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Comparative Analysis of the Role of CD4⁺ and CD8⁺ T Cells in Severe Asthma Development¹

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Abstract—The role of CD8⁺ T cells in asthma has not been fully discussed. The mechanisms of CD4⁺ and CD8⁺ cells in severe asthma (SA) development were compared. The microarray data (GSE31773) was downloaded from the Gene Expression Omnibus (GEO) database, including 20 samples of CD4⁺ and CD8⁺ T cells, which were collected from 8 health controls (HC), 4 non-severe asthma (NSA) and 8 SA patients. DEGs of CD4⁺ and CD8⁺ T cells in the HC vs. NSA and HC vs. SA groups were identified using the limma package in R. GO and pathway enrichment analysis of the common DEGs between the two groups were analyzed using DAVID. The interactive network of DEGs and significant modules were further explored. In CD4⁺ cells, there were 168 DEGs in HC vs. NSA group and 685 DEGs in HC vs. SA group, while for CD8⁺ T cells there were 719 DEGs in the HC vs. NSA groups and 1255 DEGs in the HC vs. SA groups. Besides, 80 common DEGs from CD4⁺ samples were enriched in the MAPKKK cascade and molecular metabolism, and 385 common DEGs of CD8⁺ T cells were significantly related with cell apoptosis and transformation. Moreover, two significant modules of DEGs in CD4⁺ were found to be involved with *MPO* and *BPI*. One module of CD8⁺ T cells containing *PDHA1* and *MRPL42* was identified to be related with glycolysis. In conclusion, *MPO* and *BPI* in CD4⁺, and *PDHA1* and *MRPL42* in CD8⁺ T cells might be used as specific biomarkers of SA progression. Therapy targeting the functions of CD4⁺ and CD8⁺ T cells may provide a novel perspective for SA treatment.

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INTRODUCTION

Asthma is a chronic airway inflammatory disease that is characterized by bronchospasm, mucosal edema, airway hyper-responsiveness and airway secretion increase [1]. Patients with non-severe asthma (NSA) can be treated using a combination of anti-inflammatory drugs and bronchodilators while severe asthma (SA) cases cannot be managed with conventional methods [2]. The morbidity of asthma varies from 0.3 to 17.0% in different countries and asthma causes about 180000 deaths around the world every year [3]. The disease brings a huge economic burden on both the patients' families and the society [4]. The mechanism of asthma is complicated and remains largely unknown, therefore, it is of great significance to explore the pathogenesis of asthma.

Previous studies have focused on the immune effects on asthma progression. Lots of inflammatory cells, like T lymphocytes [5], mast cells [6] and macrophages [7], and the inflammatory medium produced by these cells, as well as the structural cells, such as epithelial cells [8], vascular endothelial cells [9] and

smooth muscle cells [10], all participate in the progression of asthma. Vascular endothelial growth factor (VEGF) can promote the hyperplasia of smooth muscle cells and blood vessels to cause the airway stenosis in asthma [11]. Activated neutrophils have been found to play a crucial role in SA development via secreting lots of enzymes, inflammatory medium and cytokines [12]. Akbari et al. proved that the CD4⁺ natural killer T cells play a crucial role in human asthma development [13]. Also, CD4⁺ T cells can promote the exacerbation of chronic allergic asthma by producing interleukin-17 (IL-17) [14]. Helper T cells (Th2), one of the CD4⁺ T cell subgroups, play an important role in the treatment of SA [15]. In addition, the functions of transcription growth factor- β (TGF- β) and IL-5, IL-8 in remodeling asthmatic lung tissues have been confirmed [16–18].

Although studies have demonstrated the roles of CD4⁺ T cells in the development of the inflammatory response in asthma, little is discussed about the functions of CD8⁺ T cells in asthma. Former works have shown that the activation of CD8⁺ T cells is associated with SA formation [19]. Additionally, CD8⁺ T cells can promote the development of allergen-induced air-

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way hyper-responsiveness and pulmonary inflammation [20].

Microarray analysis is an effective approach to monitor the global alterations of gene expression and identifying genes that are important for disease development pathways. In this study, microarray analysis was used to detect differentially expressed genes (DEGs) in CD4⁺ and CD8⁺ T cells in NSA and SA samples as compared to the healthy controls (HC). Comprehensive bioinformatics analysis was used to construct an interactive network and to analyze the information on the significant modules to provide a deeper insight on the biological mechanisms of involvement of CD4⁺ T cells and CD8⁺ T cells in asthma development. This approach was a benefit for predicting the hub genes that are most likely associated with SA and identifying the molecular mechanisms of involvement of CD8⁺ T cells in SA that could serve as a novel therapeutic basis.

