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Aberrantly expressed lncRNAs identified by microarray analysis in CD4⁺T cells in asthmatic patients

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ABSTRACT

The aim of the study was to determine the expression profiles of message RNAs and long non-coding RNAs in CD4⁺T cells of asthmatic patients and to explore the clinical value and biological function. Expression profiles in CD4⁺T cells of asthmatic patients and healthy controls were analyzed by microarray. We found 2725 lncRNAs and 3167 mRNAs differentially expressed. The data were validated by quantitative real time polymerase chain reaction, with 3 up-regulated (ENST00000444682, ENST00000566098, ENST00000583179) and 1 down-regulated (ENST00000579468) lncRNAs found. Receiver operating characteristic curve analysis showed the area under the curve was 0.7058, 0.9026, 0.8361, 0.8316, respectively. Spearman correlation analysis showed that ENST00000566098 was positively related with IL-13 and ENST00000579468 was positively related with peak expiratory flow. Bioinformatics analyses were performed to explore the function of lncRNAs. Specific lncRNAs aberrantly expressed in CD4⁺T cells may take part in the development of asthma and may be used as biomarkers for diagnosis.

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1. Introduction

Bronchial asthma is a common chronic airway inflammatory disease. A large array of cells, cytokines, chemokines, and other pro-inflammatory mediators are involved in the progression of asthma [1]. As a critical component of lymphocytes, CD4⁺T cells remain at the forefront of asthma pathogenesis because of their myriad effects on inflammatory responses in the respiratory tract [2].

Long noncoding RNAs (lncRNAs) are at least 200 nucleotides in length [3], differing from other smaller noncoding RNA. They express in specific cell types, and have less conservative compared with protein-coding RNAs. Notably, previous studies show that

some lncRNAs may be potential biomarkers for the diagnosis and prognostic prediction of many diseases because of the aberrantly expression during the onset and development of diseases [4–6]. Recent evidence indicates that lncRNAs play important roles in many physiological and pathological processes, such as genomic imprinting [7], cell differentiation [8], apoptosis [9], proliferation [10], chromosome modification [11] and transcriptional activation [12]. Thus, the role of lncRNA in inflammatory diseases has attracted considerable attention. The current studies have reported lncRNAs controlled the development of specific immune cell-type, including dendritic cells [13], macrophage [14], eosinophils [15], monocytes [15], and CD4⁺ Th1 cells [16]. Wang et al. found that lncDC contributed to the activation status of DCs by regulating STAT3 and controlling the STAT3 transcriptional program in DCs [13]. lncRNA-Cox2 was found to associate with the switch/sucrose nonfermentable chromatin-remodeling complex, to control the expression of late-primary NK-KB-regulated gene in macrophage [14]. And lncRNA Rmrp modulated Th17 effector function with DDX [17].

However, little is known about the role of lncRNAs in asthma, a disease involving distinct T cell-driven processes. Therefore, we

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performed microarray to examine lncRNAs and mRNAs expression profiles in CD4⁺T cells from peripheral blood of asthmatic patients and healthy controls in the present study. Consequently, we validated them by qRT-PCR, hoping the results could provide further insights in the pathogenesis of asthma.

2. Material and methods

2.1. Subjects

Samples from 108 patients with asthma and 45 healthy controls were collected in this study. Adult asthmatic patients between the ages of 18 and 65 years were recruited from Tongji Hospital (Wuhan, China). The diagnosis of asthma was based on the Global Initiative for Asthma (GINA) guidelines [18]. All subjects had a doctor's diagnosis of symptomatic asthma and demonstrated evidence of airways hyperresponsiveness (PD20 methacholine < 2.5 mg) and/or bronchodilator responsiveness (>12% improvement in FEV1% predicted following inhalation of 200 µg salbutamol). Participants were excluded if they were current smokers or ex-smokers with a history more than 10 pack-years, had a course of oral corticosteroids, or a respiratory tract infection in the previous 4 weeks. In total 10 ml blood sample were collected from each subject. The clinical information, including lung function, FeNO, plasma IgE, allergic history, etc., were collected from all participants at their first visit of the study.

Our study was conducted in compliance with the institutional policy regarding the protection of patients' private information and approved by the Ethical Committee of Tongji Hospital, Huazhong University of Science and Technology (IRB ID: 20140415). Written informed consent was obtained from all participants. Sample collection and all the experimental methods in our study were carried out in accordance with the approved guidelines.

2.2. CD4⁺ T cell isolation and total RNA extraction

CD4⁺ T cells from each participant were isolated using density centrifugation and magnetic beads isolation. Briefly, the diluted blood was carefully layered onto Ficoll-Hypaque gradient (TBD, Tianjin, China) after the peripheral blood was mixed with PBS. The peripheral blood mononuclear cells (PBMCs) layer was obtained after centrifugation at 800g for 25 min. CD4⁺ T cells were isolated from PBMCs by negative selection using MagCelect Human CD4⁺ T Cell Isolation Kit (R&D systems, Minneapolis, USA) according to the manufacture's instruction. PBMCs were incubated with Magcellet Streptavidin Ferrofluid after labeled with negative selection biotinylated antibodies. CD4⁺ T cells were enriched by depletion of unwanted cells using magcellect magnet (R&D systems, Minneapolis, USA). Then the cells were frozen in TRIzol agent (Takara, Dalian, China) at –80 °C. Total RNA was extracted from CD4⁺ T cells using TRIzol reagent according to the manufacturer's instruction. The RNA quantity was measured with a NanoDrop ND-2000 (Thermo, Waltham, USA).

