

lncRNA profiling in NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts

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Lilei Wang¹, Ying Zhou² and Yubao Cui³ 

Abstract

Airway epithelium cells are the first line of defense against airborne allergens. When cultured, epithelial cells can be exposed to various allergens, providing an ideal model to investigate allergic disorders. This study sought to characterize the profile of long noncoding (lnc) RNAs, which can regulate gene expression and exert functions in diverse cellular processes, in airway epithelial cells exposed to house dust mite allergens. NCI-H292 cells were exposed to house dust mite extract for 24 h. RNA expression was profiled in exposed and unexposed cells. There were 270 lncRNAs that were differentially expressed (fold change ≥ 2 , $P < 0.05$) in NCI-H292 cells after stimulation with *Dermatophagoides farinae* (house dust mite) extracts. Furthermore, 119 lncRNAs and 22 messenger RNAs were co-expressed. Gene Ontology analysis showed that these under-regulated and up-regulated lncRNAs were associated with biological process, cellular component, and molecular function. After bioinformatic analysis of significantly regulated signaling pathways, we found these lncRNAs may target 16 gene pathways, including glycolysis, axon guidance, ErbB signaling, and mitogen-activated protein kinases (MAPK) signaling. The identification of differentially regulated lncRNAs in NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts, as well as their target gene pathways, can provide insight to the etiology and pathogenesis of allergy.

Keywords

airway epithelium, allergen, house dust mite, long noncoding (lnc) RNAs, microarray

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Introduction

Asthma is a complex lung disorder provoked by environmental stimuli, including allergens. Since airborne allergens disturb the airway epithelium, epithelial abnormalities, such as epithelial damage and basement membrane thickening, are detectable before clinical symptoms of asthma.¹ Cultured airway epithelial cells provide a tractable model to asthma pathology. For example, NCI-H292 cells (squamous cell carcinoma) exposed to the major allergen Phl p 1 purified from an extract of Timothy grass pollen extract (GPE) exhibit different gene expression compared to cells exposed to complete GPE.² Similarly, in response to house dust mite

allergen—one of the most common causes of allergic asthma—NCI-H292 cells exhibit a greater number of differentially expressed genes than either healthy or allergic epithelium.³ While some

¹Shandong Institute of Parasitic Diseases, Shandong Academy of Medical Sciences, Jining, China

²Department of Pediatrics Laboratory, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi, China

³Department of Clinical Laboratory, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi, China

Corresponding author:

Yubao Cui, Department of Clinical Laboratory, Wuxi People's Hospital Affiliated to Nanjing Medical University, No. 299, Qingyang Road, Wuxi 214023, Jiangsu, China.
Email: ybcui1975@hotmail.com



of the expression changes may derive from the instability of a cultured cancer cell line, expression differences were also noted between stimulated and unstimulated cells.³ Thus, cultured epithelial cells exposed to various allergens remain useful as a model for investigating allergy to better understand the genetic contributions to the disease.

Long noncoding RNAs (lncRNAs) are non-protein-coding RNA transcripts that exert transcriptional and post-transcriptional regulation on messenger RNAs (mRNAs) and microRNAs.⁴ Abnormal lncRNA expression can cause cell dysfunction and promote disease progression by dysregulating their targets.^{5–7} In a study of mRNA and noncoding RNA expression in circulating CD8⁺ T cells in patients with severe asthma, CD8⁺ T-cell function appeared to be regulated by changes in the expression of multiple species of lncRNA.⁸ However, little is known about the role of lncRNA in asthma. To determine the contribution of lncRNAs to asthma pathology, here NCI-H292 cells were exposed to an extract from house dust mites. House dust mites are a major source of allergic disorders, such as allergic asthma, chronic rhinitis, atopic dermatitis, and atopic eczema. The most important house dust mites are *Dermatophagoides pteronyssinus* worldwide, *D. farinae* in drier areas, and *Blomia tropicalis* in subtropical and tropical regions.⁹ *D. farinae* extracts were used here given their important contribution to allergic asthma.

