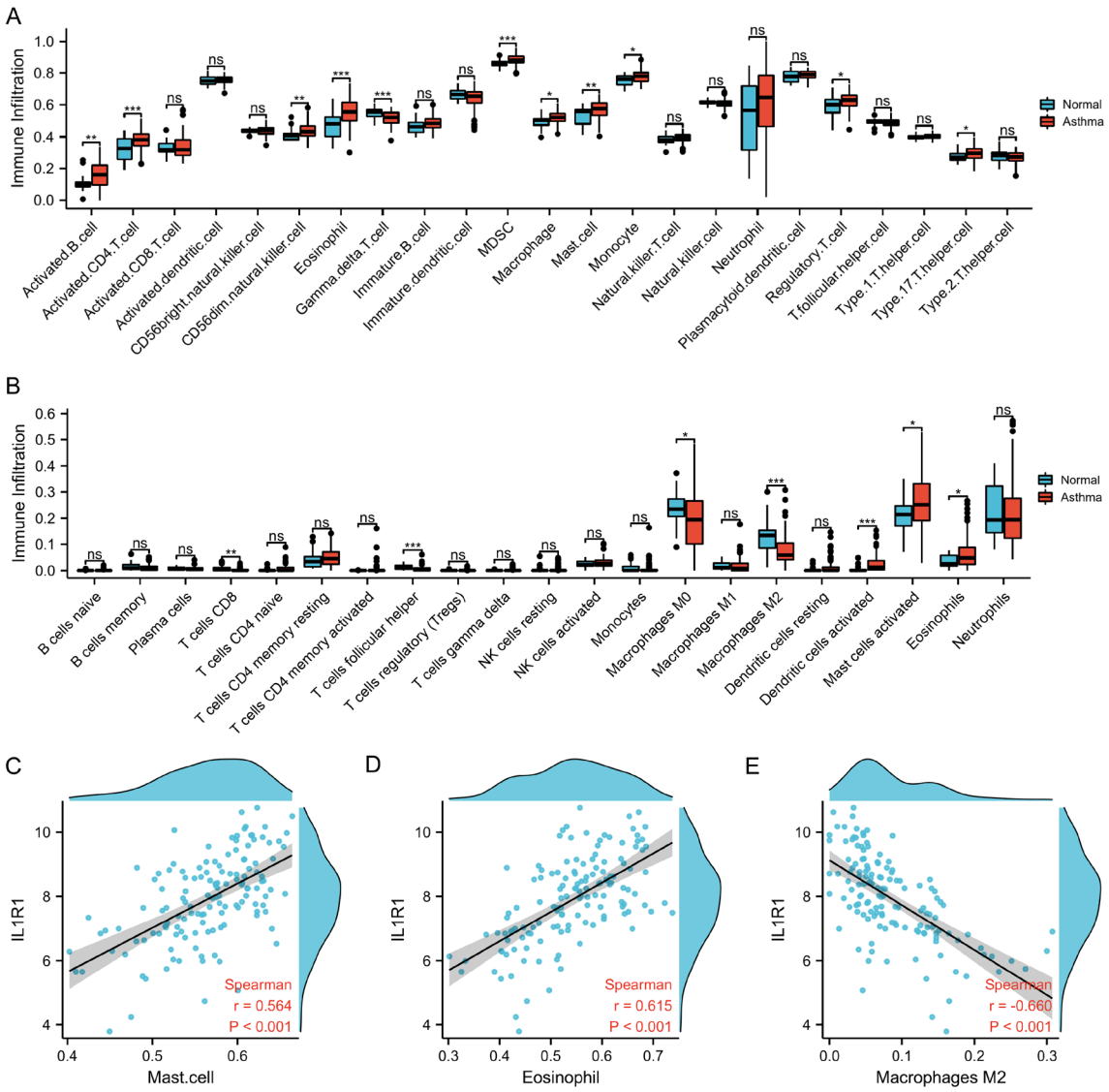

Figure 6. Immune cell subtypes differences between asthma and control groups. A. Differences in immune infiltration levels between asthma and control groups by ssGSEA algorithm analysis. B. Distribution of the immune cells by CIBERSORT analysis. C-E. IL1R1 expression was positively correlated with infiltration levels of mast cells and eosinophil, and negatively correlated with macrophages M2.

