Identification and Functional Profiling of Differentially Expressed Long Non-Coding RNAs in Nasal Mucosa with Allergic Rhinitis

Zhiqi Ma,^{1,*} Yaoshu Teng,^{1,*} Xiang Liu,¹ Jing Li,¹ Jiangwei Mo,¹ Min Sha¹ and Yong Li¹

¹Department of Otorhinolaryngology Head and Neck Surgery, Hangzhou First People's Hospital, Nanjing Medical University, Hangzhou, Zhejiang, P.R. China

Long non-coding RNAs (IncRNAs) have been proved to play important roles in a variety of human immune diseases. However, their pathological effects on the development of allergic rhinitis (AR) have not been clearly understood. The aim of this study was to determine the expression profile of IncRNAs in nasal mucosa of AR patients by IncRNA microarray and to predict potential roles of specific IncRNAs in the pathogenic mechanisms of AR by analysis of IncRNA-mRNA co-expression network, Gene Ontology (GO) and pathway. The IncRNA microarray analysis showed that a total of 2,259 IncRNAs (1,033 up-regulated and 1,226 down-regulated) and 704 mRNAs (157 up-regulated and 547 down-regulated) were significantly differentially expressed in the nasal mucosa samples from 4 AR patients as compared to those from 4 non-allergic subjects (fold change > 2; P < 0.05). In addition, the lncRNA-mRNA co-expression network contained 143 network nodes including 76 IncRNAs and 67 mRNAs, in which 117 significant correlation pairs presented as positive, and 108 pairs presented as negative. The results from GO and pathway analysis indicated that the IncRNAs-coexpressed mRNAs were enriched in several biological processes and cellular signaling pathways related to AR development, such as positive regulation of interleukin-13 secretion, Fc epsilon RI signaling pathway and NF-kappa B signaling pathway. To summary, our study provides important information on the molecular mechanisms and biological functions of these AR-related IncRNAs, which could be utilized for developing novel therapeutic strategies for AR.

Keywords: allergic rhinitis; bioinformatics; expression profile; long non-coding RNA; microarray Tohoku J. Exp. Med., 2017 June, **242** (2), 143-150. © 2017 Tohoku University Medical Press

Introduction

The incidence of allergic rhinitis (AR) is remarkably increasing recent years in industrial countries, which seriously affects the quality of life of AR patients (Meltzer 2016). AR is characterized by chronic inflammation of the nasal mucosa with hypersensitivity, hypersecretion and remodeling, which results from seasonal or perennial responses to specific allergens, such as pollens, dust mites, pets, pests, and molds. The pathological changes of their nasal mucosa are closely related to the massive infiltration and aberrant activation of many immune cells and the abnormal production of various inflammatory mediators (Baumann et al. 2013; Scadding 2014; Kamekura et al. 2016). Although numerous efforts have been exerted to verify the potential mechanisms following these changes, the treatment of AR is still quite difficult. Thus, it is very urgent to identify the key molecules that are involved in the AR pathologies, which may eventually lead us to better understand the underlying molecular mechanisms of AR pathogenesis and to further discover novel targets for the treatment of AR.

Long non-coding RNAs (lncRNAs), a subset of nonprotein-coding RNAs, are defined as the transcripts of more than 200 nucleotides in length. Emerging evidence has postulated that lncRNAs play important roles in transcriptional, post-transcriptional, or epigenetic regulation of gene expression and are involved in a variety of biological processes, such as cell differentiation, embryonic development and metabolism (Ponting et al. 2009; Kung et al. 2013; Pagani et al. 2013; Spurlock et al. 2016). Aberrantly expressed lncRNAs have been detected in the development and progression of many human and animal diseases, which as endogenous regulatory molecules may affect the mRNA expression levels of critical genes and modulate pathologic signaling pathways (Kazemzadeh et al. 2015). However, the expression and function of lncRNAs in the pathogenesis of AR remain unclear.

Received February 16, 2017; revised and accepted May 31, 2017. Published online June 14, 2017; doi: 10.1620/tjem.242.143. *These two authors contributed equally to this work.