Materials and methods

Cell culture

NCI-H292 human airway epithelial cells (Cell Bank, Shanghai Institute for Biological Science, Chinese Academy of Science, Shanghai, China) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, Breda, the Netherlands) supplemented with 1.25 mM of L-glutamine, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 10% (v/v) of fetal bovine serum (HyClone, Logan, UT, USA). Cells were grown in fully humidified air containing 5% CO₂ at 37°C and were subcultured weekly, as was done in a previous study.³

Induction experiment

Induction was performed in accordance with a previous report in which the authors found that a

24-h exposure to *D. farinae* extract evoked an inflammatory cytokine response.³ Cells were cultured for 2 weeks, until reaching 80% confluence. Cells were incubated with Hanks' balanced salt solution (HBSS) for 24 h, then culture medium was removed. *D. farinae* extract diluted in HBSS (2 µg/mL) or HBSS alone (control condition) was applied to cells for 24 h. Subsequently, cells were harvested by centrifugation and used for RNA extraction. Each treatment was performed in triplicate.

Dermatophagoides farinae extract

Dermatophagoides farinae extract was produced by Greer Laboratories (Lenoir, NC, USA) as a lyophilized powder. It was dissolved in phosphate buffer saline (PBS), and then dialyzed against PBS and diluted to a stock concentration of 8 µg/mL.

RNA extraction and purification

Total RNA was isolated using TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. Samples were checked for integrity using an Agilent Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) and an RNA Integrity Number with cut-off > 7.0. Total RNA was further purified by RNeasy micro kit (Qiagen GmbH, Hilden, Germany) and RNase-Free DNase Set (Qiagen GmbH).

RNA amplification and labeling

Total RNA was amplified and labeled by Low Input Quick Amp Labeling Kit, One-color (Agilent), following the manufacturer's instructions. Labeled complementary RNA (cRNA) were purified by RNeasy mini kit (Qiagen GmbH).

Array hybridization

lncRNAs were analyzed using Agilent's human lncRNA 4 × 180 K array (design ID: 045142), which contains 175,906 probes with length of 60 nt each. The probes cover more than 41,053 lncRNAs from NCBI RefSeq (7055), NCBI other (3436), UCSC (2340), ensemble (3675), Homolog (1110), LNCRNA-DB (56), ncRNA-SCAN (1181), Agilent-G3 (13,740), Agilent Other (1828), and other Database (6632), as well as 29,417 mRNAs from SurePrint G3 Human Gene (27,958 mRNA)

and other. For the array, each transcript is represented using 1–5 probes.

Each slide was hybridized with 1.65- μ g Cy3-labeled cRNA using a Gene Expression Hybridization Kit (Agilent) and a hybridization oven (Agilent) according to the manufacturer's instructions. After a 17-h hybridization, slides were washed in staining dishes (Thermo Shandon, Waltham, MA, USA) with Gene Expression Wash Buffer Kit (Agilent), following the manufacturer's instructions.

Data acquisition

Microarrays were scanned using an Agilent Microarray Scanner (Agilent) with default settings of dye channel green, scan resolution = 3 μ m, 20 bit. Scanned images were imported as TIFFs into Feature Extraction software 10.7 (Agilent). Raw data were normalized by a quantile normalization and the robust multichip average (RMA) algorithm in the Gene Spring Software 11.0 (Agilent). Probe-level files and mRNA-level files were generated after normalization. Differentially expressed lncRNAs and mRNAs were identified through fold-change filtering. Data have been submitted to the Gene Expression Omnibus (GEO) database.

Gene function analysis

The predicted target genes were input into the Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov/>) for Gene Ontology (GO) analysis of the molecular functions. Additionally, KEGG (Kyoto Encyclopedia of Genes and Genomes) database (<http://www.genome.ad.jp/kegg/>) and BioCarta (<http://www.biocarta.com>) were used to analyze the target genes and their potential functions in predicted pathways. The recommended *P* value cut-off was 0.05.

Quantitative real-time polymerase chain reaction

Total RNA was extracted using TRIzol Reagent following the manufacturer's instructions and checked for RNA integrity. Qualified total RNA was further purified by RNeasy micro kit and RNase-Free DNase Set and then reverse-transcribed using iScript cDNA

synthesis kit (Bio-Rad, Hercules, CA, USA) in accordance with the manufacturer's instructions. lncRNA expression in cells was measured by quantitative polymerase chain reaction (qPCR) using ABI Power SYBR Green PCR Master Mix (ABI, Foster City, CA, USA) on a 7900HT Sequence Detection System (ABI).

