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Original Article

Effect of PM2.5 on MicroRNA Expression and Function in Nasal Mucosa of Rats With Allergic Rhinitis

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Abstract

Background: Particulate matter 2.5 (PM2.5) refers to particulate matter with aerodynamic equivalent diameter less than or equal to 2.5 μm, which is an important component of air pollution. PM2.5 aggravates allergic rhinitis (AR) and promotes AR nasal mucosa inflammation. Therefore, the influence of PM2.5 inhalation exposure on microRNA (miRNA) expression profiles and function in the nasal mucosa of AR rats was investigated.

Methods: Female Sprague Dawley rats were distributed randomly to 2 groups: AR model PM2.5 exposure group (ARE group) and AR model PM2.5-unexposed control group (ARC group). The rats of ARE group were made to inhale PM2.5 at a concentration of 200 $\mu g/m^3$, 3 h/day, for 30 days. miRNA expression profiles of the nasal mucosa from both groups were determined using an miRNA gene chip and were verified by quantitative real-time PCR (qRT-PCR). Gene function enrichment analysis was performed using bioinformatics analysis.

Results: The ARE group revealed 20 significantly differentially expressed miRNAs, including 4 upregulated and 16 downregulated miRNAs (fold change > 1.5 or < 0.66, $P < .05$). Of these, 9 selected miRNAs were verified by qRT-PCR, and the results of 8 miRNAs were in accordance with the miRNA gene chip results, with highly positive correlation ($r = .8583$, $P = .0031$). Numerous target genes of differentially expressed miRNAs were functionally enriched in high-affinity immunoglobulin E receptor signaling, ErbB signaling, mucin O-glycans biosynthesis, transforming growth factor β signaling, mitogen-activated protein kinase signal transduction, phosphatidylinositol signaling, mucopolysaccharide biosynthesis, mammalian target of rapamycin signaling, T cell receptor signaling, Wnt signaling, chemokine signal transduction, and natural killer cell-mediated cytotoxicity pathways.

Conclusions: PM2.5 causes significant changes in miRNA expression in the nasal mucosa of AR rats. miRNA plays an important role in regulating PM2.5 effects in AR rat biological behavior and mucosal inflammation. This study provides a theoretical basis for the prevention and treatment of AR from the effects of environmental pollution on the gene regulation mechanism.

Keywords

particulate matter 2.5, allergic rhinitis, microRNA, gene expression, target gene

Allergic rhinitis (AR) is a chronic inflammatory disease of the nasal mucosa and is mainly caused by IgE-mediated type I allergic inflammation, multiple immune-competent cells releasing inflammatory factors, and cytokines. In the rapidly developing society with industrialization and urbanization, air pollution and other environmental changes have caused a significant increase in AR prevalence and symptom severity.[1]

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Particulate matter 2.5 (PM2.5) obviously aggravates AR patients' condition and is an important cause of AR incidence; it promotes the progress of AR nasal mucosa inflammation.[2] Therefore, the impact and serious harm by air pollutants such as PM2.5 should be emphasized during the prevention and treatment of AR. Despite various treatments such as antihistamine therapy, corticosteroid therapy, and specific desensitization, the therapeutic effects still remain limited, and so, AR is currently considered a refractory disease.[3] Intensive study of the cause of advanced AR incidence, and investigation of AR pathogenesis, is especially important for further improvement of AR diagnosis and treatment, as well as to improve the quality of life of AR patients.

MicroRNAs (miRNA/miR) are 22-nucleotide-long noncoding RNAs that exist widely in multicellular organisms and affect mRNA stability and translation, thus playing an important role in posttranscriptional regulation of gene expression.[4] The regulatory effect of miRNA is reflected in gene expression; cell development, differentiation, and apoptosis; and ontogenesis. miRNA genes are transcribed by RNA polymerase II to generate primary miRNA transcripts (pri-mRNA), which form the mature miRNAs after a series of processing steps. The mature miRNA complementarily pairs with the 3′ untranslated region of the target gene (complete or incomplete pairing), to form the RNA-induced silencing complex (RISC), which induces translational suppression or degradation of the target gene mRNA, thus negatively regulating protein expression at the posttranscript level. Currently, thousands of miRNA sequences have been discovered in mammals, most of which are unknown in function. Therefore, searching for miRNAs and their target genes, and exploring their regulatory functions, is of significant importance in understanding the gene regulatory mechanisms of diseases.