EXPERIMENTAL

Affymetrix microarray data. The microarray and other forms of high flux data produced by the scientific community are archived and freely released from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI) [21], which is the biggest completely public data storage. We extracted the gene expression profiles (GSE31773) from the study of Tsitsiou et al. [19], which were deposited in the GEO database [22] based on the platform of GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. The study contained 20 samples of circulating CD4⁺ cells and 20 CD8⁺ T cell specimens, which were isolated from 8 healthy controls (HC), 4 patients with NSA and 8 patients with SA. Data derived from the samples of CD4⁺ cells and specimens of CD8⁺ T cells were separated into two groups: HC vs. NSA and HC vs. SA group.

Probes were transformed into the corresponding gene names based on the annotation information. The mean expression value of each gene was calculated to act as the single expression value, and then the log₂ transformation was performed to normalize the data from the skewed normal distribution to an approximate normal distribution [23]. The Limma package in R language was used to select the differentially expressed genes (DEGs) in the two groups respectively. The p -value < 0.05 and $\log_2$ Fold Change (FC) > 1.5 or $|\log_2 \text{FC}| < 1/1.5$ ($\log_2$ Fold Change $|\text{FC}| > 0.585$) were chosen as the cut-off criteria.

Common DEGs in three stages. In order to identify the common DEGs in the HC vs. and NSA vs. SA group, we compared the expression values of the selected DEGs from samples in the HC vs. NSA and the HC vs. SA groups respectively [24] and reflected them in three sets of the Venn diagram [25].

Gene ontology (GO) analysis of DEGs. GO analysis has become a commonly used approach for functional

studies of large-scale genomic or transcription data [26]. DAVID bioinformatics resources consist of an integrated biological knowledgebase and analytical tools aimed at a systematical extraction of the biological meaning from large gene or protein lists [27]. We used DAVID to enrich the functions and pathways of the common DEGs in each gene set in the GSEA network with the p value < 0.05 .

Pathway analysis of common DEGs in each group.

To compare the pathways involving the common DEGs in HC vs. NSA and HC vs. SA both in CD4⁺ and CD8⁺ T cells, a Web-based Gene Set Analysis Tool kit (WebGestalt) [28, 29] was used to annotate the functions and pathways of DEGs based on the cumulative hypergenometric distribution [30]. The adj p value < 0.05 was chosen as the threshold.

Interaction network construction of common DEGs.

The variety of physiological cell activities and reactions to the external and internal environments are all connected by a protein interaction network [31]. Therefore, a further investigation of protein interactions is necessary to recognize and understand the biological phenomena [32]. The target genes that interact with the DEGs were selected using the Search Tool of the Retrieval of Interacting Genes (STRING) database based on the information such as sequence characters and structures [33]. Finally, interaction patterns with a degree > 0.4 were selected.

Functional analysis of significant modules in the interaction network.

Gene Set Enrichment Analysis (GSEA) can be used to calculate the variation of biological functions represented by the gene via recognizing the significance of the gene expression values in the whole gene set [34]. The interaction network was visualized using the Cytoscape software and the functional modules in the interaction network were divided and annotated using the Mcode plug-ins [35] with a threshold > 2 (the degrees of each node in the modules were chosen as threshold) and BiNGO [36] based on a hypergenometric distribution. The adj $p < 0.05$ was chosen as the threshold value.

RESULTS

DEGs Screening

Samples of CD4⁺ and CD8⁺ T cells were separated into two groups respectively, HC vs. NSA and HC vs. SA. A total of 168 DEGs in the HC vs. NSA group and 985 DEGs in the HC vs. SA group of CD4⁺ T cells (Notion 1-1, see Supplementary http://www.molecbio.com/downloads/2015/3/supp_WangX_en.pdf), and 719 DEGs in the HC vs. NSA group and 1255 DEGs in the HC vs. SA group of CD8⁺ T cells were screened (Notion 1-2, see Supplementary). There were more DEGs in SA samples than that in NSA samples from the perspective of comparison within groups, while the total number of DEGs from the two kinds of samples

of CD8⁺ T cells was greater than that from CD4⁺ T cells.

Besides, we got 80 common DEGs for the two groups in CD4⁺ T cells for all three stages (from HC to NSA and then to SA) and 385 DEGs common for all three stages between the two groups in CD8⁺ T cells (Fig. 1).