Eight lncRNAs that were significantly up-regulated according to microarray analysis were evaluated by real-time polymerase chain reaction (RT-PCR). The first strand complementary DNA (cDNA) was synthesized in a total reaction volume of 20 μ L, including 0.5 μ g total RNA, 4 μ L reaction mix (5 \times), 1 μ L iScript Reverse transcriptase, and 14.5 μ L H₂O. RT reaction was performed for 5 min at 25°C, 30 min at 42°C, 5 min at 30°C, denaturation for 5 min at 85°C, and held at 4°C. Subsequently, the SYBR Green qPCR was performed in a total reaction volume of 10 μ L, including 5 ng of the first cDNA strand template, 5- μ L 2 \times SYBR Green PCR buffer, 0.5 μ L forward primer (10 μ mol), 0.5 μ L reverse primer (10 μ mol), and double-distilled water. The reaction was performed as follows: 50°C for 2 min; an initial denaturation step of 95°C for 10 min; 40 cycles of 95°C (15 s) and 60°C (1 min); and a final extension step at 72 °C for 5 min. All experiments were done in triplicate. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primers used in this study are listed in Supplemental Table 1. For quantitative results, the expression of each lncRNA was represented as a fold change using the 2^{- $\Delta\Delta$ Ct} method.

Statistical analyses

Cell samples were processed in triplicate, and all data are expressed as mean \pm standard deviation. Student's t-test was used to compare two variables of microarray data. Expression differences were analyzed by fold change, and a difference with *P* < 0.01 was considered statistically significant. The false discovery rate was also calculated to correct the *P* value. Furthermore, a differential expression of each lncRNA or mRNA in NCI-H292 cells compared before and after stimulation with *D. fariniae* extracts was analyzed using Student's t-test with SPSS (Version 17.0 SPSS, Chicago, IL, USA). *P* < 0.05 was considered significant.

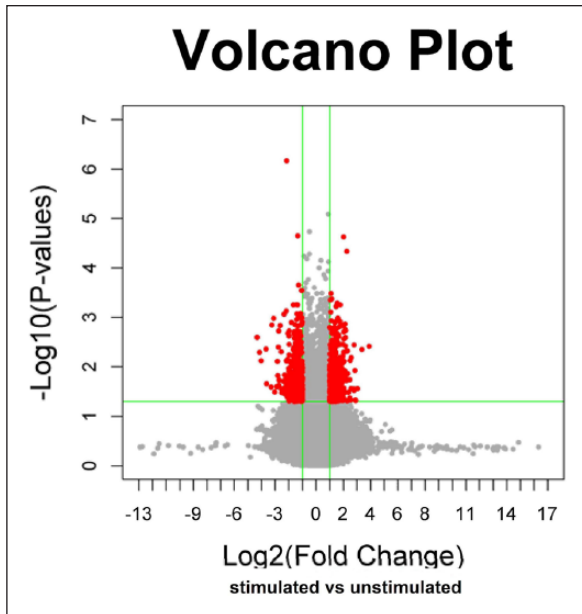


Figure 1. The volcano plot for differential lncRNAs expressed in NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts. x-axis, Log_2 (fold change); y-axis, $-\log_{10}$ (P value). The line in parallel with x-axis, P value = 0.05; the line in parallel with y-axis, fold change = 2. The area in red was drawn for lncRNAs expressed differentially in NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts. Expression was determined as the mean of three replicates.

Results

Differentially expressed lncRNAs in NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts

NCI-H292 cells either stimulated with *D. farinae* extract diluted in HBSS or treated with HBSS alone (unstimulated) were analyzed for RNA expression differences by microarray. We identified 270 lncRNAs significantly differentially expressed (fold change ≥ 2 , $P < 0.05$); specifically, 142 were up-regulated and 128 down-regulated after stimulation with *D. farinae* extracts (Figure 1). Table 1 shows 38 up-regulated and 38 down-regulated at a higher significance level ($P \leq 0.01$, and a fold change ≥ 2 or fold change ≤ 0.5). To verify the results from the microarray experiments, the expression of eight lncRNAs was validated by quantitative real-time polymerase chain reaction (qRT-PCR). The expression levels of these lncRNAs determined by qRT-PCR were consistent with those by microarray (Supplemental Figure 1).