miRNA participates in PM2.5-induced pathological changes such as inflammation, endothelial dysfunction, and blood coagulation.[5] Cells from endobronchial brushings of a diesel exhaust gas allergen exposure-sensitized population showed significant upregulation of miR-183-5p, miR-324-5p, and miR-132-3p. Associated target gene prediction and pathway research revealed close relationships with cell cycle control, increased epithelial cell apoptosis, and proinflammatory pathways.[6] PM2.5 aggravates AR possibly because of mechanisms like enhanced allergen sensitization, increased eosinophil infiltration, and nasal mucosa structural remodeling. However, the effect of PM2.5 on AR nasal mucosa miRNA expression still lacks in research. It is thus necessary to further investigate the regulatory network of miRNAs and their target genes that reflect the effect of PM2.5 in AR nasal mucosa.

In this study, daily PM2.5 exposure of 3 h was applied on rats continuously for 30 days. Differentially expressed miRNAs were screened using miRNA gene chip to detect miRNA expression changes in the nasal mucosa of AR rat models with and without PM2.5 exposure and were verified by quantitative real-time PCR (qRT-PCR). Target genes and the regulatory network of differentially expressed miRNAs in AR with and without PM2.5 exposure were predicted and analyzed by combining bioinformatics methods to determine the differentially expressed miRNAs and to provide a theoretical basis for further studies on specific miRNA functions. miRNA studies will help better comprehend the potential gene regulation mechanism of PM2.5-mediated induction and aggravation of AR and also increase the number of targets reported for AR prevention and treatment.

Materials and Methods

PM2.5 Collection

PM2.5 collection was performed using a TSP/PM10/PM2.5-2 sampler for atmospheric particulates (Dickel Inc, Beijing, China) through the Whatman 41 cellulose filter membrane (Whatman Inc, Maidstone, UK), and the sampling point was at Center for Atmospheric Chemistry Study, Department of Environmental Science and Engineering, Fudan University, Shanghai, P. R. China (31.3°N, 121.5°E), about 20 meters above the ground. The filter membrane was placed in a constant temperature and humidity box before and after sampling, at 20 ± 0.1°C, 40 ± 2% humidity, and was weighed by an electronic balance (Sartorius 25S BT, precision: 10 μg, Sartorius Co, Germany), after at least 48 h. The PM2.5 sample was ultrasonic-oscillatory eluted from the sample filter membrane for 45 min. The eluent was collected and filtered. To determine the PM2.5 concentration, 100 μL of the PM2.5 sample was placed on a filter membrane and air dried and weighed on an electronic balance after at least 48 h at 20 ± 0.1°C and 40 ± 2% humidity to calculate the concentration. The PM2.5 sample was preserved at –20°C until use.

AR Rat Model Preparation and PM2.5 Inhalation Exposure

Eight female Sprague Dawley rats aged 4 to 5 weeks and weighing 180 to 200 g were bought and raised at the animal experiment center of Fudan University, and all experimental steps followed the requirements of animal ethics committee of Fudan University (certificate number: SYXK-Hu-2014-0029). Animals were distributed randomly to 2 groups: AR model PM2.5 exposure group (ARE group) and AR model PM2.5-unexposed

control group (ARC group); each group contained 4 rats. The AR rat models were prepared as reported.[7,8] A suspension of 0.3 mg ovalbumin (OVA, class V; Sigma-Aldrich Company, St. Louis, Missouri) as antigen and 30 mg aluminum hydroxide (Sigma-Aldrich Company) as adjuvant in 1 mL normal saline was used for sensitization. In the basic sensitization phase, 1 mL of suspension was administered to the rats (ARE group and ARC group) every other day through intraperitoneal injection, 7 times in total. Irritation was induced by intranasal administration of 1% OVA at 100 μL drops once each day for 7 days continuously, starting from 1 week after finishing the intraperitoneal injection.