The NF-kappa B signaling pathway is a crucial mediator of asthma inflammation, and inhibition of this pathway by targeted molecules can attenuate airway inflammation [24]. In allergic asthma, patients with high group 2 ILC2s can show activation of eosinophils, mast cells, fibroblasts and epithelial cells through cytokines to initiate and amplify airway inflammation, requiring NF-kappa B signaling to fully activate and stably produce type 2 cytokines [25]. In this study, six IRGs were involved in this pathway; namely, TNFAIP3, IL1R1, PLAU, NFKBIA, NFKB1, and ICAM1 were upregulated in the asthma samples. It has been reported that the activation of IL-1 signaling, including IL1R1 in sputum, is associated with airway inflammasome activation and asthma exacerbation [26]. PLAU, the uPA-encoding gene, interacts with its receptor and play essential roles in inflammatory cell activation, cell adhesion and extracellular matrix degradation in the asthma [27, 28]. These cumulative findings suggest that the six IRGs involved in NF-kappa B signaling pathway participate in asthma progression via different mechanisms related to allergic inflammation and barrier dysfunction.

We also assessed the diagnostic value of the NF-kappa B pathway IRG-characteristic genes and other asthma-related IRGs (IL8, IL6R). As expected, all of these IRGs had moderate diagnostic value in patients with asthma based on analysis of the GSE76262 gene expression datasets, while the diagnostic value of IL8 was relatively lower. Routine clinical cytokine assays for TNF-α, IL-1β, IL-8 and IL-6 are closely related to 4 asthma-related IRGs (TNFAIP3, IL1R1, IL8 and IL6R, respectively). We also tested the cytokines in serum samples to further confirm their diagnostic efficacies. TNF-α, IL-1β and IL-6 had moderate diagnostic values, whereas the diagnostic value of IL-8 was significantly.

An essential feature of asthma is chronic airway wall inflammation, characterized by the immune infiltration and activation, including mast cells, neutrophils, eosinophils, lymphocytes, and DCs [29]. The complex interactions among these immune cell types promotes asthma progression. By analyzing their expressions from asthma datasets using the ssGSEA algorithm and CIBERSORT analysis, we were able to provide deep insights into the regulatory network among immune cells in asthma. Indeed, we found that follicular helper T cells, activated DCs, activated mast cells and eosinophils were significantly higher in asthma, whereas macrophages M0 and macrophages M2 were significantly reduced, in sputum samples from patients with asthma, suggesting that M2-like polarization of macrophages may reduce the asthma progression via inhibition of inflammatory response cells.

This study had several limitations. We identified the diagnostic values of only some IRGs-related cytokines in serum. Analysis of larger, prospective samples will be required to strengthen the evidence for a diagnostic value of these IRGs in asthma. Functional experiments are also needed to further describe the pathogenesis of asthma.

In summary, through bioinformatics analysis we were able to identify 75 potential IRGs that are associated with asthma, most of which are involved in the NF-kappa B signaling pathway. These findings also identified hub genes in the pathway with moderate diagnostic value for asthma. We also identified IRGs-related cytokines (TNF-α, IL-1β, IL-8 and IL-6) in serum samples to further confirm their diagnostic efficacy, finding that the cumulative AUC of these 4 related cytokines for asthma diagnosis was 0.998. Infiltration levels of immune cell subtypes were also identified to elucidate the associations between asthma and its immune environment. Cumulatively, these newly identified hub genes and immune infiltration provided clues about the mechanisms underlying asthma development.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 81901632, 82001576, 81971509, and 81971513); Medical College of Soochow University Students' Extracurricular Scientific Research Project (2022YXYKWKY061); The Primary Research & Development Plan of Jiangsu Province (BE2022736).