2.3. Microarray and quantitative real-time polymerase chain reaction(qRT-PCR)

Arraystar human lncRNA microarray V3.0 (Array-Star, Rockville, USA) was designed for the global profiling of human lncRNAs and protein-coding transcripts. The lncRNAs contained in this microarray were from authoritative public transcription database. The microarray work was completed by KangChen Bio-tech, Shanghai, China.

QRT-PCR was performed for validation. Primescript RT reagent kit (TaKaRa, Dalian, Japan) was used in the reverse transcriptase (RT)

reaction. The qRT-PCR was performed to quantify lncRNA and mRNA levels using an ABI Prism 7900 Real-Time System (Applied Biosystems, Foster City, USA) with SYBR Premix Ex Taq (Takara, Dalian, China). The primers used in qRT-PCR were designed by Ribo Bio-tech, Guangzhou, China (Shown in [Supplementary Table S1](#)). β -actin was used as an internal control. The data were analyzed using the $2^{-\Delta\Delta C_t}$ method and presented as relative expression level.

2.4. Gene ontology (GO) and Kyoto Encyclopedia of genes and Genomes (KEGG) pathway analysis

GO analysis is a functional analysis associating differentially expressed mRNAs with GO categories. The GO categories are derived from Gene Ontology (www.geneontology.org). Besides, based on the latest KEGG database (<http://www.kegg.jp>), we made pathway analysis for differentially expressed mRNAs. KEGG analysis provides the biological pathways which have significant enrichment of differentially expressed mRNAs.

2.5. Analysis of the lncRNA-mRNA co-expression network

lncRNA-mRNA-network was built to identify the co-expression relationship between mRNA and lncRNA according to the normalized signal intensity. We calculated the Pearson correlation coefficient and *P* value for each pair of mRNA-lncRNA. Significant correlation pairs (*P* < 0.05, Pearson correlation coefficient > 0.8) were chosen to construct the network.

2.6. Statistical analysis

Data were presented as means $\pm$ SEM. Statistical analyses were performed using Prism 5 software (GraphPad Software Inc., San Diego, USA). Normally distributed data were analyzed using Student's *t*-test for two groups. Data which did not meet normally distribution were evaluated by Mann–Whitney *U* test. Association between parameters was tested with Spearman ranking correlation. Receiver-operating characteristic (ROC) curves were performed to evaluate the diagnostic value of lncRNAs that were dysregulated in the CD4⁺ T cells of asthmatic patients compared to healthy controls. All data were analyzed using two-tailed tests, *P* < 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Information of the subjects

We recruited 108 asthmatic patients and 45 healthy controls in this study. In this study, asthmatic group were obviously worse in terms of pulmonary function, including FEV1/FVC, FEV1, FEV1%, and PEF than the healthy controls, while the FVC and FVC% showed no difference between the two groups. The FeNO and IgE in the plasma were significantly higher in the asthmatic group compared with the controls (Shown in [Supplementary Table S2](#)).

3.2. lncRNA expression profiling in CD4⁺T cells from patients with asthma

Microarray analysis were performed in the CD4⁺T cells from 5 patients with asthma and 5 age- and sex-matched healthy controls using an Arraystar Human lncRNA Microarray Version 3.0. Compared with healthy controls, we found that 863 lncRNAs were up-regulated and 1862 were down-regulated in patients with asthma (*P* < 0.05). A heat map was created to group the lncRNAs based on their expression levels among the samples ([Fig. 1A](#)). And

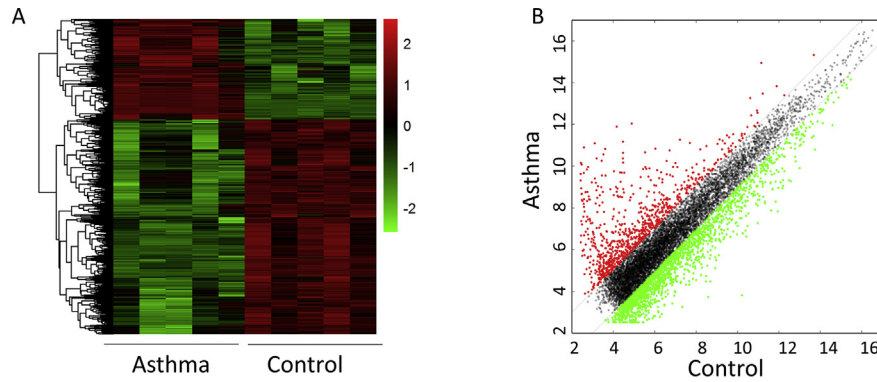


Fig. 1. Differentially expressed lncRNAs in CD4⁺T cells of asthmatic patients and healthy controls. (A) Hierarchical cluster analysis was performed to show the differentially expressed lncRNAs between asthmatic patients and healthy controls. Red represents higher expression level and green represents lower expression level. (B) After normalization, the Scatter-Plot shows difference in the expression of lncRNAs. Red: up-regulated, green: down-regulated, gray: not differentially expressed. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Scatter-Plot was built to show the differentially expressed lncRNAs between the two groups (Fig. 1B).

3.3. Validation of differentially expressed lncRNAs by qRT-PCR

To validate the microarray expression data, 8 dysregulated lncRNAs, including 3 up-regulated lncRNAs (ENST00000444682, ENST00000534789, ENST00000530263) and 5 down-regulated lncRNAs (ENST00000566098, ENST00000583179, ENST00000579468, ENST00000580420, ENST00000444374) were selected for further study based on their *P* value (<0.05), fold changes (>2) (Shown in Supplementary Table S3). An independent set of samples from 108 asthma patients and 45 healthy controls was used to validate the 8 candidate lncRNAs by qRT-PCR (Fig. 2). The relative expression levels of ENST00000444682 in the CD4⁺T cells of patients with asthma were significantly higher than the healthy controls (Fig. 2D), while the expression of ENST00000566098, ENST00000583179 and ENST00000579468

were significantly lower (Fig. 2A–C).