The PM2.5-animal-inhalation exposure system was used in this study, including an aerodynamic device (HSENG AS18-2; Ningbo Haosheng Pneumatic Machinery Co Ltd., Ningbo, China), aerosol generator (HRH-WAG6; Beijing HuiRongHe Technology Co Ltd), a flow valve, PM2.5 exposure cabin, PM2.5 monitoring system (PC-3A; Jiangsu Laser Research Institute Co Ltd.), and PM2.5 filtration system.[2] The system can provide air replacement inside and outside the exposing cabin. In this study, a PM2.5 saline solution of 10 mg/mL was placed in the aerosol generator, and PM2.5 was maintained at effective controllable concentrations and was continuously present in the exposure cabin by application of the aerodynamic device. The ARE group was exposed to PM2.5 at 200 $\mu g/m^3$, for 3 h each day and 30 days continuously, followed by nasal irritation. PM2.5 was replaced with normal saline with the same procedure in the ARC group.

RNA Extraction and Quality Inspection

After the last PM2.5 inhalation exposure, 3% pentobarbital sodium at 30 mg/kg body weight was injected intraperitoneally. Upon complete anesthesia, the nasal mucosa of both sides was harvested immediately into RNAlater®. Total RNA extraction was performed following the instructions provided with the mirVana™ miRNA Isolation Kit (Cat#AM1560; Ambion, Austin, Texas).

miRNA Chip Experiment

miRNA molecules in the sample RNA were fluorescence labeled as per the instructions provided with the Agilent miRNA chip, miRNA Complete Labeling, and Hyb Kit (Cat# 5190-0456; Agilent Technologies, Santa Clara, California). Sample hybridization was processed following the hybridizing part of the standard operation procedure, using the correlative reagent kit for the Agilent miRNA chip, miRNA Complete Labeling, and Hyb Kit (Cat# 5190-0456; Agilent Technologies). Hybridization continued for 20 h in the hybridization oven (Cat# G2545A; Agilent Technologies), at 55°C and 20 rpm. Developing was processed in staining dishes (Cat# 121; Thermo Shandon, Waltham, Massachusetts) after hybridization. After fluorescence labeling, the miRNA molecules were spotted on Agilent miRNA chip slides, covered with cover glass, and then fixed.

The chip result was scanned using an Agilent Microarray Scanner (Cat# G2505C; Agilent Technologies), software setting Scan resolution = 5 μm, PMT 100%. Data were read using the Feature Extraction software 10.7.1.1 (Agilent Technologies).

RNA Reverse Transcription to cDNA

The instructions of the Script II RT Kit were followed for reverse transcription.

The reaction solution was prepared in a 0.2-mL PCR cuvette. Table 1 shows the reagents for reverse transcription.

After mixing the reaction solution, the PCR cuvette was placed in the PCR instrument with the program: 37.0°C for 60 min, 95.0°C for 5 min, and 4.0°C hold.

qRT-PCR (SYBR Green)

qRT-PCR was performed following the instructions of the miRcute miRNA qPCR Detection Kit.

qRT-PCR Verification of miRNA Chip Screened Genes

In this study, according to the following 3 conditions: (1) the average expression of samples being more than 3, (2) the fold change being relatively large, and (3) the miRNA function being related to the study, 9 miRNAs (rno-miR-130b-3p, rno-miR-141-3p, rno-miR-195-5p, rno-miR-199a-3p, rno-miR-200a-3p, rno-miR-214-3p, rno-miR-96-5p, rno-miR-199a-5p, and rno-miR-338-3p) were selected from 20 differentially expressed miRNAs screened by the miRNA chip. Expression in the nasal mucosa tissue of both the ARE group and ARC group was determined by qRT-PCR to verify the reliability of miRNA chip results.

Table 1. Reagents for Reverse Transcription.

Reagent	Volume (μL)
5× miScriptHiFlex Buffer	4.0
10× miScriptNucleics Mix	2.0
miScript Reverse Transcriptase Mix	2.0
Template RNA+H	12.0
Total	20.0

Bioinformatics Analysis

miRNA target gene prediction. Target gene prediction was performed using the miRNA target gene prediction software miRDB 5.0 (http://mirdb.org/miRDB/).

Target gene function enrichment analysis. Candidate target genes of differentially expressed miRNAs were processed for gene function enrichment analysis by gene ontology (GO) and for Pathway enrichment using the online bioinformatics analysis software, DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/). The GO analysis includes 3 parts: biological process, cellular component, and molecular function. The parameter setting was as follows: enrichment test P value $< .01$ and P value corrected false discovery rate (FDR) value $< .01$.

Construction of regulation network map for miRNAs and target genes. The target regulation network map among miRNAs and candidate target genes was constructed based on the results of target gene prediction and functional enrichment analysis, by combining Cytoscape and Adobe Illustrator CS6 software.