Correspondence: Yong Li, Department of Otorhinolaryngology Head and Neck Surgery, Hangzhou First People's Hospital, Nanjing Medical University, No. 261, Huansha Road, Hangzhou, Zhejiang 310006, P.R. China.

e-mail: leeyung828@126.com

In this study, we examined the expression profile of lncRNA in nasal mucosa tissues from AR patients via lncRNA microarray analysis in order to identify differentially expressed lncRNAs. Subsequently, we also predicted and analyzed the potential functions of these lncRNAs by bioinformatics methods in order to find novel insights into AR pathogenesis and new clues of gene therapy for AR.

Materials and Methods

Patients and sample collection

This study was approved by the Ethics Committee of Hangzhou First People's Hospital, Nanjing Medical University, Hangzhou, China, and informed consent was obtained at enrollment from each participating subject. Nasal mucosal tissue samples were obtained surgically from the inferior turbinates from 19 patients with perennial AR (AR group, 8 males and 11 females; mean age 31.5 years; range 22-58 years) and 14 non-allergic patients with nasal septum deviation (Control group; 6 males and 8 females; mean age 35.8 years; range 23-62 years), who were admitted to the Department of Otolaryngology, Hangzhou First People's Hospital, Nanjing Medical University between 2013 and 2015. Each nasal cavity was locally treated with diluted epinephrine cotton tablets 10 min before sampling to reduce intraoperative nasal bleeding. All samples were immediately preserved in RNAlater Solution (Ambion, USA) after resection and then stored at -20°C until use. All patients in the AR group had a positive skin-prick test (SPT) to dust mites, animal dander, cockroaches, and/or molds; and a positive screening of specific IgE. Each AR patient was diagnosed based on his/her medical history, nasal endoscopic examination, an allergen skin-prick test, and a serum specific IgE assay. None of the patients had received topical or systemic corticosteroid therapy for 2 weeks prior to study recruitment. The subjects with a history of smoking and/or other immune system disorders, such as rheumatoid arthritis, systemic lupus erythematosus and scleroderma, were not including in this study.

RNA extraction

Total RNA was isolated from nasal mucosal tissues using TRIzol Reagent (Life technologies, Carlsbad, CA, US) following the manufacturer's instructions, and then quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The RNA integrity was inspected by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). Qualified RNA (RNA Integrity Number \geq 7.0, 28S/18S \geq 0.7) was further purified by RNeasy micro kit (QIAGEN, GmBH, Germany) and RNase-Free DNase Set (QIAGEN).

Microarray assay

Human lncRNA Microarray (4×180 K; v6.0) was manufactured at Shanghai Biotechnology Corporation (Shanghai, China), which contains 95,956 capture probes for 77,103 lncRNAs and 18,853 RNAs based on the most authoritative databases such as GENCODE v21, Ensembl, LNCipedia v3.1, Lncrnadb, Noncode v4 and UCSC. Microarray assay was carried out according to the manufacturer's protocols. Briefly, total RNA was amplified and labeled by Low Input Quick Amp WT Labeling Kit (Agilent technologies). Labeled cRNAs were purified using RNeasy mini kit (QIAGEN), and then they were hybridized with each slide using Gene Expression Hybridization Kit (Agilent technologies) in Hybridization Oven (Agilent technologies). The slides were washed by Gene Expression Wash Buffer Kit (Agilent technologies) after 17 hours hybridization. They were scanned by Agilent Microarray Scanner (Agilent technologies). Data were acquired with Feature Extraction software 10.7 (Agilent technologies). Raw data were normalized by Quantile algorithm, GeneSpring Software 11.0 (Agilent technologies). Differentially expressed lncRNAs and mRNAs with statistical significance between two groups were identified through volcano plot filtering (fold change ≥ 2.0 and P < 0.05). The Gene Cluster (v3.0) and Java TreeView software programs were used to perform the hierarchical cluster analysis of these differentially expressed lncRNAs and mRNAs.

Quantitative real-time reverse transcription PCR (qRT-PCR) validation

Total RNA was reversely transcribed into cDNA using PrimeScript RT Master Mix (TaKaRa, Dalian, China) following the manufacturer's instructions. qRT-PCR was performed by using SYBR Premix Ex Taq II (TaKaRa) on the 7900 HT Sequence Detection System (ABI, USA). The primer sequences were listed in Table 1 and were synthesized by Invitrogen (Shanghai, China). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control and relative quantitation of lncRNA expression was determined using the comparative threshold cycle (2^{-ddCt}) method.