Additionally, we identified 1438 mRNAs significantly differentially expressed (fold change ≥ 2 ,

$P < 0.05$), with 987 up-regulated and 451 down-regulated in *D. farinae*-stimulated cells versus unstimulated cells (Supplemental Table 2). Our result is similar to a previous report.³

GO and pathway analyses

GO analysis through several bioinformatics tools, included DAVID and KEGG, was performed to begin dissecting the potential contributions of the lncRNAs differentially expressed following stimulation of NCI-H292 cells with *D. farinae* allergens. We found that differentially expressed lncRNAs were associated with cellular process (ontology: biological process), cell (ontology: cellular component), and binding (ontology: molecular function) (Figure 2). Pathway analysis determined that these lncRNAs can target 16 gene pathways (Table 2). Based on the differentially expressed lncRNAs and their targeted pathways, we established an interaction network (Supplemental Figure 2). This analysis identified *PFKL* and *GAPDH*, components of the glycolysis pathway, which were predicted to be targets of the lncRNAs AK074162 and ENSTD0000499202, respectively. The network for axon guidance includes genes *PTK2*, *KRAS*, *GNAI2*, *EFNA2*, *SEMA3E*, and *ITGB1*. *PTK2* appears to be targeted by HMLincRNA565; *KRAS* by Uc001rgr; *GNAI2* by DA201452; *EFNA2* by AK024373; *SEMA3E* by uc001pry, ENST00000417751, BC008015, DB497821, and ENST00000509669; and *ITGB1* by AF147315.

Construction of lncRNA:mRNA association networks

We performed a bioinformatics analysis to predict potential lncRNA targets in the database, using target prediction programs. Subsequently, interactions between the lncRNAs and their targets were drawn by Cytoscape software (v2.8.3); these comprised 506 lncRNAs and 123 mRNAs (Supplemental Table 2). Using the lncRNA:mRNA association networks, we found that 119 lncRNAs and 22 mRNAs were co-expressed (Figure 3).

Discussion

This study reports the first lncRNA profiling in NCI-H292 cells in response to *D. farinae* extracts. We identified 270 lncRNAs that were either up- or

Table 1. List of lncRNA transcripts differentially expressed in NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts meeting the criteria of $P \leq 0.01$, and a fold change ≥ 2 or fold change ≤ 0.5 .