Statistical Analysis

Statistical analysis was performed using SPSS software (Version 18.0; SPSS, Inc, Chicago, Illinois). The experimental data are expressed as means ± standard deviation (means ± SD). Multigroup mean comparison was performed using 1-way analysis of variance, and $P < .05$ was considered to indicate differences with statistical significance.

Results

Total RNA Extraction and Quality Inspection Results

Total RNA was quantified with the NanoDrop spectrophotometer, and the A_{260}/A_{280} ratios were between 1.8 and 2.0, indicating no contamination with DNA, protein, or phenol in the RNA samples. The results of 1% denaturing agarose gel electrophoresis showed RNA integrity number ≥ 6.0, and $28S/18S > 0.7$, indicating no degradation of RNA samples, complying with the requirements of the miRNA chip or quantitative experiment (Table 2).

The primary samples of the chip experiment were total RNAs. Total RNAs were quality inspected with the NanoDrop ND-2000 spectrophotometer and Agilent Bioanalyzer 2100 (Agilent Technologies). Follow-up experiments were carried out only in quality inspection-qualified RNAs.

PM2.5 Affects miRNA Expression Profiles in the Nasal Mucosa of AR Rats

The miRNA gene chip detection analysis revealed 20 significant differentially expressed miRNAs in the PM2.5 exposure group of the AR model, including 4 upregulated miRNAs that were rno-miR-199a-3p, rno-miR-214-3p, rno-miR-195-5p, and rno-miR-199a-5p (fold change >1.5, $P < .05$) and 16 downregulated miRNAs that were rno-miR-301a-3p, rno-miR-31a-3p, rno-miR-204-5p, rno-miR-141-3p, rno-miR-598-3p, rno-miR-96-3p, rno-miR-9a-3p, rno-miR-200b-3p, rno-miR-200a-3p, rno-miR-130b-3p, rno-miR-338-3p, rno-miR-137-3p, rno-miR-429, rno-miR-183-3p, rno-miR-96-5p, and rno-miR-182 (fold change <0.66, $P < .05$) (Table 3).

Many target genes of these differentially expression miRNAs are functionally enriched in the high-affinity immunoglobulin E receptor (FcεRI) signaling pathway, in response to stimulus, and exocytosis (Figure 1).

qRT-PCR Verification of miRNA Chip Screening Gene

In this study, 9 miRNAs were selected from 20 differentially expressed miRNAs screened by the miRNA chip. Their expression in the nasal mucosa of the ARE and ARC groups was determined by qRT-PCR to verify the reliability of miRNA chip results. The qRT-PCR results

Table 2. RNA Quality Inspection Results.

Sample	Concentration (ng/μL)	Volume (μL)	Total Amount (μg)	A_{260}/A_{280}	RIN	28S/18S	Result
ARC1	1103.7	55	60.70	2.15	8.8	1.5	A1
ARC2	1165.7	55	64.11	2.15	8.5	1.4	A1
ARC3	606.2	55	33.34	2.16	8.8	1.2	A1
ARC4	471.3	55	25.92	2.13	9.0	1.7	A1
ARE1	597.4	55	32.86	2.15	9.0	1.6	A1
ARE2	668.8	55	36.78	2.16	9.1	1.7	A1
ARE3	422.1	55	23.22	2.13	9.2	1.7	A1
ARE4	324.3	55	17.84	2.15	8.7	1.4	A1

Abbreviations: ARC, allergic rhinitis model PM2.5 control; ARE, allergic rhinitis model PM2.5 exposure; RIN, RNA integrity number. Indicator of RNA integrity ranged from 0 to 10, and higher value indicates better RNA integrity.

Table 3. Details of the Differentially Expressed miRNAs.