3.4. ROC curve analysis of confirmed lncRNAs

ROC curve analysis was used to identify the diagnostic value of lncRNAs (Fig. 3). We found the highest AUC was for ENST00000583179 (AUC = 0.9026, 95% CI: 0.8442–0.9610, $P < 0.0001$). Furthermore, we combined the confirmed lncRNAs with each other and defined the ratio as a new individual to achieve better diagnostic efficiency. And ROC curve analysis of these new individuals showed the highest AUC was for ENST00000444682/ENST00000583179 (AUC = 0.9441, 95% CI: 0.9093–0.9789, $P < 0.0001$) (Fig. 3E–G).

3.5. Correlation of clinical data and validated lncRNAs

Spearman correlation test was performed to assess the correlation between asthma-related clinical data and lncRNAs in the

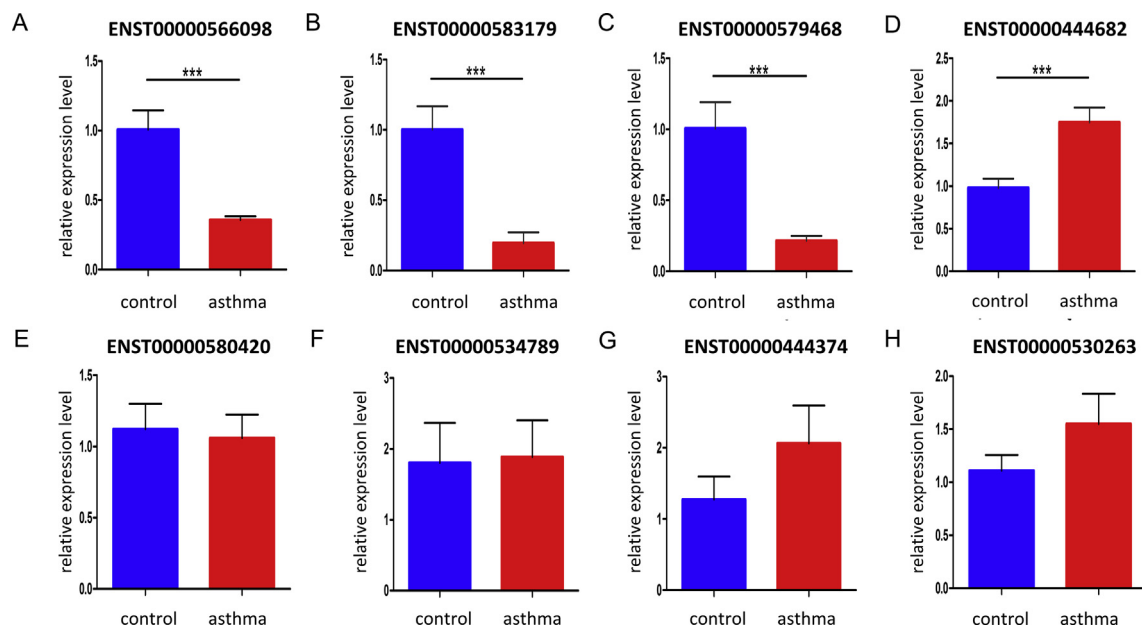


Fig. 2. Validation of the selected lncRNAs in CD4⁺T cells in asthmatic patients compared with healthy controls by qRT-PCR. A–H shows the relative expression level of ENST00000566098, ENST00000583179, ENST00000579468, ENST00000444682, ENST00000580420, ENST00000534789, ENST00000444374, ENST00000530263, respectively. ***: $P < 0.001$.