Analysis of IncRNA-mRNA coexpression network

According to the normalized signal intensity of specific expression lncRNAs or mRNAs from our microarray assay, Pearson correlation coefficient (PCC) was calculated to evaluate the correlation between the differentially expressed lncRNAs and mRNAs. PCC ≥ 0.8 was considered as a statistically significant correlation pair. The co-expression network shown the significant pairs were constructed by using the Cytoscape software (The Cytoscape Consortium, San Diego, CA, USA).

Gene function analysis

The co-expression mRNAs were imported into the Database for Annotation, Visualization, and Integrated Discovery v6.8 (DAVID; http://david.abcc.ncifcrf.gov), which utilized Gene Ontology (GO) and pathway analysis to identify the enriched GO themes and cell signaling pathways of these mRNAs. The significant GO terms and pathways were retained in accordance with P < 0.05 and false discovery rate (FDR) < 0.05.

Statistical analysis

Statistical analyses were performed using the SPSS software for Windows (version 16.0; SPSS, Inc., Chicago, IL, USA). The differences between the two groups were determined using a two-tailed Student's *t*-test if the data are normally distributed. P < 0.05 was considered statistically significant.

Results

Overview of the expression profile of lncRNAs in AR nasal mucosa

To identify the differentially expressed lncRNAs associated with perennial AR, we examined the expression pattern of lncRNAs in the four nasal mucosa tissue samples from the AR group and the other four samples from the Control group using a microarray assay. To prepare for pre-

Table 1. Primer sequences used for qR1-

IncRNA	Forward primer (5' to 3')	Reverse primer (5' to 3')	Product (bp)
Inc-STRC-1:1	CGTGTAATCCTAGCACTGTGG	GGCTGGTCTTGAACTCCTGA	69
Inc-UBLCP1-4:2	GGAGTGTGAGGGTCCAGAAA	CTCTCTCCAGCACAGCACAC	131
Inc-RTL1-8:1	ACCTTGGAGCCTGCTTACG	GGTTCTTCCATGTCCAGCAT	75
NR_121637	AGATGCTGGGATGTGGATTTA	GGGAAACAGACTGAGGGAATC	67
Inc-GABPA-9:1	GGCTTGCTCTGTCACCTCAT	TGGCAATACAACTCTGACCTG	125
NR_103763	GCCAAACCTGGTCAAAGAAC	GGTTGCTGCCATTTTTCTTC	134
Inc-FRG2-3:1	CCTCTACTGTGTGTCCAACACC	GTGTGTTGCCTGTTTTTCTCC	69
CCL21	ATCCCAGCTATCCTGTTCTTGC	GTGAGGGGCTGACTCTTGCAG	209
APOA2	GACCGTGACTGACTATGGCA	GGCTGTGTTCCAAGTTCCAC	174
GAPDH	TGTTGCCATCAATGACCCCT	CTCCACGACGTACTCAGCG	201



Fig. 1. Alterations in lncRNA and mRNA expression profiles between the AR and Control groups. Volcano plots show differentially expressed lncRNAs (A) and mRNAs (B) in the nasal mucosa tissues from the AR group relative to those from the Control group. The horizontal line represents a *p* value of 0.05 and the vertical lines correspond to 2.0-fold up and down. Heat maps indicate hierarchical clustering results of differentially expressed lncRNAs (C) and mRNAs (D). Each row represents a lncRNA or mRNA, and each column represents a sample. Red and green colors indicate up-regulated and down-regulated expression, respectively. C: Control group. AR: AR group.

dicting target genes of lncRNAs, the expression levels of mRNAs in AR nasal mucosa were simultaneously analyzed using this microarray. Our data showed that a total of 2,259 lncRNAs (1,033 up-regulated and 1,226 down-regulated) and 704 mRNAs (157 up-regulated and 547 down-regulated) were significantly differentially expressed in the nasal mucosa tissue samples from the AR group relative to those from the Control group (fold change > 2; P < 0.05), as indicated by the volcano plots and heat maps (Fig. 1). The top