Function Annotation Analysis of Common DEGs

To investigate the functional changes of selected common DEGs, we used DAVID to identify the significant GO categories in biological processes. Twenty GO terms were enriched among the 80 DEGs in CD4⁺ T cells, of which several significant functions were related to the MAPKKK cascade, neuron migration, regulation of apoptosis and regulation of cell motion (Table 1). Besides, another 20 GO terms of the 385 selected DEGs in CD8⁺ T cells were also enriched, which mainly function in biological adhesion, neuron development, transcription and regulation of transcription (Table 2).

Pathway Enrichment Analysis

To gain further insight into the pathways involving the 80 common DEGs in CD4⁺ T cells and the 385 common DEGs in CD8⁺ T cells, we used DAVID to identify the significant pathways. Common DEGs in CD4⁺ T cells were enriched in such pathways like phagosome, Jak-STAT signaling pathway, metabolic pathway and hepatitis C (Table 3a), while the TGF- β signaling pathway was significantly enriched in CD8⁺ T cells (Table 3b).

Gene–gene Interactive Pairs of DEGs

We imported the 80 common DEGs of three time points in CD4⁺ T cells and the other 385 common DEGs in CD8⁺ T cells into the STRING database to identify the gene–gene interactive pairs. Finally, 21 gene–gene interactive pairs in the CD4⁺ T cell group and 143 DEG patterns in the CD8⁺ T cell group were obtained from the database (Notion 3, see Supplementary http://www.molecbio.com/downloads/2015/3/supp_WangX_en.pdf).

Functional Analysis of Modules in the Interaction Network

Two modules with the 80 common DEGs in CD4⁺ T cells and one module with the 385 common DEGs in the CD8⁺ T cell group were obtained using the Mcode and BiNGO plug-ins respectively (Fig. 2). Module A with DEGs like *RNASE3* (ribonuclease, RNase A family, 3), *MPO* (myeloperoxidase) and *BPI* (bactericidal/permeability-increasing protein) (Fig. 2a), module B with the DEGs like *MPO*, *BPI* and *AZU1* (azurocidin 1) (Fig. 2b), and module C with the DEGs

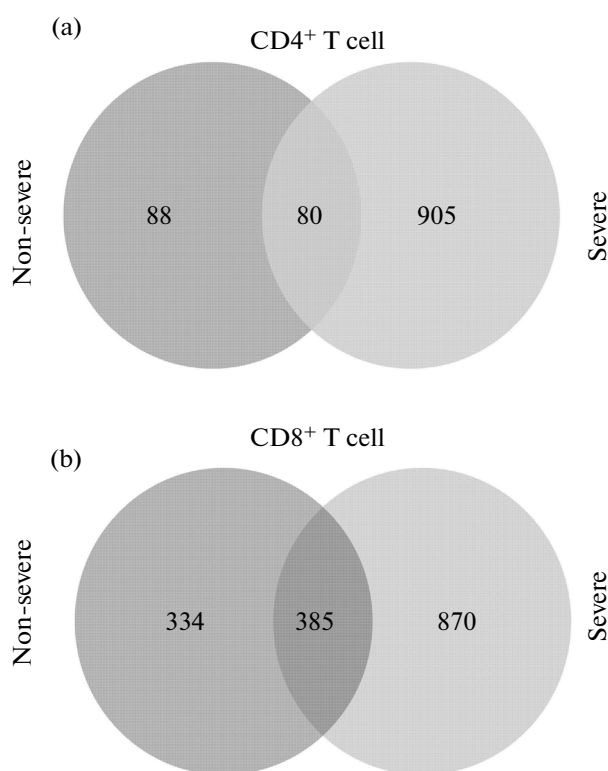


Fig. 1. Comparison of DEGs in the two kinds of T cells. a—DEGs in CD4⁺ T cell samples of non-severe and severe asthma; b—DEGs in CD8⁺ T cell samples of non-severe and severe asthma.