Systematic name	*P*	Fold change	Regulation	Mean of ARE	Mean of ARC
rno-miR-199a-3p	.04	1.67	Up	11.00	10.30
rno-miR-214-3p	.02	1.60	Up	9.03	8.38
rno-miR-195-5p	.05	1.57	Up	8.93	8.32
rno-miR-199a-5p	.02	1.55	Up	9.23	8.61
rno-miR-301a-3p	.02	0.63	Down	5.14	5.82
rno-miR-31a-3p	.01	0.62	Down	3.27	3.97
rno-miR-204-5p	.03	0.60	Down	7.53	8.28
rno-miR-141-3p	.00	0.60	Down	9.77	10.50
rno-miR-598-3p	.04	0.56	Down	3.87	4.80
rno-miR-96-3p	.03	0.56	Down	2.67	3.57
rno-miR-9a-3p	.05	0.52	Down	6.44	7.49
rno-miR-200b-3p	.04	0.52	Down	11.83	12.88
rno-miR-200a-3p	.01	0.51	Down	11.47	12.49
rno-miR-130b-3p	.04	0.50	Down	3.95	5.07
rno-miR-338-3p	.02	0.44	Down	6.65	7.83
rno-miR-137-3p	.01	0.44	Down	2.91	4.18
rno-miR-429	.03	0.43	Down	11.38	12.75
rno-miR-183-3p	.04	0.40	Down	3.09	4.68
rno-miR-96-5p	.05	0.34	Down	9.54	11.62
rno-miR-182	.05	0.33	Down	6.76	8.88

Abbreviations: ARC, allergic rhinitis model PM2.5 control; ARE, allergic rhinitis model PM2.5 exposure.

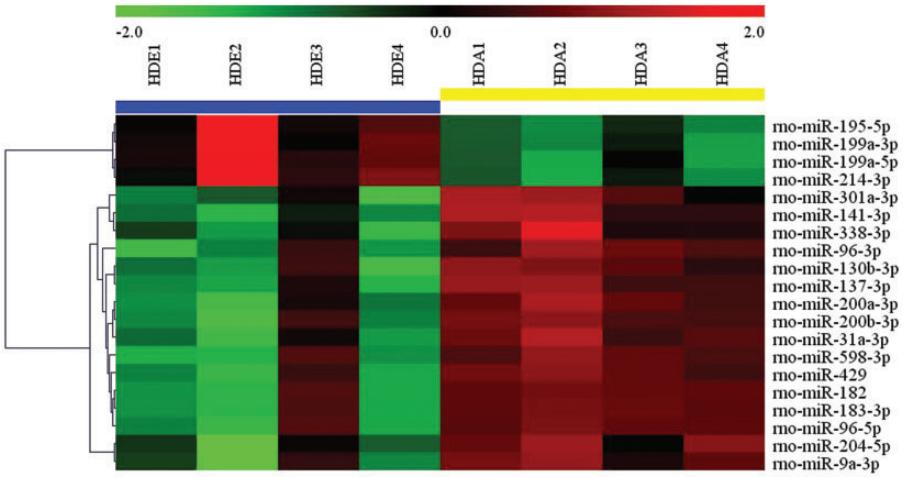

Figure 1. Cluster analysis diagram of significant differentially expressed miRNAs in the nasal mucosa of PM2.5-exposed AR rats. Abscissas for sample name; ordinates for differentially expressed miRNAs; red and green labels indicate upregulated and downregulated miRNAs, respectively.

of 8 miRNAs (rno-miR-130b-3p, rno-miR-141-3p, rno-miR-195-5p, rno-miR-199a-3p, rno-miR-200a-3p, rno-miR-214-3p, rno-miR-96-5p, and rno-miR-338-3p) were in accordance with the miRNA gene chip detection results. Only 1 miRNA (rno-miR-199a-5p) showed a qRT-PCR result that was not in accordance with the miRNA gene chip detection (see Figure 2). The coincidence rate of the 2 results reached 88.9% (8/9). The above results indicate that the miRNA chip results had good reliability.

Functional Enrichment Analysis Results by GO and Pathway of miRNA Candidate Target Genes in PM2.5-Affected AR Rat Nasal Mucosa

First, miRNA target gene prediction software was utilized to analyze the differentially expressed miRNA candidate target genes in the nasal mucosa of AR rats. Gene function annotation software DAVID Bioinformatics Resources 6.7 was then used for GO and Pathway function enrichment and to annotate the associating