20 differentially expressed lncRNAs and mRNAs were listed in Tables 2 and 3. Among these lncRNAs, lnc-RAD9B-1:4 (\log_2 Fold change: 4.575409) and ENST00000505668 (\log_2 Fold change: -2.48488) were the most up-regulated and down-regulated lncRNAs, respectively. In addition, APOA2 (\log_2 Fold change: 4.681268) and PKDCC (\log_2 Fold change: -2.5797) were the most up-regulated and down-regulated mRNAs, respectively. Our data suggest that these 4 aberrantly expressed RNAs may play critical

Table 2. Top 20 differentially expressed lncRNAs in AR nasal mucosa tissues compared with non-allergic nasal mucosa tissues.

Up-regulated IncRNAs			Down-regulated IncRNAs				
IncRNA	Chromosome	log ₂ Fold change	P value	IncRNA	Chromosome	log ₂ Fold change	P value
Inc-RAD9B-1:4	chr12	4.575409	0.024898	Inc-KLF15-2:6	chr3	-3.56609	0.013021
Inc-CLMP-7:1	chr11	4.205477	0.003242	Inc-WDR1-8:2	chr4	-3.48981	0.044533
Inc-RELL1-3:1	chr4	3.262546	0.025392	Inc-JAKMIP2-1:1	chr5	-3.41471	0.01277
Inc-IKBKB-1:2	chr8	3.218508	0.048599	Inc-C2orf67-1:1	chr2	-3.3909	0.002839
Inc-FRG2-3:1	chr4	3.132287	0.045141	Inc-REXO1L1-3:1	chr8	-3.37594	0.009661
NR_103763	chr4	3.083562	0.000857	Inc-FAM114A1-3:1	chr4	-3.25897	4.49E-05
NR_039731	chr15	2.968512	0.001578	Inc-STRC-1:1	chr15	-3.16891	1.34E-05
ENST00000510351	chr4	2.960223	0.044065	Inc-ZNF862-1:7	chr7	-3.14145	5.52E-05
Inc-BLID-1:15	chr11	2.89539	0.023426	Inc-ACYP2-8:1	chr2	-2.94739	0.001316
Inc-NKX6-2-3:2	chr10	2.773267	0.043759	NR_003503	chr2	-2.89954	0.002232
ENST00000591257	chr9	2.544614	0.000136	Inc-G3BP1-2:3	chr5	-2.69427	3.62E-05
ENST00000441026	chr2	2.539452	4.16E-06	Inc-AP4S1-4:1	chr14	-2.68226	0.001085
Inc-NKX6-2-3:2	chr10	2.52588	0.041713	Inc-SLC29A1-2:1	chr6	-2.65483	0.000191
ENST00000420153	chr14	2.521062	0.034219	Inc-LEF1-3:6	chr4	-2.64904	0.00459
Inc-FAM172A-12:1	chr5	2.513385	0.000732	Inc-ANKRD54-1:1	chr22	-2.64417	0.04786
ENST00000424208	chr15	2.361368	0.000406	NR_110263	chr2	-2.53255	0.044538
Inc-CAMSAP2-4:1	chr1	2.341801	0.000166	Inc-C15orf41-3:1	chr15	-2.50798	0.004958
Inc-CCT5-16:1	chr5	2.327405	0.026981	Inc-RRM2B-7:3	chr8	-2.50151	0.014329
Inc-A1CF-2:1	chr10	2.277211	0.00047	Inc-ST8SIA5-2:1	chr18	-2.48548	0.015751
ENST00000591184	chr9	2.271016	0.031533	ENST00000505668	chr5	-2.48488	1.59E-05

Table 3. Top 20 differentially expressed mRNAs in AR nasal mucosa tissues compared with non-AR nasal mucosa tissues.