Down-regulation				Up-regulation			
ProbeName	P values	Fold change	Target_id	ProbeName	P values	Fold change	Target_id
pl026699	0.002551688	0.049921026	ENST00000509669	pl007022	0.009383119	2.01778346	HMlincRNA798
pl061602	0.005120169	0.056673363	NR_027029	pl059882	0.007343201	2.018204385	gnl UG Hs
pl017117	0.007571792	0.061278659	AK094780	pl105723	0.005503883	2.027408248	
agiseq31014	0.004362928	0.078377219	ENST00000450888	pl054765	0.009685332	2.039516058	
pl091245	0.001435942	0.106812681		agiseq5432	0.00167986	2.062459916	NR_003044
CUST_2634_	0.001054225	0.115789584	ENST00000424650	CUST_4265_	0.004198792	2.097687638	ENST00000433843
PI428631609				PI428631609			
pl020462	0.007716195	0.140473576	BG200518	pl032158	0.008620776	2.098155888	BQ329856
pl020040	0.00401442	0.146714341	uc002syn	pl011122	0.008576561	2.114286718	AK123210
pl014049	0.001883852	0.150308921	ENST00000443163	pl040961	0.000326657	2.132671666	exon2259
CUST_8793_	0.001477988	0.162226874	ENST00000473817	pl055914	0.009005299	2.152046694	uc003mwg
PI428631609							
pl014845	0.00497862	0.170017052	ENST00000498832	pl026475	0.006796269	2.164882047	ENST00000417751
pl096179	0.000738371	0.220587397	uc.339+	pl014137	0.005425761	2.176143984	
pl047191	0.00212464	0.239165609		pl059861	0.005664215	2.179985169	DB497821
pl012156	0.001895924	0.25497346	NR_002866.2	pl011724	0.004918058	2.257942593	
pl084196	0.001278383	0.28681123		pl050170	0.005283143	2.333246789	
pl001657	0.00621968	0.32267286	AK001097	pl093238	0.002179301	2.362686367	uc003nhj
pl017960	0.004759181	0.328738173	AF283773	pl064493	0.008823399	2.378147082	
pl077611	0.002601467	0.331224127	ENST00000508968	pl095902	0.003680506	2.603789027	AK023737
pl089052	0.007509207	0.348607813	uc001vii	pl072670	0.001222418	2.746266733	BC008015
pl010623	0.005593987	0.362295994		pl015027	0.005104226	2.755761838	BC041848
pl086672	0.004424856	0.363072373		pl030151	0.002481112	2.811623767	uc002xhi
pl083437	0.001858985	0.381160003		pl105889	0.002298616	2.849014704	
agiseq16053	0.008589558	0.383083212	TCONS_00025464	pl102233	0.001698029	2.86941163	gnl UG Hs
agiseq45528	0.001976147	0.386914318	ENST00000444965	pl076787	0.000517801	2.944129898	gnl UG Hs
pl051020	0.008337345	0.388942509		pl039966	0.001029728	3.017443067	
agiseq3212	0.000556772	0.389554515	ENST00000447194	CUST_12878_	0.008274064	3.072069923	ENST00000499202
				PI428631609			
pl083817	0.005655209	0.401352547	uc003ysg	agiseq4061	0.008792142	3.41887713	TCONS_00020732
pl023688	0.001488721	0.401700308		pl023141	0.003531875	3.875856107	
CUST_12586_	0.009223068	0.405738984	ENST00000525363	pl111117	0.005065994	4.035506777	
PI428631609							
pl006223	0.001030314	0.409740637		pl097869	0.002734214	4.091041131	DA201452
pl073808	0.006070873	0.410454638	uc010jpk	pl029486	0.001740854	4.358524277	
pl071245	0.000220397	0.41240135		pl035448	0.00495818	4.647281738	AF288406
agiseq23303	0.004613261	0.41305524	TCONS_00024373	pl003369	0.004790425	4.921086088	HMlincRNA1577
pl033918	0.005921428	0.420285246		agiseq11240	0.007493107	5.649317013	BG687505
pl026204	0.003727625	0.452881012	AY129027	pl007816	0.006468404	5.999920175	
agiseq46954	0.005254003	0.453181225	TCONS_00025779	agiseq20969	0.003597954	6.88518551	ENST00000435643
pl040561	0.003160344	0.467211366		pl012345	0.00445474	10.20455567	
pl017025	0.001255417	0.48981737	ENST00000432142	agiseq17394	0.003842145	14.90923206	ENST00000481004

down-regulated by more than twofold. Furthermore, we predicted the potential lncRNA targets to derive an interaction network between lncRNAs and mRNAs. Co-expression was found for 119 lncRNAs and 22 mRNAs. These interactions should be confirmed in patients with asthma or in animal models to better understand their contribution to asthma pathology. The findings should also be validated in other models to rule out the potential

contributions of accumulated mutations in a cancer cell line.

Pathway analysis identified 16 gene pathways in which the differentially expressed lncRNAs provide regulation; many of these pathways are related to development of asthma or allergy. For example, the allergen-induced early asthma response in rats is associated with glycolysis, calcium binding, and mitochondrial activity.¹⁰