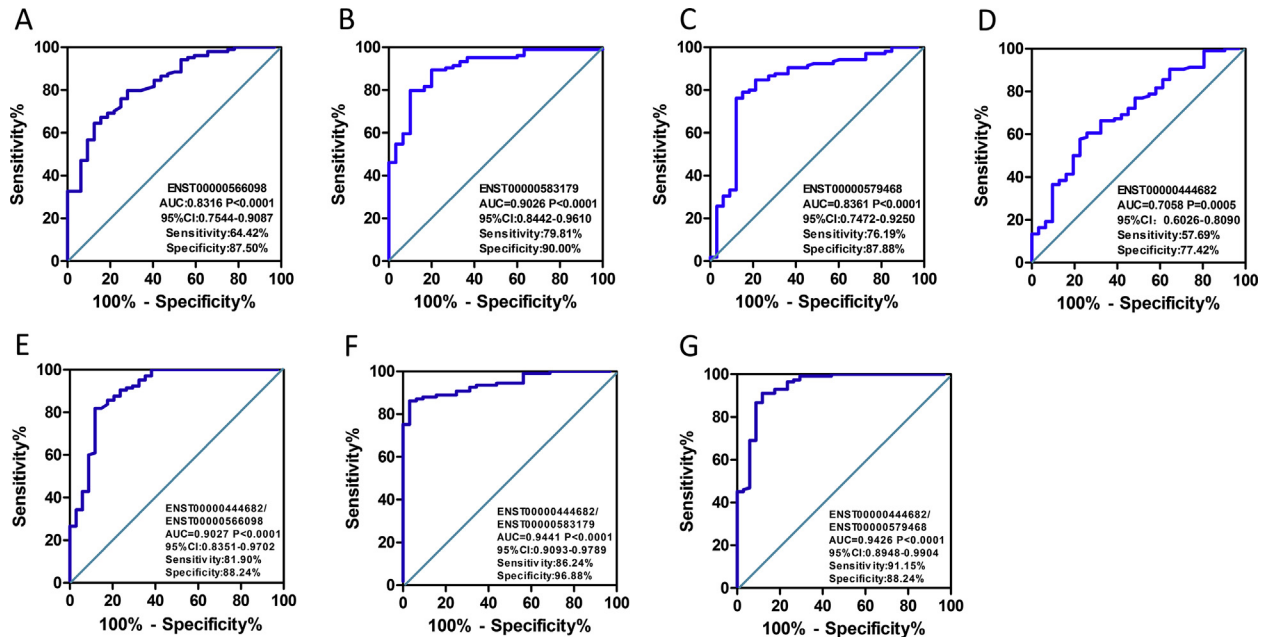


Fig. 3. Receiver operating characteristic (ROC) curve of lncRNAs. A-G shows the diagnostic value of ENST00000566098, ENST00000583179, ENST00000579468, ENST00000444682, ENST00000566098/ENST00000444682, ENST00000583179/ENST00000444682, ENST00000579468/ENST00000444682, respectively.

CD4⁺T cells from asthmatic patients to determine the efficiency of significantly and differentially expressed lncRNAs as biomarkers for the disease. The level of ENST00000566098, ENST00000579468, ENST00000444682 were correlated with FEV1/FVC ($P < 0.05$) (Shown in [Supplementary Table S4](#)).

3.6. Gene Ontology (GO) and Kyoto Encyclopedia of genes and Genomes (KEGG) pathway analysis of differentially expressed mRNAs

GO analysis was performed to investigate the biological processes, cellular components, and molecular function associating protein-coding mRNAs, since no comprehensive annotation database is available for categorizing lncRNAs. A total of 3168 mRNAs were included in GO analyses, including 1491 up-regulated and 1677 down-regulated mRNAs.

KEGG pathway analysis identified associated pathways by up- and down regulated mRNAs. In KEGG pathway analysis of the up-regulated mRNAs, the top enriched network was “rheumatoid arthritis”, “cytokine-cytokine receptor interaction”, and “tuberculosis”. As for pathway analysis of the down-regulated transcripts, the top pathways were “HTLV-1 infection”, “vascular smooth muscle contraction”, and “proteoglycans in cancer” (Shown in [Supplementary Figure S1–3](#)).

3.7. Correlation of cytokines at mRNA level and validated lncRNAs

As mentioned in previous test 3.6, “cytokines and receptor activity” was highly enriched in up-regulated mRNAs, we tested the relative expression level of classical cytokines of Th1 (IL-6, TNF- α) and Th2 type (IL-4, IL-5, IL-13) immune response in CD4⁺T cells from asthmatic patients by qRT-PCR. Spearman correlation test was done between the cytokines and lncRNAs and spearman correlation coefficients were shown in [Table 1](#). ENST00000566098 ($r = 0.4002$, $P < 0.001$), ENST00000583179 ($r = 0.2704$, $P < 0.05$), ENST00000579468 ($r = 0.3971$, $P < 0.001$) and ENST00000444682 ($r = 0.3068$, $P < 0.001$) were positive associated with IL-13 which played important role in asthma. Besides, ENST00000583179 was

negatively related with IL-4 ($r = -0.2407$, $P < 0.01$) and IL-6 ($r = -0.2342$, $P < 0.01$). ENST00000579468 correlated with IL-5 ($r = 0.2538$, $P < 0.01$) positively. And ENST00000444682 was positively associated with IL-5 ($r = 0.1867$, $P < 0.05$) and negatively associated with IL-6 ($r = -0.1869$, $P < 0.05$).

3.8. lncRNA-mRNA co-expression network

Co-expression network analysis was performed between the 4 lncRNAs and the 3167 differentially expressed mRNAs, with the threshold $P < 0.05$ and pearson correlation coefficient > 0.8 ([Fig. 4](#)). In total 304 mRNAs and 4 lncRNAs were included in the network, with 193, 56, 46, and 11 mRNAs co-expressing with ENST00000583179, ENST00000579468, ENST00000566098, and ENST00000444682, respectively.