Up-regulated mRNAs			Down-regulated mRNAs				
mRNAs	Chromosome	log ₂ Fold change	P value	mRNAs	Chromosome	log ₂ Fold change	P value
APOA2	chr1	4.681268	0.01584	MEPE	chr4	-6.27932	0.000298
SERPINB2	chr18	2.443513	0.048449	IBSP	chr4	-6.08862	0.002319
ZC3H7B	chr22	2.407443	0.000492	SFRP5	chr10	-5.0718	0.012203
CCL21	chr9	2.325714	0.010102	SPINK14	chr5	-4.41066	0.009646
HELB	chr12	2.133718	0.039189	WIF1	chr12	-3.91077	0.00353
PBX2	chr6	2.116748	0.001016	PCSK1	chr5	-3.81344	0.008932
ALB	chr4	1.982338	0.003931	ZNF385B	chr2	-3.51136	0.003086
CCR4	chr3	1.95775	0.043851	FAM69C	chr18	-3.30767	0.004597
PEX6	chr6	1.928499	0.000525	MYOC	chr1	-3.2336	0.003157
MPRIP	chr17	1.914924	0.002887	SPINK1	chr5	-3.20929	0.009015
ALB	chr4	1.910788	0.001807	NOG	chr17	-3.00486	0.035256
KLK8	chr19	1.900787	0.007661	NPTX2	chr7	-2.99658	0.0016
CTSZ	chr20	1.811372	0.000327	APCDD1L	chr20	-2.9795	0.02735
USP29	chr19	1.784777	0.018706	MUC7	chr4	-2.93041	0.0368
PPAPDC1A	chr10	1.744907	0.017946	KCNK2	chr1	-2.90289	0.014477
TSSK1B	chr5	1.730168	0.005969	PRSS21	chr16	-2.85916	0.014673
IL7R	chr5	1.682561	0.019464	ISM1	chr20	-2.84051	0.037677
ALB	chr4	1.629744	0.006815	KRBA2	chr17	-2.71138	0.000556
MAPK11	chr22	1.610458	0.007483	PLA2G2A	chr1	-2.61712	0.025083
GBP4	chr1	1.609842	0.002851	PKDCC	chr2	-2.5797	0.01212

roles in the maintaining a normal function of the nasal mucosa.

QRT-PCR validation

To validate our microarray data, qRT-PCR was carried out to detect the expression levels of 7 lncRNAs and 2 mRNAs randomly selected from the 2,259 differentially expressed lncRNAs and 704 mRNAs in the 19 nasal mucosa tissue samples from the AR group and the 14 samples from the Control group, of which lnc-UBLCP1-4:2, Inc-RTL1-8:1 and NR_121637 were down-regulated, and Inc-GABPA-9:1, NR_103763, CCL21 and APOA2 were up-regulated in the nasal mucosa from the AR group. These results of qRT-PCR were consistent with those of the micro-array assay, which indicated a concordance rate of 77.8% (7/9) (Fig. 2).

Construction of lncRNA-mRNA co-expression network

To predict the target genes of lncRNAs and to investigate the potential interaction between the lncRNAs and



Fig. 2. Validation of lncRNA microarray data by qRT-PCR. Up-regulated and down-regulated lncRNAs are indicated by bars above and below the horizontal axis in the chart, respectively. The heights of the bars represent the values of log₂ fold changes.

mRNAs in nasal mucosa of AR patients, we analyzed the correlation between the top 300 differentially expressed IncRNAs and mRNAs from our microarray data by calculating PCC. Based on the value of PCC (PCC ≥ 0.8), the co-expression network were constructed and visualized by using the Cytoscape software. The network contained 143 network nodes including 76 lncRNAs and 67 mRNAs, in which 117 significant correlation pairs presented as positive, and 108 pairs presented as negative. The network also indicated that a single lncRNA could regulate the mRNA expression of multiple coding genes and some lncRNAs could co-regulate the expression of the same gene. For example, three coding genes (SRGAP2C, CBS and PKIB) were regulated by one lncRNA (NR_110169). NR_125386, together with ENST00000569912, regulated the mRNA expression of IL17RE (Fig. 3).