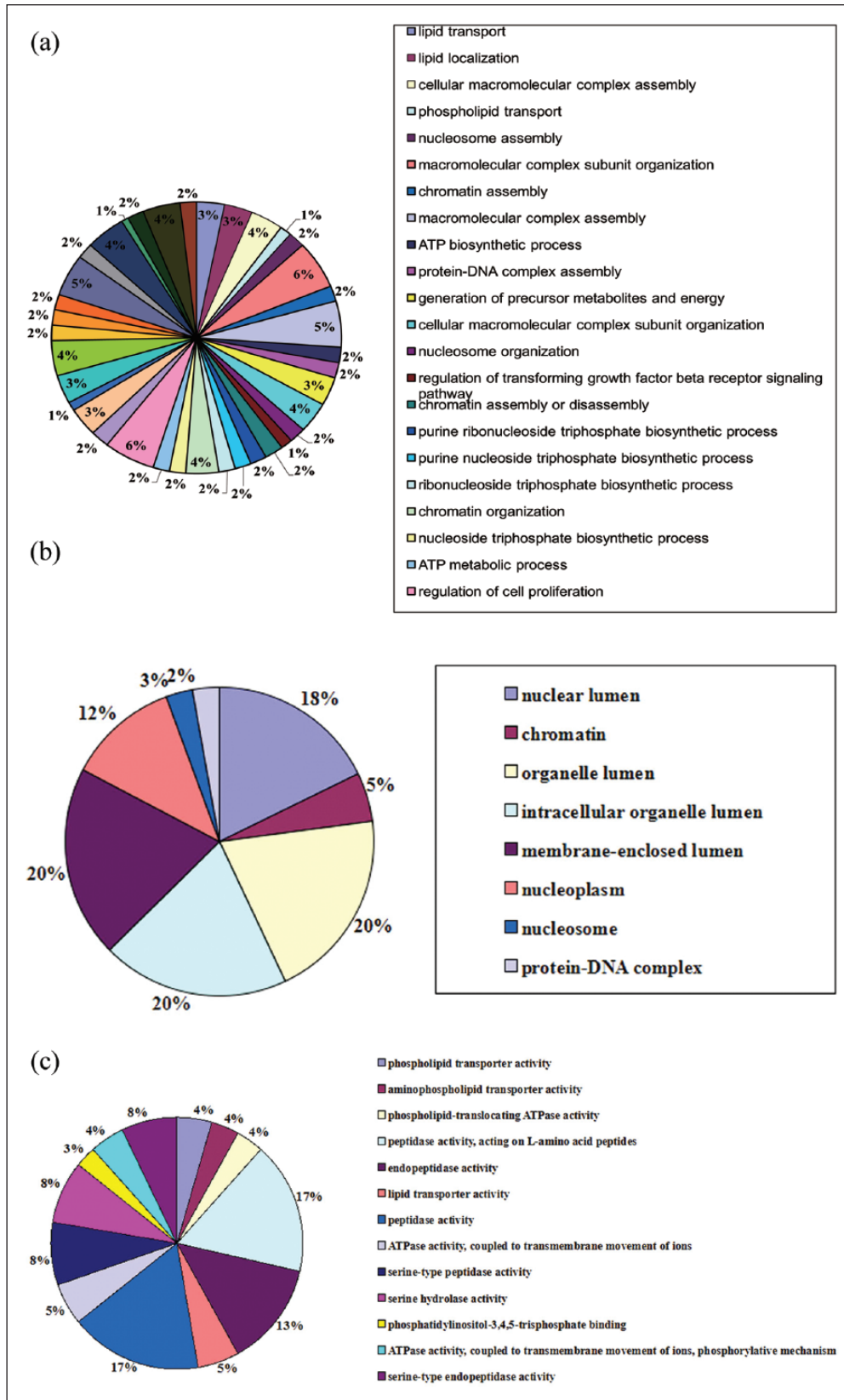


Figure 2. Gene Ontology (GO) enrichment analysis of lncRNA targets ($P < 0.01$): (a) GO analysis of lncRNA-target genes according to biological process, (b) GO analysis of lncRNA-target genes according to cell component, and (c) GO analysis of lncRNA-target genes according to molecular function.

Table 2. Pathway analysis of the predicted target genes for differentially expressed lncRNAs.