4. Discussion

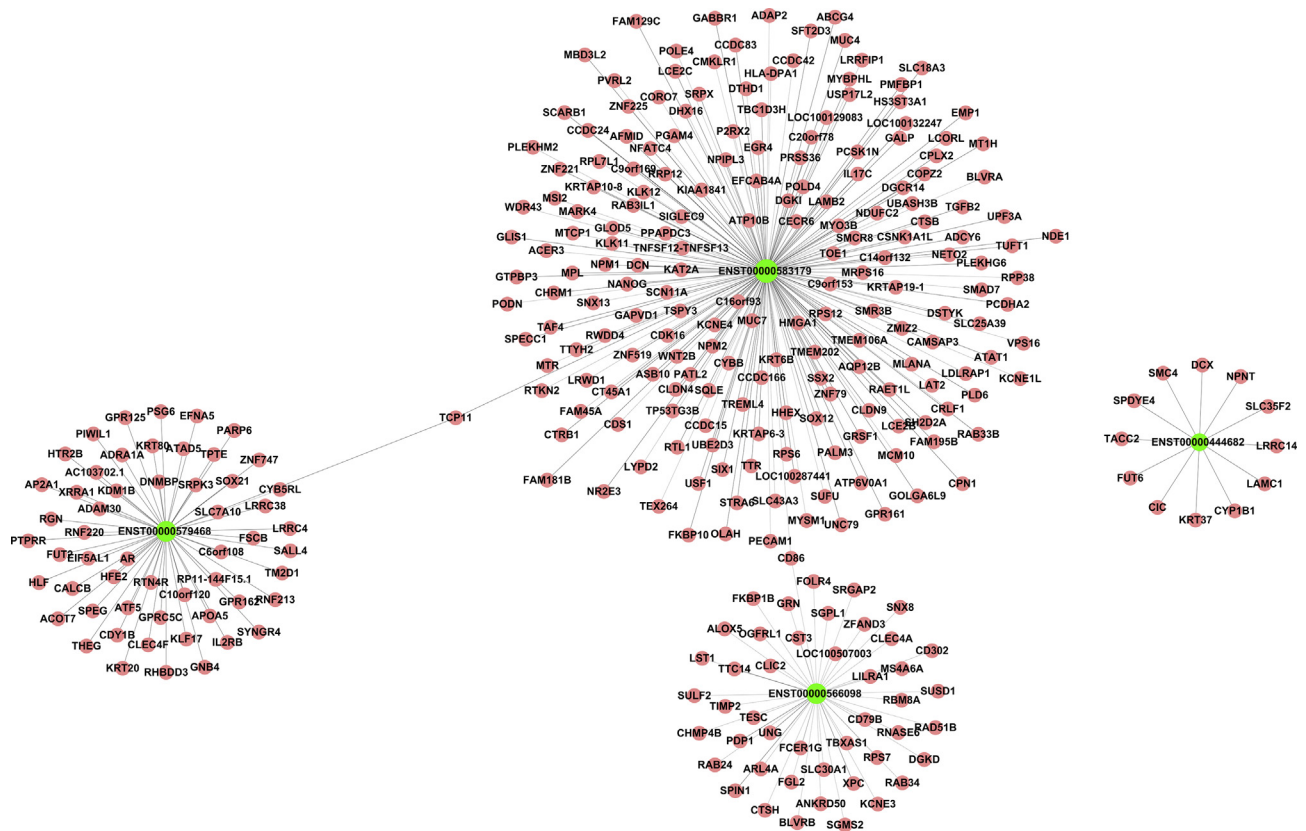
In previous study, Austin et al. revealed that one lncRNA (PVT1) was associated with proliferation and IL-6 release in ASMCs [19]. Additionally, Zhang et al. found another lncRNA (BCYRN1) was regulated by Schisandrin B through miR-150 in asthmatic rat model [20]. Wang’s group found the differentially expressed lncRNAs in asthmatic mouse after receiving the induced pluripotent stem cell (iPSC)-mesenchymal stem cells (MSCs) treatment [21]. Zhu et al. also found the different lncRNA expression profiles between asthmatic patients and controls [22]. However, CD4⁺T cells were selected as research objective in our study, which were not discussed in previous study. We found that some lncRNAs expressed aberrantly in CD4⁺T cells.

In this study, spearman correlation analysis of lncRNAs and clinical data was performed. The correlation between lncRNAs and FEV1/FVC was found. Meanwhile, no correlation between the lncRNAs and eosinophil count, IgE level in plasma, or FeNO was found.

Further GO and KEGG pathway analyses were utilized to study the differentially expressed mRNAs to explore the function of differentially expressed lncRNAs. The result of GO analysis showed

Table 1Spearman rank correlation coefficients of cytokines and qRT-PCR-confirmed lncRNAs in CD4⁺T cells from asthmatic patients.

	ENST00000566098	ENST00000583179	ENST00000579468	ENST00000444682
IL-4	0.06209	−0.2407**	−0.1179	−0.0148
IL-5	0.1469	0.1223	0.2538**	0.1867*
IL-13	0.4002***	0.2704*	0.3971***	0.3068***
IL-6	−0.02898	−0.2342**	−0.001691	−0.1869*
TNF- α	0.06973	−0.1627	−0.1359	0.00231

lncRNAs: long noncoding RNAs; IL-4: interleukin-4; IL-5: interleukin-5; IL-13: interleukin-13; IL-6: interleukin-6; TNF- α : tumor necrosis factor- α .*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.**Fig. 4.** lncRNA-mRNA co-expression network. Green: lncRNA; red: mRNA; solid line: positive correlation; dotted line: negative correlation. (Threshold: $P < 0.05$ and Pearson correlation coefficient > 0.8). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

that the up-regulated gene profile was closely related with biological process such as immune system process, defense response and leukocyte activation. These items were in accordance with the function of CD4⁺T cell in asthma [22]. As for the molecular function classification, the top three GO terms were protein binding, cytokine activity, and cytokine receptor binding in the up-regulated gene profile. This result suggests that lncRNAs may correlate with cytokine, cytokine receptor. Then we hypothesized that lncRNAs might be related with immune system process and expression of cytokines. Four lncRNAs were positively associated with IL-13. ENST00000583179 was negatively related with IL-4 and IL-6. ENST00000579468 correlated with IL-5 positively. And ENST00000444682 was positively associated with IL-5 and negatively associated with IL-6. These results indicated that the four lncRNAs might be involved in Th2 immune response, coincided with the GO analysis. Th2 cells are characterized by secretion of IL-4, IL-5 and IL-13. IL-4 plays a critical role in the differentiation of Th2 cells from uncommitted Th0 cells and the isotype switching of B cells from producers of IgG to producers of IgE [23]. IL-13 mimics

IL-4 in inducing IgE secretion but does not promote Th2 cell differentiation. And it not only induces airway hyperresponsiveness but also produces several of the structural changes seen in chronic asthma, including goblet cell hyperplasia, airway smooth muscle proliferation, and subepithelial fibrosis [24]. IL-5 is critically involved in the differentiation of eosinophils from bone marrow precursor cells and also prolongs eosinophil survival [25].