Enrichment analysis of lncRNAs-coexpressed mRNAs

To explore the potential functions of the differentially expressed lncRNAs in the progression of AR, we analyzed the functional enrichment (GO and pathway annotations) of their coexpressed mRNAs. The GO analytical data showed that several significantly over-represented GO terms were included in biological process, molecular function and cellular component (P < 0.05; FDR < 0.05). Among these GO terms, some were associated with inflammation response and immune dysfunction, such as positive regulation of inflammatory response (GO: 0050729), positive regulation of interleukin-13 secretion (GO: 0050808), secretory granule (GO: 0030141) and neuropeptide receptor activity (GO: 0008188). Pathway analysis indicated that 33 pathways were significantly enriched among the lncRNAs–coexpressed mRNAs. Many of these pathways were linked to activation or repression of immune cells, such as Fc epsilon RI signaling pathway, NF-kappa B signaling pathway, Tolllike receptor signaling pathway, T cell receptor signaling pathway and cytokine-cytokine receptor interaction (Fig. 4).

Discussion

AR, an airway allergic disease, is caused by hypersensitivity to one or multiple antigens resulting in chronic allergic inflammation of the nasal mucosa. The pathogenesis of AR is rather complex. Although many studies have shown that a large number of genes are involved in the pathogenesis of AR, it is unclear how these genes are regulated. With the rapid development of genomics technology, thousands of non coding RNAs such as miRNAs and IncRNAs have been discovered, and their roles in regulating coding genes have been intensively studied. Abnormal expression of non coding RNAs may contribute to a number of human allergic disorders. Using a miRNA microarray assay, Yu et al. (2011) showed that the miRNA expression profile was altered in the nasal mucosa of AR patients. And also, miR-143 was found to inhibit interleukin-13-induced inflammatory cytokine and mucus production in nasal epithelial cells from AR patients by targeting IL13R α 1 (Teng et al. 2015). These studies have suggested that the non-coding RNAs are involved in the regulation of AR-related gene expression and the pathological process of AR.

LncRNAs, an emerging member of non-coding RNAs, were simply considered as transcriptional noise in the beginning. However, recent studies have postulated that lncRNAs are also involved in important biological processes and are tightly related to the development of human diseases through the complex molecular mechanisms (Thai et al. 2013; McKiernan et al. 2014). To explore the expression levels of lncRNAs in airway allergic diseases, Tsitsiou et al. (2012) found a change in expression of lncRNAs, in circulatin CD8+ T cells from patients with severe asthma. Another report showed that lncRNAs BCYRN1 was important to regulate the protein level of transient receptor potential 1 (TRPC1) by increasing the stability of TRPC1. As a result, BCYRN1 was able to promote the proliferation and migration of airway smooth muscle cells in asthma (Zhang et al. 2016). Taken together, these studies have provided evidence that lncRNAs may play a critical role in the development of asthma. In the present study, we are the first to assess lncRNA expression profile in AR and non-allergic nasal mucosa using high throughput lncRNA microarray. Our data showed a total of 2,259 lncRNAs that were significantly expressed in the nasal mucosa from AR patients as compared to the same tissues obtained from non-allergic patients, with fold changes of 2 or more, which will help us understand the AR-related global transcriptome in depth.

Although without protein coding capability, increasing



Fig. 3. LncRNA-mRNA co-expression network. The rounded rectangle nodes represent lncRNAs, and the cycle nodes represent mRNAs. The red and green colors denote high and low expression, respectively. The lines with arrowheads or blunt ends represent the positive or negative regulation, respectively. The quantitative regulatory relationship between lncRNAs and mRNAs is indicated by the different areas of these nodes.

evidence has shown that lncRNAs could function as an emerging class of modulators in the regulation of coding gene expression and cellular activity. The major mechanisms include chromatin remodeling, complex assembly scaffolding, *cis* or *trans*- gene expression regulating, demethylation promoting, transcriptional regulation of target genes, and mRNA processing control, *etc* (Sone et al. 2007; Wahlestedt 2013; Qiao et al. 2016). In order to dissect the functions of lncRNAs, we used a lncRNA microarray assay and lncRNA-mRNA co-expression analysis to predict the potential functions of the differentially expressed

IncRNAs in the nasal mucosa of AR patients. Our data showed that these differentially expressed IncRNAs might contribute to the development and progression of AR by altering the mRNA expression levels of numerous target coding genes, within which we have specified and further confirmed 5 randomly selected lncRNAs.