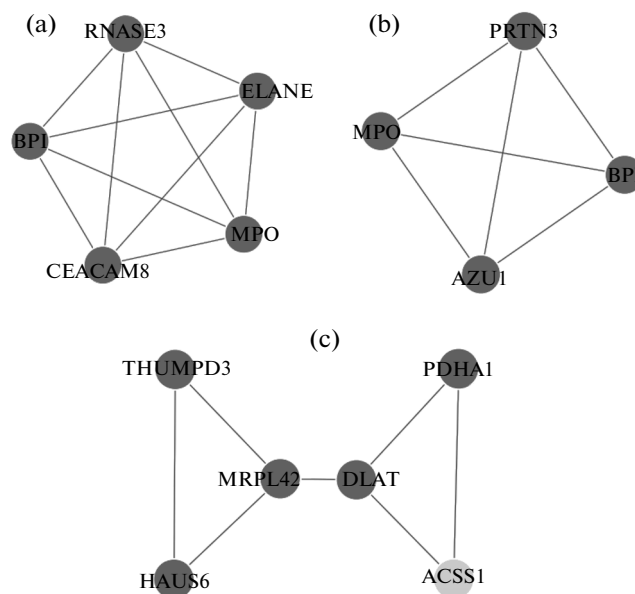


Fig. 2. Modules with DEGs in the interactive network. (a) Module A with interactive DEGs in CD4⁺ T cells; (b) Module B with interactive DEGs in CD4⁺ T cells; (c) Module C with interactive DEGs in CD8⁺ T cells.

Table 1. Terms of DEGs in NSA and SA samples of CD4⁺ T cell

Term	Count	P-value
GO terms of DEGs in NSA samples of CD4⁺ T cell		
GO:0000165 ~ MAPKKK cascade	7	2.48E-04
GO:0001764 ~ neuron migration	5	2.48E-04
GO:0060348 ~ bone development	6	3.06E-04
GO:0040012 ~ regulation of locomotion	7	3.11E-04
GO:0051270 ~ regulation of cell motion	7	3.20E-04
GO:0051130 ~ positive regulation of cellular component organization	6	0.00175148
GO:0030030 ~ cell projection organization	8	0.00186764
GO:0006928 ~ cell motion	9	0.00189714
GO:0051173 ~ positive regulation of nitrogen compound metabolic process	10	0.00349014
GO:0007519 ~ skeletal muscle tissue development	4	0.00390481
GO terms of DEGs in SA samples of CD4⁺ T cell		
GO:0009057 ~ macromolecule catabolic process	59	9.6495E-05
GO:0042981 ~ regulation of apoptosis	58	0.00037359
GO:0043067 ~ regulation of programmed cell death	58	0.0004689
GO:0045449 ~ regulation of transcription	150	0.00051335
GO:0010941 ~ regulation of cell death	58	0.00052035
GO:0043068 ~ positive regulation of programmed cell death	36	0.00053843
GO:0030163 ~ protein catabolic process	47	0.00056239
GO:0010942 ~ positive regulation of cell death	36	0.00058319
GO:0044265 ~ cellular macromolecule catabolic process	52	0.000914
GO:0043065 ~ positive regulation of apoptosis	35	0.00094211

MRPL42 (mitochondrial ribosomal protein L42), *PHDA1* (pyruvate dehydrogenase (lipoamide) $\alpha 1$) (Fig. 2c) were annotated respectively. Module A and B in CD4⁺ T cells were directly involved in the immune defense response, while module C in CD8⁺ T cells was involved in the glycolysis metabolism (Table 4).

DISCUSSION

Asthma is a chronic airway inflammatory disease that involves lots of inflammatory cells and inflammatory mediators and is characterized by bronchial hyper-responsiveness and airway remodeling [1]. In this work, the gene expression profiles in CD4⁺ and CD8⁺ T cells from HC samples and patients with NSA and SA were compared using microarray analyses. Several DEGs in CD4⁺ T cells were shown to be related with the immune response to asthma, what is in accordance with the former studies that have also implicated that CD8⁺ T cells can take part in asthma progression at the perspective of inflammatory response. Otherwise, the total number of DEGs in CD4⁺ was greater than that in the CD8⁺ T cell in asthma samples. Also, there was a variety of DEGs in CD8⁺ T cells obtained from patients with SA involved in such processes as cell apoptosis, suggesting that

CD8⁺ T cells might be associated with SA via the indirect immune response.