We conducted co-expression network analysis of validated lncRNAs and differentially expressed mRNAs. Many genes with important regulatory functions were co-expressed with lncRNA, such as SMAD7, WNT2B, ATF5, ZNF225, EGR4. SMAD7 regulates the function of regulatory T cells in the cAMP signaling pathway [26]. WNT2B takes part in the differentiation of leukemic cells [27]. Stabilization of ATF5 regulates the IL-1 β -stimulated C/EBP signaling pathway [28] which is responsible for the proliferation of bronchial smooth-muscle cells in asthmatic patients [29]. ZNF225 binding to the promoter of T-bet regulates the T helper cell differentiation [30]. EGR4 interacts with NF- κ B and controls transcription of genes encoding inflammatory cytokines in T cells [31].

There are however limitations in the present study. Firstly, these data may be confirmed with more population from different regions. Secondly, findings of our study were presented mainly based on the bioinformatics analysis. So, further experiments are needed to verify these findings.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.bbrc.2018.07.079>.

Transparency document

Transparency document related to this article can be found online at <https://doi.org/10.1016/j.bbrc.2018.07.079>.

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Supplementary Table S1. Primers used in qRT-PCR analysis.

Primer Name	Sequence
ENST00000566098-forward	ACCCACCTTGACCTCACTTACA
ENST00000566098-reverse	GCCTCTCTTGTGTCTAGGCATAA
ENST00000583179-forward	CCTGTGCTTTTACATCTACTCTTGG
ENST00000583179-reverse	GGAAGTGGAACAGACGCTTAGAGT
ENST00000579468-forward	GAACTACCCTTTCTCCCTCCC
ENST00000579468-reverse	ATTGGTGCTTCAGCCCTGTCT
ENST00000444682-forward	CAACTGACCCCTGAGGCACA
ENST00000444682-reverse	CACCATCTTCCAGACGGCAT
ENST00000580420-forward	TGTGGGCAACCGATTTGGT
ENST00000580420-reverse	TCTCCGAGCAGGTGCCACTA
ENST00000534789-forward	TTCAGTGTGCCCTGCTTGTG
ENST00000534789-reverse	CTCCTCAGTGGGTCCTACGAT
ENST00000444374-forward	TGATCCAGGTGTTGAGACAGAAT
ENST00000444374-reverse	TCTACCAGGTCAGCCCAAATC
ENST00000530263-forward	AAACTGGCTCCCAGATAAGGC
ENST00000530263-reverse	GAAGCTGAAATCCCTGGAGGT
IL-4-forward	ATGGGTCTCACCTCCCAACT
IL-4-reverse	GATGTCTGTTACGGTCAACTCG
IL-5-forward	TCTACTCATCGAACTCTGCTGA
IL-5-reverse	CCCTTGACAGTTTGACTCTC

IL-6-forward	ACTCACCTCTTCAGAACGAATTG
IL-6-reverse	CCATCTTTGGAAGGTTTCAGGTTG
IL-13-forward	CCTCATGGCGCTTTTGTTGAC
IL-13-reverse	TCTGGTTCTGGGTGATGTTGA
TNF- α -forward	GGAGGGGTCTTCCAGCTGGAGA
TNF- α -reverse	CAATGATCCCAAAGTAGACCTGC
β -actin-forward	AGAAAATCTGGCACCACACCT
β -actin-reverse	GATAGCACAGCCTGGATAGCA

Supplementary Table S2 Characteristics of subjects providing peripheral blood.

	Microarray analysis		Validation	
	Asthma n = 5	Control n = 5	Asthma n = 103	Control n = 40
Age (y)	39.00±9.76	37.80±10.28	40.53±12.16	36.83±10.83
Sex (male/female)	1/4	1/4	35/68	12/28
FEV1/FVC (%)	66.39±10.34*	84.21±7.03	68.09±13.68**	86.99±6.54
FEV1 (L)	2.48±0.83*	3.42±0.58	2.31±0.73**	3.32±0.48
FEV1 (%)	77.36±14.24*	101.45±10.42	80.36±16.49**	100.40±11.12
FVC (L)	3.52±0.69	3.80±0.72	3.60±0.87	3.83±0.56
FVC (%)	95.35±17.68	117.34±15.26	103.64±18.57	108.72±15.82
PEF (L)	5.52±2.10*	7.91±1.83	5.36±1.91**	8.97±2.37
FeNO (ppb)	87.47±60.07*	13.28±8.74	90.42±62.56**	15.33±7.24
IgE (IU/mL)	187.00±98.80*	58.32±26.43	198.33±104.23**	56.52±29.39

FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; PEF: peak expiratory flow.

*: $P < 0.05$ compared asthma with control in microarray analysis group; **: $P < 0.05$ compared asthma with control in validation group.

Supplementary Table S3. Information of selected differentially expressed lncRNAs in asthmatic patients compared with healthy controls.

Number	Sequence name	Gene symbol	Regulation	Length	Chromosome	Type	Fold change	P value
1	ENST00000566098	RP11-105C19.1	Down	375	Chr16	Intergenic	2.0090815	0.025220822
2	ENST00000583179	RP11-19P22.5	Down	2110	Chr17	Intergenic	6.3681843	0.003663574
3	ENST00000579468	AC01076.10	Down	287	Chr17	Natural antisense	7.7148307	0.000571327
4	ENST00000444682	RP11-96L14.7	Up	623	Chr1	Natural antisense	3.6558839	1.92729E-05

lncRNA: long non-coding RNA

Supplementary Table S4. Spearman rank correlation coefficients of clinical variables and qRT-PCR-confirmed lncRNAs in CD4⁺T cells from asthmatic patients.