To further understand the biological functions and molecular mechanisms of AR-related lncRNAs, we utilized GO and pathway analysis to identify the biological functions and signaling networks enriched among the lncRNAscoexpressed mRNAs. Notably, these mRNAs were



Fig. 4. Enrichment analysis of lncRNA co-expressed mRNAs. GO functional enrichment analysis (A-C) and pathway annotation (D) are performed, respectively. The significantly GO terms are included in biological process (A), cellular component (B) and molecular function (C). Ordinate is the significant GO/pathway term and the abscissa is the p value of the negative logarithm (P < 0.05).

involved in multiple immune regulations or responses, such as promoting interleukin-13 secretion, facilitating leukocyte migration, and enhancing inflammatory response. Pathway analysis profiling also showed that several signaling pathways in immune cells have been regulated by lncRNAscoexpressed mRNAs, including T cell receptor signaling pathway, Toll-like receptor signaling pathway, Fc epsilon RI signaling pathway, cytokine-cytokine receptor interaction, and NF-kappa B signaling pathway, etc. Interestingly, accumulated reports have showed that these biological processes, molecular functions, cellular components and signaling pathways are tightly associated to the pathogenesis of AR (Oliver et al. 2000; Wang and Zheng 2011; Couto Alves et al. 2013).

To validate the accuracy of high through put microarray assay, we further performed qRT-PCR to examine our microarray data, and found that the concordance rate was 77.8% (7/9), strongly suggesting the reliability of the microarray results. As we have completed the bioinformatics studies of lncRNAs in the development and progression of AR, more studies clarifying the exact molecular mechanisms of specific lncRNAs implicated in AR through the implementation of a series of functional experiments will be needed. These studies will provide us with a more complete understanding of lncRNA-mediated AR pathogenesis.

In conclusion, our study suggests that a portion of lncRNAs may participate in some specific biological processes and signaling pathways involved in the pathogenesis of AR through regulating target genes. Thus, our study will lay the foundation for future functional and mechanism studies of AR-related lncRNAs, even provide novel therapeutic targets for AR patients.

Acknowledgments

This work was supported by grants from the Science and Technology Development Project of Hangzhou (No. 20140733Q05), Medical and Health Technology Program of Zhejiang (No. 2013RCA041) and Zhejiang Provincial Natural Science Foundation of China (No. LY17H130001).

Conflict of Interest

The authors declare no conflict of interest.

References

- Baumann, R., Rabaszowski, M., Stenin, I., Tilgner, L., Scheckenbach, K., Wiltfang, J., Schipper, J., Chaker, A. & Wagenmann, M. (2013) Comparison of the nasal release of IL-4, IL-10, IL-17, CCL13/MCP-4, and CCL26/eotaxin-3 in allergic rhinitis during season and after allergen challenge. *Am. J. Rhinol. Allergy*, 27, 266-272.
- Couto Alves, A., Bruhn, S., Ramasamy, A., Wang, H., Holloway, J.W., Hartikainen, A.L., Jarvelin, M.R., Benson, M., Balding, D.J. & Coin, L.J. (2013) Dysregulation of complement system and CD4+ T cell activation pathways implicated in allergic response. *PLoS One*, 8, e74821.
- Kamekura, R., Yamashita, K., Jitsukawa, S., Nagaya, T., Ito, F., Ichimiya, S. & Himi, T. (2016) Role of Crosstalk between Epithelial and Immune Cells, the Epimmunome, in Allergic

Rhinitis Pathogenesis. Adv. Otorhinolaryngol., 77, 75-82.