Disclosure of conflict of interest

None.

Address correspondence to: Yuting Liang, Center for Clinical Laboratory, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China. E-mail: liangyuting666@126.com; Longwei Qiao, Center for Reproduction and Genetics, School of Gusu, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Nanjing Medical University, Suzhou 215002, Jiangsu, China. E-mail: qiaolongwei1@126.com; Renxin Ji, The School of International Medical Technology of Shanghai Sanda University, Shanghai 201209, China. E-mail: jirenxin@qq.com

References

[1] Chen G, Chen D, Feng Y, Wu W, Gao J, Chang C, Chen S and Zhen G. Identification of key signaling pathways and genes in eosinophilic asthma and neutrophilic asthma by weighted gene co-expression network analysis. Front Mol Biosci 2022; 9: 805570.

[2] Papi A, Brightling C, Pedersen SE and Reddel HK. Asthma. Lancet 2018; 391: 783-800.

[3] Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, Cruz AA, Duijts L, Drazen JM, FitzGerald JM, Fleming LJ, Inoue H, Ko FW, Krishnan JA, Levy ML, Lin J, Mortimer K, Pitrez PM, Sheikh A, Yorgancioglu AA and Boulet LP. Global initiative for asthma strategy

2021: executive summary and rationale for key changes. Am J Respir Crit Care Med 2022; 205: 17-35.
[4] Wang R, Murray CS, Fowler SJ, Simpson A and Durrington HJ. Asthma diagnosis: into the fourth dimension. Thorax 2021; 76: 624-631.
[5] Kim MH, Rhee CK, Shim JS, Park SY, Yoo KH, Kim BY, Bae HW, Sim YS, Chang JH, Cho YJ and Lee JH. Inhaled corticosteroids in asthma and the risk of pneumonia. Allergy Asthma Immunol Res 2019; 11: 795-805.
[6] Amin S, Soliman M, McIvor A, Cave A and Cabrera C. Usage patterns of short-acting β_2-agonists and inhaled corticosteroids in asthma: a targeted literature review. J Allergy Clin Immunol Pract 2020; 8: 2556-2564, e2558.
[7] Anagnostou K, Harrison B, Iles R and Nasser S. Risk factors for childhood asthma deaths from the UK Eastern Region Confidential Enquiry 2001-2006. Prim Care Respir J 2012; 21: 71-77.
[8] Hammad H and Lambrecht BN. The basic immunology of asthma. Cell 2021; 184: 2521-2522.
[9] Shamji MH, Sharif H, Layhadi JA, Zhu R, Kishore U and Renz H. Diverse immune mechanisms of allergen immunotherapy for allergic rhinitis with and without asthma. J Allergy Clin Immunol 2022; 149: 791-801.
[10] Zhang S, Lin K, Qiu J, Feng B, Wang J, Li J, Peng X, Ji R, Qiao L and Liang Y. Identification of potential key autophagy-related genes in asthma with bioinformatics approaches. Am J Transl Res 2022; 14: 7350-7361.
[11] Zhou L, Liu J, Wang L, He Y and Zhang J. Carbocistein improves airway remodeling in asthmatic mice. Am J Transl Res 2022; 14: 5583-5590.
[12] Wu Q, Liu J, Deng J and Chen Y. Long non-coding RNA HOTTIP induces inflammation in asthma by promoting EFNA3 transcription by CCCTC-binding factor. Am J Transl Res 2022; 14: 8903-8917.
[13] Pelaia C, Deng J and Chen Y. Long non-coding RNA HOTTIP induces inflammation in asthma by promoting EFNA3 transcription by CCCTC-binding factor. Am J Transl Res 2022; 14: 8903-8917.