It was shown that the DEGs *MPO* and *BPI* appeared both in module A and module B in the CD4⁺ T cells. *MPO* (encoded by the *MPO* gene) is a heme peroxidase which is produced by neutrophils and macrophages [37]. Studies demonstrated that *MPO* has the ability to destroy external materials by transforming H₂O₂ into HClO which can destroy the cellular membrane and then damage the body of the cells of the defense system [37, 38]. It has been reported that *MPO* can activate the metal matrix proteinase (MMP) and affect the airway remodeling function in asthma [39]. Also, higher *MPO* levels have been found in patients with SA what indicates that it can act as an index for the degree of asthma severity [40]. The *MPO* gene in CD4⁺ T cells in SA was up-regulated as compared with that in NSA, suggesting that *MPO* plays an important role in SA formation. Meanwhile, *BPI* (encoded by the *BPI* gene) is a cation membrane bound type antimicrobial protein that can affect the galanz negative bacilli and neutralize endotoxins [41]. The phagocytic ability of neutrophils could be increased by *BPI* by activating the alexin activity in the serum [42]. VEGF takes part in the airway remodeling in SA via contributing to vascular proliferation and

Table 2. GO terms of DEGs in NSA and SA samples of CD8⁺ T cell

Term	Count	<i>P</i> -value
GO terms of DEGs in NSA samples of CD8⁺ T cell		
GO:0000902 ~ cell morphogenesis	22	3.05E-07
GO:0032989 ~ cellular component morphogenesis	23	4.59E-07
GO:0000904 ~ cell morphogenesis involved in differentiation	17	2.12E-06
GO:0007155 ~ cell adhesion	30	2.75E-06
GO:0022610 ~ biological adhesion	30	2.83E-06
GO:0031589 ~ cell-substrate adhesion	10	2.82E-05
GO:0048666 ~ neuron development	17	1.23E-04
GO:0048667 ~ cell morphogenesis involved in neuron differentiation	13	1.49E-04
GO:0048858 ~ cell projection morphogenesis	14	1.72E-04
GO:0048812 ~ neuron projection morphogenesis	13	1.78E-04
GO terms of DEGs in SA samples of CD8⁺ T cell		
GO:0006350 ~ transcription	137	4.58E-07
GO:0016568 ~ chromatin modification	32	1.01E-06
GO:0045449 ~ regulation of transcription	154	2.10E-05
GO:0006325 ~ chromatin organization	34	1.08E-04
GO:0006338 ~ chromatin remodeling	11	1.28E-04
GO:0051276 ~ chromosome organization	38	6.20E-04
GO:0051603 ~ proteolysis involved in cellular protein catabolic process	44	8.41E-04
GO:0044257 ~ cellular protein catabolic process	44	9.39E-04
GO:0030163 ~ protein catabolic process	45	9.49E-04
GO:0019941 ~ modification-dependent protein catabolic process	42	0.001169

Table 3. KEGG pathway analysis of common DEGs in samples of CD⁺ T cells

ID	Pathway	Parameters
a. Pathways of common DEGs in samples of CD4⁺ T cell		
4145	Phagosome	O = 2; raw <i>P</i> = 0.0330; adj <i>P</i> = 0.0406
4630	Jak-STAT signaling pathway	O = 2; raw <i>P</i> = 0.0338; adj <i>P</i> = 0.0406
1100	Metabolic pathways	O = 6; raw <i>P</i> = 0.0186; adj <i>P</i> = 0.0406
5160	Hepatitis C	O = 2; raw <i>P</i> = 0.0259; adj <i>P</i> = 0.0406
4640	Hematopoietic cell lineage	O = 2; raw <i>P</i> = 0.0117; adj <i>P</i> = 0.0406
b. Pathways of common DEGs in samples of CD8⁺ T cell		
4350	TGF-beta signaling pathway	O = 5; raw <i>P</i> = 0.0008; adj <i>P</i> = 0.0020
4722	Neurotrophin signaling pathway	O = 5; raw <i>P</i> = 0.0047; adj <i>P</i> = 0.0078
4144	Endocytosis	O = 5; raw <i>P</i> = 0.0293; adj <i>P</i> = 0.0293
4010	MAPK signaling pathway	O = 6; raw <i>P</i> = 0.0279; adj <i>P</i> = 0.0293