- Kazemzadeh, M., Safaralizadeh, R. & Orang, A.V. (2015) LncRNAs: emerging players in gene regulation and disease pathogenesis. J. Genet., 94, 771-784.
- Kung, J.T., Colognori, D. & Lee, J.T. (2013) Long noncoding RNAs: past, present, and future. *Genetics*, **193**, 651-669.
- McKiernan, P.J., Molloy, K., Cryan, S.A., McElvaney, N.G. & Greene, C.M. (2014) Long noncoding RNA are aberrantly expressed in vivo in the cystic fibrosis bronchial epithelium. *Int. J. Biochem. Cell Biol.*, **52**, 184-191.
- Meltzer, E.O. (2016) Allergic Rhinitis: Burden of Illness, Quality of Life, Comorbidities, and Control. *Immunol. Allergy Clin. North Am.*, 36, 235-248.
- Oliver, J.M., Kepley, C.L., Ortega, E. & Wilson, B.S. (2000) Immunologically mediated signaling in basophils and mast cells: finding therapeutic targets for allergic diseases in the human FcvarepsilonR1 signaling pathway. *Immunopharma*cology, 48, 269-281.
- Pagani, M., Rossetti, G., Panzeri, I., de Candia, P., Bonnal, R.J., Rossi, R.L., Geginat, J. & Abrignani, S. (2013) Role of microRNAs and long-non-coding RNAs in CD4(+) T-cell differentiation. *Immunol. Rev.*, 253, 82-96.
- Ponting, C.P., Oliver, P.L. & Reik, W. (2009) Evolution and functions of long noncoding RNAs. *Cell*, **136**, 629-641.
- Qiao, J., Yao, H., Xia, Y., Chu, P., Li, M., Wu, Y., Li, W., Ding, L., Qi, K., Li, D., Xu, K. & Zeng, L. (2016) Long non-coding RNAs expression profiles in hepatocytes of mice after hematopoietic stem cell transplantation. *IUBMB Life*, 68, 232-241.
- Scadding, G. (2014) Cytokine profiles in allergic rhinitis. *Curr: Allergy Asthma Rep.*, **14**, 435.
- Sone, M., Hayashi, T., Tarui, H., Agata, K., Takeichi, M. & Nakagawa, S. (2007) The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons. J. Cell Sci., 120, 2498-2506.
- Spurlock, C.F., Crooke, P.S. & Aune, T.M. (2016) Biogenesis and Transcriptional Regulation of Long Noncoding RNAs in the Human Immune System. J. Immunol., 197, 4509-4517.
- Teng, Y., Zhang, R., Liu, C., Zhou, L., Wang, H., Zhuang, W., Huang, Y. & Hong, Z. (2015) miR-143 inhibits interleukin-13-induced inflammatory cytokine and mucus production in nasal epithelial cells from allergic rhinitis patients by targeting IL13Rα1. Biochem. Biophys. Res. Commun., 457, 58-64.
- Thai, P., Statt, S., Chen, C.H., Liang, E., Campbell, C. & Wu, R. (2013) Characterization of a novel long noncoding RNA, SCAL1, induced by cigarette smoke and elevated in lung cancer cell lines. *Am. J. Respir. Cell Mol. Biol.*, **49**, 204-211.
- Tsitsiou, E., Williams, A.E., Moschos, S.A., Patel, K., Rossios, C., Jiang, X., Adams, O.D., Macedo, P., Booton, R., Gibeon, D., Chung, K.F. & Lindsay, M.A. (2012) Transcriptome analysis shows activation of circulating CD8+ T cells in patients with severe asthma. J. Allergy Clin. Immunol., 129, 95-103.
- Wahlestedt, C. (2013) Targeting long non-coding RNA to therapeutically upregulate gene expression. *Nat. Rev. Drug Discov.*, **12**, 433-446.
- Wang, W. & Zheng, M. (2011) Nuclear factor kappa B pathway down-regulates aquaporin 5 in the nasal mucosa of rats with allergic rhinitis. *Eur. Arch. Otorhinolaryngol.*, 268, 73-81.
- Yu, S., Zhang, R., Liu, G., Yan, Z., Hu, H., Yu, S. & Zhang, J. (2011) Microarray analysis of differentially expressed microRNAs in allergic rhinitis. *Am. J. Rhinol. Allergy*, 25, e242-e246.
- Zhang, X.Y., Zhang, L.X., Tian, C.J., Tang, X.Y., Zhao, L.M., Guo, Y.L., Cheng, D.J., Chen, X.L., Ma, L.J. & Chen, Z.C. (2016) LncRNAs BCYRN1 promoted the proliferation and migration of rat airway smooth muscle cells in asthma via upregulating the expression of transient receptor potential 1. *Am. J. Transl. Res.*, 8, 3409-3418.