[14] Li X, Abdel-Maksoud MA, Iqbal I, Mubarak A, Farrag MA, Haris M, Alghamdi S, Ain QU and Almekhlafi S. Deciphering cervical cancer-associated biomarkers by integrated multi-omics approach. Am J Transl Res 2022; 14: 8843-8861.
[15] Ren Z, Gao M and Jiang W. Prognostic role of NLGN2 and PTGDS in medulloblastoma based on gene expression omnibus. Am J Transl Res 2022; 14: 3769-3782.
[16] Xi X, Cao T, Qian Y, Wang H, Ju S, Chen Y, Chen T, Yang J, Liang B and Hou S. CDC20 is a novel biomarker for improved clinical predictions in epithelial ovarian cancer. Am J Cancer Res 2022; 12: 3303-3317.
[17] Hou F, Liu H, Gong Q and Xu H. Network pharmacology of Huatan Tongluo decoction and clinical effects of its combination with acupuncture in the treatment of stroke. Am J Transl Res 2022; 14: 8215-8224.
[18] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.
[19] Tan M, Huang G, Chen J, Yi J, Liu X, Liao N, Hu Y, Zhou W and Guo Q. Construction and validation of an eight pyroptosis-related lncRNA risk model for breast cancer. Am J Transl Res 2022; 14: 2779-2800.
[20] Pan X, Jin X, Wang J, Hu Q and Dai B. Placenta inflammation is closely associated with gestational diabetes mellitus. Am J Transl Res 2021; 13: 4068-4079.
[21] Hodeib M, Taha G, Mohamed M, Maarek E and Doudar NA. IL-8 gene expression and bronchial asthma phenotypes in children. Egypt J Immunol 2021; 28: 138-144.
[22] Chen S, Chen Z, Deng Y, Zha S, Yu L, Li D, Liang Z, Yang K, Liu S and Chen R. Prevention of IL-6 signaling ameliorates toluene diisocyanate-induced steroid-resistant asthma. Allergol Int 2022; 71: 73-82.
[23] Adikusuma W, Chou WH, Lin MR, Ting J, Irham LM, Perwitasari DA, Chang WP and Chang WC. Identification of druggable genes for asthma by integrated genomic network analysis. Biomedicines 2022; 10: 113.
[24] Ma B, Athari SS, Mehrabi Nasab E and Zhao L. PI3K/AKT/mTOR and TLR4/MyD88/NF-κB signaling inhibitors attenuate pathological mechanisms of allergic asthma. Inflammation 2021; 44: 1895-1907.
[25] Lertnimitphun P, Zhang W, Fu W, Yang B, Zheng C, Yuan M, Zhou H, Zhang X, Pei W, Lu Y and Xu H. Safranal alleviated OVA-induced asthma model and inhibits mast cell activation. Front Immunol 2021; 12: 585595.
[26] Wei W, Huang J, Ma Y, Ma X, Fang L, Fang W and Hao C. IL-1 signaling pathway molecules as key markers in childhood asthma. Pediatr Allergy Immunol 2021; 32: 305-313.
[27] Bégin P, Tremblay K, Daley D, Lemire M, Claveau S, Salesse C, Kacel S, Montpetit A, Becker A, Chan-Yeung M, Kozyrskyj AL, Hudson TJ and Laprise C. Association of urokinase-type plasminogen activator with asthma and atopy.

Am J Respir Crit Care Med 2007; 175: 1109-1116.

[28] Azouz NP, Ynga-Durand MA, Caldwell JM, Jain A, Rochman M, Fischesser DM, Ray LM, Bedard MC, Mingler MK, Forney C, Eilerman M, Kuhl JT, He H, Biagini Myers JM, Mukkada VA, Putnam PE, Khurana Hershey GK, Kottyan LC, Wen T, Martin LJ and Rothenberg ME. The antiprotease SPINK7 serves as an inhibitory checkpoint for esophageal epithelial inflammatory responses. Sci Transl Med 2018; 10: eaap9736.

[29] Hammad H and Lambrecht BN. The basic immunology of asthma. Cell 2021; 184: 1469-1485.