	ENST00000566098	ENST00000583179	ENST00000579468	ENST00000444682
gender	-0.01655	-0.05138	0.0393	0.08515
age	-0.1114	-0.006391	0.0373	-0.09267
FEV1%	0.1626	0.05439	0.2334*	0.2291*
FVC%	-0.03449	-0.0541	0.07127	0.06519
FEV1/FVC	0.2883*	0.1042	0.2725*	0.2355*
PEF	0.1232	0.184	0.2953**	0.2389*
PD20	0.2398	0.1346	0.2823*	0.2878*
eosinophil count	-0.03506	-0.05971	-0.08075	-0.1412
eosinophil rate	-0.1191	-0.05522	-0.1321	-0.2314
plasma IgE	-0.05906	0.09725	0.09825	-0.02886
FeNO	0.04363	0.02666	0.008317	-0.1173
allergic rhinitis	-0.0566	-0.1355	-0.2124*	0.2628*

FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; PEF: peak expiratory flow; FeNO: fractional exhaled nitric oxide; lncRNA: long non-coding RNA. *: $P < 0.05$, **: $P < 0.01$.

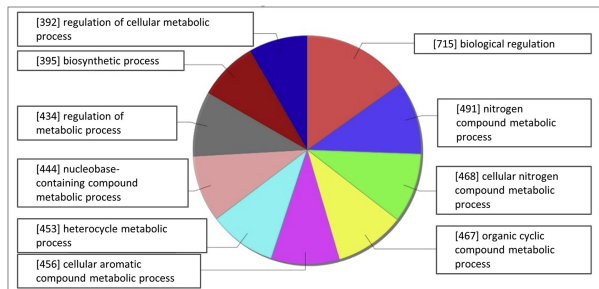
Supplementary Figure S1. Gene Ontology (GO) analysis of up-regulated mRNAs. The classification of GO terms targeted in biological process (A), cellular component (C) and molecular function (E). The significant enriched GO terms targeted in biological process (B), cellular component (D) and molecular function (F).

Supplementary Figure S2. Gene Ontology(GO) analysis of down-regulated mRNAs. The classification of GO terms targeted in biological process (A), cellular component (C) and molecular function (E). And the significant enriched GO terms targeted in biological process (B), cellular component (D) and molecular function (F).

Supplementary Figure S3. The KEGG analysis of differentially expressed mRNAs. The top 10 pathways of up-regulated mRNAs (A and B) and down-regulated mRNAs (C and D).