Pathway terms	Counts	Percentage (%)	P value	Genes
Systemic lupus erythematosus	7	2.430555556	0.007801038	HIST1H2BK, HIST1H2AG, HIST1H2BI, HIST1H2BJ, ELANE, HIST1H2AH, HIST1H4I, HIST1H3G, HIST1H4H
Alzheimer's disease	9	3.125	0.007807456	ATF6, ATP5D, NDUFS7, UQCRI1, ATP2A1, ATP5G2, NDUFC1, ITPR3, GAPDH
Internal ribosome entry pathway	3	1.041666667	0.008845197	SNORD10, PTBP1, EIF4A1, SNORA67
Glycolysis pathway	3	1.041666667	0.021952	PFKL, GAPDH, ENO1
Axon guidance	6	2.307692308	0.040864977	PTK2, KRAS, GNAI2, EFNA2, SEMA3E, ITGB1
Small-cell lung cancer	5	1.736111111	0.060748942	AKT1, PTK2, PIAS2, ITGB1, MYC
Endometrial cancer	4	1.388888889	0.063670713	AKT1, KRAS, APC2, MYC
ErbB signaling pathway	5	1.736111111	0.067384111	AKT1, PTK2, KRAS, SHC2, MYC
PTEN-dependent cell cycle arrest and apoptosis	3	1.041666667	0.079626969	AKT1, PTK2, ITGB1
Trefoil factors initiate mucosal healing	3	1.041666667	0.079626969	AKT1, PTK2, ITGB1
Acute myeloid leukemia	4	1.388888889	0.082526478	AKT1, PPARD, KRAS, MYC
Insulin signaling pathway	6	2.083333333	0.090022222	AKT1, KRAS, PYGM, SOCS1, MKNK2, SHC2
Alzheimer's disease	6	2.307692308	0.091589769	ATF6, NDUFS7, UQCRI1, ATP2A1, NDUFC1, ITPR3
Regulation of eIF4E and p70 S6 kinase	3	1.041666667	0.092658214	AKT1, SNORD10, EIF4A1, SNORA67
Ubiquitin-mediated proteolysis	6	2.083333333	0.094431239	UBE2D3, SOCS1, BIRC6, PIAS2, CDC34, UBOX5
MAPK signaling pathway	9	3.125	0.098722751	AKT1, KRAS, RASGRP2, MAP4K2, MKNK2, FGF22, MYC, HSPA8, CDC25B

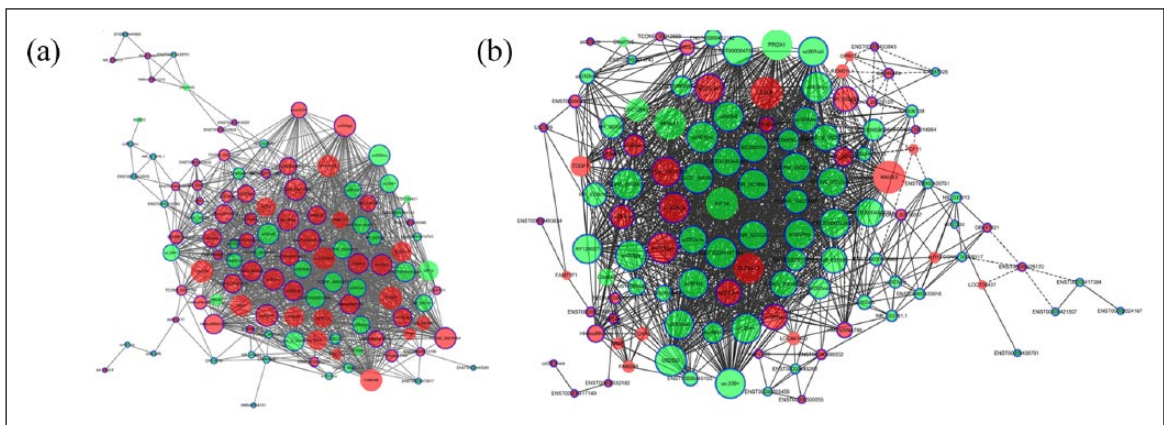


Figure 3. Co-expression network for lncRNAs and mRNAs according to array data and bioinformatic analysis using DAVID and Cytoscape (v2.8.3): (a) NCI-H292 cells before stimulation with *Dermatophagoides farinae* extracts and (b) NCI-H292 cells after stimulation with *Dermatophagoides farinae* extracts. Up-regulation is marked in red, down-regulation is marked in green, and the rings marked in blue represent lncRNA. The solid line represents positive correlations, while the dashed line represents negative correlations.