Table 4. Modules of common DEGs in interactive network

GO-ID	<i>p</i> -value	Corr <i>p</i> -value	<i>x</i>	Description	Genes in test set
Module A					
42742	0.000257	0.00499	2	Defense response to bacterium	BPI RNASE3
9617	0.000325	0.00499	2	Response to bacterium	BPI RNASE3
6952	0.000179	0.00499	3	Defense response	BPI RNASE3 MPO
51707	0.00113	0.0104	2	Response to other organism	BPI RNASE3
51869	0.00107	0.0104	4	Response to stimulus	BPI RNASE3 CEA CAM8 MPO
9607	0.0021	0.0159	2	Response to biotic stimulus	BPI RNASE3
6950	0.00242	0.0159	3	Response to stress	BPI RNASE3 MPO
51706	0.00651	0.0287	2	Multi-organism process	BPI RNASE3
44248	0.0109	0.0385	2	Cellular catabolic process	RNASE3 MPO
9056	0.0154	0.0444	2	Catabolic process	RNASE3 MPO
Module B					
42742	0.000257	0.005047	2	Defense response to bacterium	AZU1 BPI
6952	0.000179	0.005047	3	Defense response	AZU1 BPI MPO
9617	0.000325	0.005367	2	Response to bacterium	AZU1 BPI
6916	0.00061	0.007745	2	Anti-apoptosis	AZU1 MPO
51707	0.001133	0.009871	2	Response to other organism	AZU1 BPI
43066	0.001259	0.009871	2	Negative regulation of apoptosis	AZU1 MPO
43069	0.001294	0.009871	2	Negative regulation of programmed cell death	AZU1 MPO
9607	0.002104	0.012522	2	Response to biotic stimulus	AZU1 BPI
51093	0.002766	0.013039	2	Negative regulation of developmental process	AZU1 MPO
6950	0.002423	0.013039	3	Response to stress	AZU1 BPI MPO
51869	0.020489	0.043347	3	Response to stimulus	AZU1 BPI MPO
Module C					
6096	5.36E-05	0.001133	2	Glycolysis	DLAT PDHA1
6007	8.1E-05	0.001133	2	Glucose catabolic process	DLAT PDHA1
19320	0.00011	0.001133	2	Hexose catabolic process	DLAT PDHA1
46365	0.000114	0.001133	2	Monosaccharide catabolic process	DLAT PDHA1
46164	0.000129	0.001133	2	Alcohol catabolic process	DLAT PDHA1
44275	0.000201	0.001477	2	Cellular carbohydrate catabolic process	DLAT PDHA1
16052	0.000236	0.001484	2	Carbohydrate catabolic process	DLAT PDHA1
6006	0.000296	0.001627	2	Glucose metabolic process	DLAT PDHA1
19318	0.000546	0.002613	2	Hexose metabolic process	DLAT PDHA1
5996	0.000594	0.002613	2	Monosaccharide metabolic process	DLAT PDHA1

remodeling [43]. While BPI could inhibit the endogenous blood vessel formation by inducing the apoptosis of vascular endothelial cells [42]. Therefore, BPI could play a role in preventing asthma formation. Our data showed that the *BPI* gene was up-regulated in CD4⁺ T cells, indicating that *BPI* might play a crucial role in suppressing SA development.

Besides this, our data showed that *PDHA1* and *MRLP42* appeared in the module of CD8⁺ T cells in SA.

PDHA1 is an enzyme of the pyruvate dehydrogenase (PDH) system that can catalyze the pyruvate–acetyl-CoA transformation [44]. Relative functional impairment of PDHA1 in CD8⁺ T cells was described as a feature of asthma [45]. Also, defects in PDHA1 are an important cause of primary lactic acidosis, a frequent manifestation of metabolic disease like asthma [46]. The use of the β2 receptor agonist, a commonly used control drug for asthma treatment, could lead to an

increase of glycolysis characterized by hyper-lactic acidosis [47]. Thus, PDHA1 might take part in asthma progression by affecting lactic acidosis. Our data showed that the *PDHA1* gene was up-regulated, indicating that it might take part in the suppression of SA progression.

Furthermore, MRPs are enzymes responsible for the translation of mitochondrial respiratory chain complex proteins, and mutations/defects of either MRPs or other translation tools may lead to some mitochondrial diseases [48]. Epithelial cell apoptosis may contribute to airway remodeling in asthma by increasing the secretion of TGF- β , leading to epithelial cell fibrosis, stimulation of smooth muscle cell proliferation and expression of the cell division inhibitor protein p21 [49]. It has been found that MRPL41 participates in cell apoptosis mediated by p53 [50]. MRPL42 has some evolutionary relationship with MRPL41 [48]. In this work, *MRPL42* gene was shown to be up-regulated in CD8⁺ T cells, indicating that it might take part in SA development. It was proposed that CD8⁺ T cells might participate in SA progression via the indirect immune response pathway.

In summary, DEGs like *MPO* and *BPI* in CD4⁺ T cells, and *MRPL42* and *PDHA1* in CD8⁺ T cells are involved in the pathomechanisms of SA, but their role is different. These results provide a basis for the future experiments on CD8⁺ T cells of SA patients that may enhance our understanding of the pathogenesis of SA and hopefully open new therapeutic options for SA treatment.

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