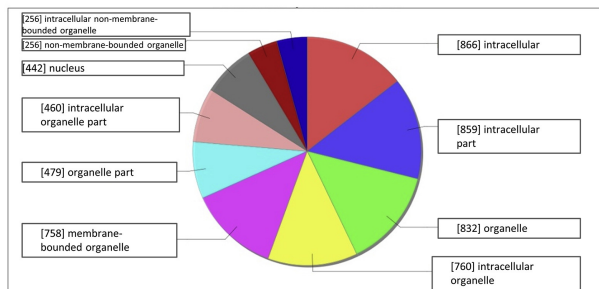
A

GO Biological Process Classification



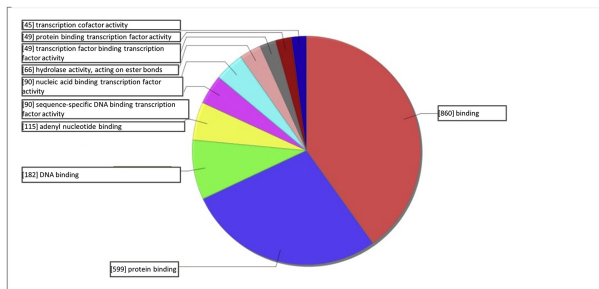
C

GO Cellular Component Classification



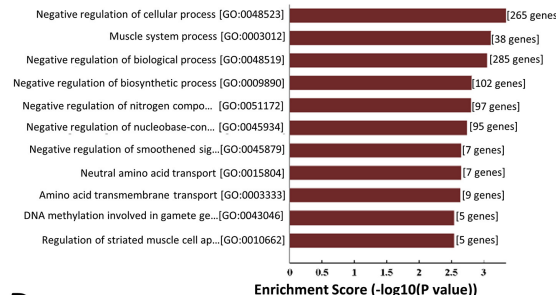
E

GO Molecular Function Classification



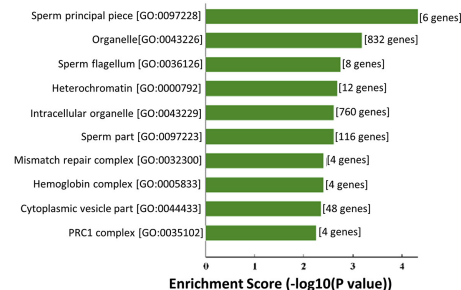
B

Sig GO terms of DE gene-BP



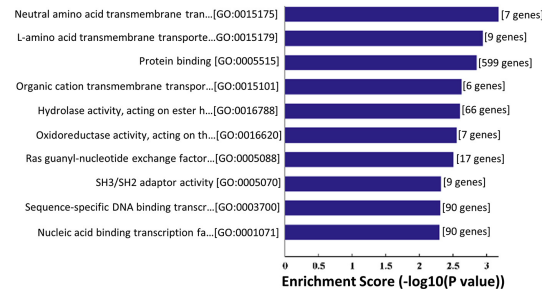
D

Sig GO terms of DE gene-CC



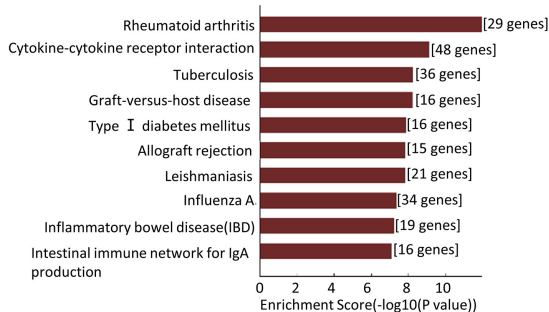
F

Sig GO terms of DE gene-MF



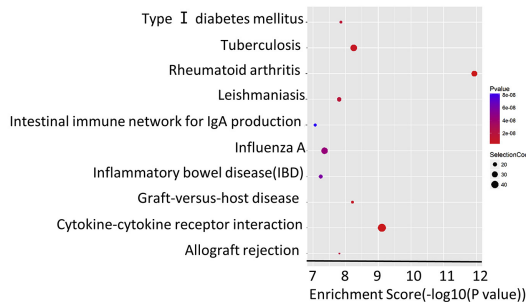
A

Sig pathway of DE gene



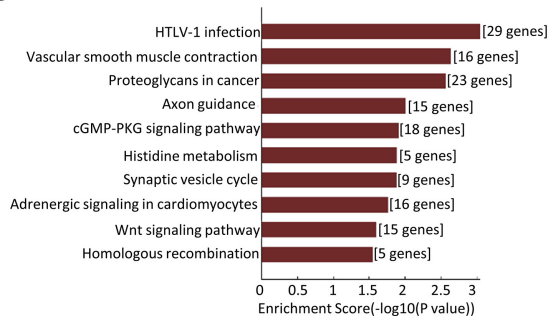
B

Sig pathway of DE gene



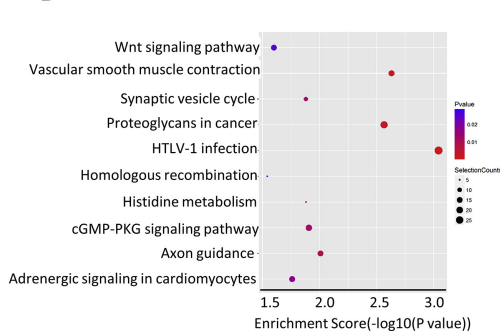
C

Sig pathway of DE gene

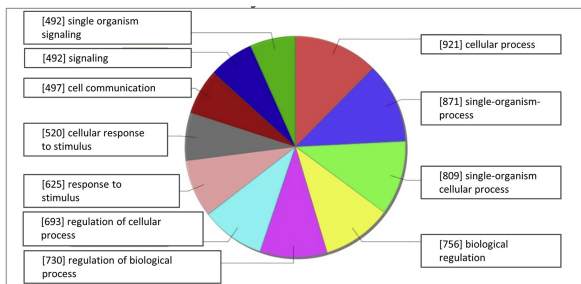


D

Sig pathway of DE gene

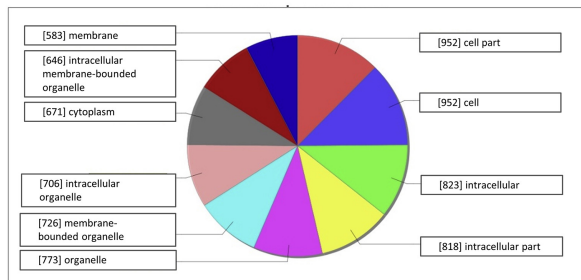


GO Biological Process Classification



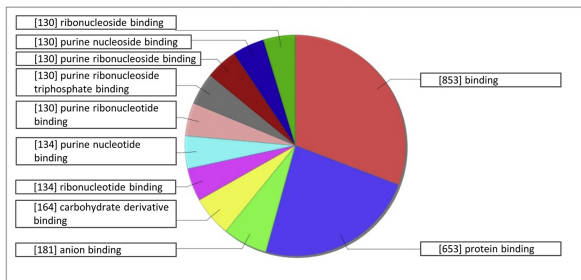
C

GO Cellular Component Classification



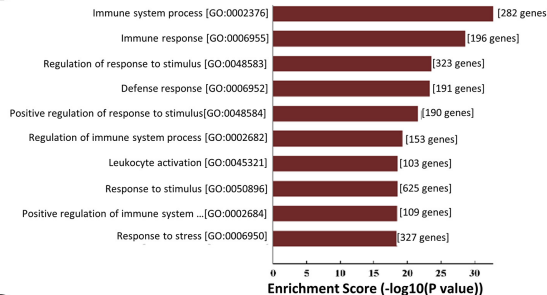
E

GO Molecular Function Classification



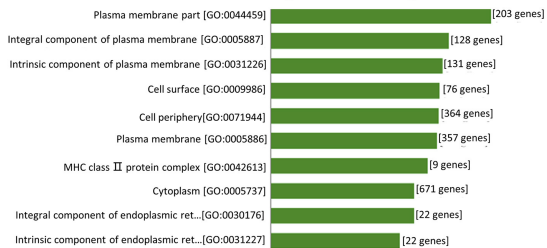
B

Sig GO terms of DE gene-BP



D

Sig GO terms of DE gene-CC



F

Sig GO terms of DE gene-MF