Similarly, aerobic glycolysis, which promotes T-cell activation, is increased in asthma.¹¹ House dust mite allergen Der p 1 exposure can activate the p38 MAPK pathway in BEAS-2B cells (bronchial epithelial cells) and eosinophils,¹² and house dust mite allergen Der p 2 induces nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and MAPK-dependent activation of bronchial epithelial cells.¹³

The predicted lncRNA interactions with mRNA (Supplemental Figure 2) appear to be involved in the regulation of some of these pathways. For example, the mRNAs related to glycolysis, *PFKL*, and *GAPDH* were regulated by the lncRNAs AK074162 and ENSTD0000499202, respectively. Semaphorin3A (*Sema3A*), an axon guidance molecule in allergy in mice,¹⁴ was regulated by 10 lncRNAs including uc001pry, ENST00000417751,

BC008015, DB497821, ENST00000509669, and five unnamed lncRNAs. These theoretical regulations will be validated experimentally.

In sum, we have obtained preliminary data that exposure of bronchial epithelial cells to house dust mite allergen results in expression changes in lncRNAs and mRNAs. The lncRNA–mRNA co-expression network and pathway analysis reveals avenues for further exploration of the contribution of lncRNAs to allergy response. Further characterization of these lncRNA genes and their modes of action will allow their use for improved diagnosis, monitoring of progression, and target therapies.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORCID iD

Yubao Cui  <https://orcid.org/0000-0001-7736-8012>

References

1. Barbato A, Turato G, Baraldo S, et al. (2006) Epithelial damage and angiogenesis in the airways of children with asthma. *American Journal of Respiratory and Critical Care Medicine* 174: 975–981.
2. Röschmann KI, van Kuijen AM, Luiten S, et al. (2012) Comparison of Timothy grass pollen extract and single major allergen-induced gene expression and mediator release in airway epithelial cells: A meta-analysis. *Clinical and Experimental Allergy* 42: 1479–1490.
3. Vroiling AB, Duinsbergen D, Fokkens WJ, et al. (2007) Allergen induced gene expression of airway epithelial cells shows a possible role for TNF-alpha. *Allergy* 62: 1310–1319.
4. Nagano T and Fraser P (2011) No-nonsense function for long RNAs. *Cell* 145: 178–181.
5. Osterlund C, Grönlund H, Polovic N, et al. (2009) The non-proteolytic house dust mite allergen Der p 2 induce NF-B and MAPK dependent activation of bronchial epithelial cells. *Clinical and Experimental Allergy* 39: 1199–1208.
6. Cheetham SW, Gruhl F, Mattick JS, et al. (2013) Long noncoding RNAs and the genetics of cancer. *British Journal of Cancer* 108: 2419–2425.
7. Pastori C and Wahlestedt C (2012) Involvement of long noncoding RNAs in diseases affecting the central nervous system. *RNA Biology* 9: 860–870.
8. Tsitsiou E, Jiang X, Booton R, et al. (2012) Transcriptome analysis shows activation of circulating CD8+ T cells in patients with severe asthma. *Journal of Allergy and Clinical Immunology* 129: 95–103.
9. Arlian LG and Morgan MS (2003) Biology, ecology, and prevalence of dust mites. *Immunology and Allergy Clinics of North America* 23: 443–468.
10. Xu YD, Cui JM, Yu W, et al. (2010) The early asthmatic response is associated with glycolysis, calcium binding and mitochondria activity as revealed by proteomic analysis in rats. *Respiratory Research* 11: 107.
11. Ostroukhova M, Goplen N, Karim MZ, et al. (2012) The role of low-level lactate production in airway inflammation in asthma. *American Journal of Physiology* 302: L300–L307.
12. Thomas WR, Smith WA and Hales BJ (2002) Characterization and immunobiology of house dust mite allergens. *International Archives of Allergy and Immunology* 129: 1–18.
13. Batista PJ and Chang HY (2013) Long noncoding RNAs: Cellular address codes in development and disease. *Cell* 152: 1298–1307.
14. Yamaguchi J, Nakamura F, Aihara M, et al. (2008) Semaphorin3A alleviates skin lesions and scratching behavior in NC/Nga mice, an atopic dermatitis model. *Journal of Investigative Dermatology* 128: 2842–2849.