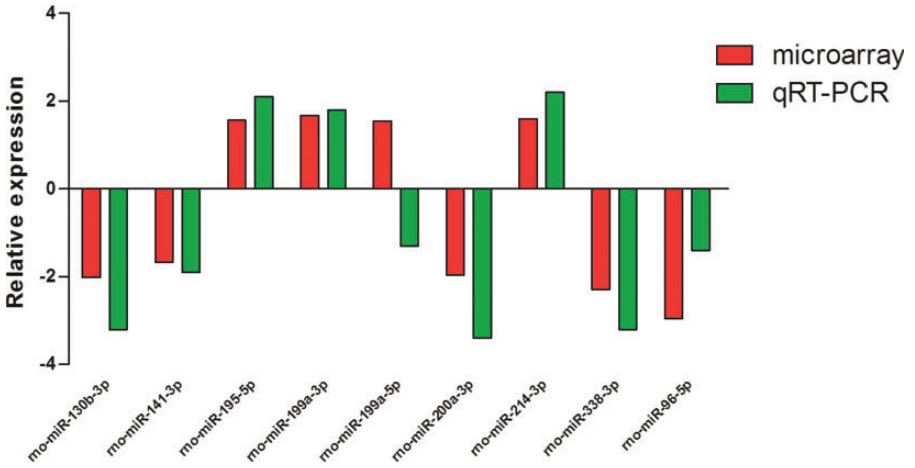

Figure 2. qRT-PCR verification of miRNA chip screened differentially expressed miRNAs. qRT-PCR, quantitative real-time polymerase chain reaction.

candidate target genes and to analyze the function of these differentially expressed miRNAs and their target genes in the AR rats mucosa.

GO analysis showed that many significant functionally enriched GO categories are associated with cell activation ($P < .01$, FDR < 0.01) including intracellular signal transduction, protein phosphorylation, metabolic process, positive regulation of gene expression, response to stimulus, and exocytosis (Figure 3; ▲Cellular component; ●Biological process; ■Molecular function). GO analysis suggests that differentially expressed miRNAs and their target genes might participate in oxidative stress, inflammatory reaction, and so on. Pathway analysis revealed several significant functional enrichment pathway categories ($P < .01$, FDR < 0.01), such as the chemokine signaling pathway, phosphatidylinositol signaling system, FcεRI signaling pathway, calcium signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway, cell migration, response to growth factor stimulus, G-protein coupled receptor activity, and so on (Figure 4).

Regulatory Network of miRNA and Candidate Target Genes in PM2.5-Affected AR Rat Nasal Mucosa

The regulation network of miRNAs and their associated candidate target genes was structured based on the result of target gene prediction and functional enrichment analysis, combining the Cytoscape and Adobe Illustrator CS6 software. Related differentially expressed miRNAs and their candidate target genes were selected based on the combination of target gene prediction and functional enrichment analysis results. The candidate target genes of miR-130b-3p include TGFBR1, CSF2, ACVR1, CBLB, ADCY7, INHBB, PTPN2, C1GALT1, ITPKB, WNT2B, MAP3K8, MAPK9, ELK4, TSC1, ULK2, and so on. The candidate target genes of miR-137-3p include DAAM1, GNGT1, GALNT18, MAPK8, DUSP8, IGF1, FGF11, PLA2G4A, WNT7A, NCK1, NCK1, NRG3, PPP3R2, GADD45A, and so on. The candidate target genes of miR-141-3p include IL1A, SERINC3, SIRT1, DUSP3, CSNK1A1, PRMT1, IFNGR2, MAPK8, STAT5A, SIAH1, MAP2K4, CDKN1B, CDKN1B, SRC, CBL, BID, STAT2, and LDLRAD4. The candidate target genes of miR-200b-3p-3p include RPS6KB1, CRKL, EGFR, BTRC, PPP2CA, MAP3K5, MAP3K5, SIAH1, DUSP1, SOCS5, ST3GAL2, ATF2, NOG, RIPK2, and NRG1. The target genes functions of differentially expressed miRNAs include participation in oxidative stress reactions, release of inflammatory mediators, immunocellular function and reaction regulation, and so on. Our study found that there is a complex regulatory network among the differentially expressed miRNAs and candidate target genes, wherein each miRNA regulates more than 10 target genes. Moreover, each target gene is regulated by multiple miRNAs. Target regulatory relationships among miRNAs and their candidate target genes in PM2.5-affected AR rats nasal mucosa are shown in Figure 5.

Discussion

AR is an allergic disease caused by many factors. It is caused by internal and external factors. Internal causes include inflammatory cells, inflammatory factors, and genes, while external causes include allergens and air pollution. The role of air pollution, especially PM2.5 exposure, in the pathogenesis of AR is gradually concerned. Our previous studies have found that in AR rat models, inhalation exposure to PM2.5 could induce a series of changes such as nasal symptom aggravation, inflammatory cytokines change of Th1/Th2 cytokine

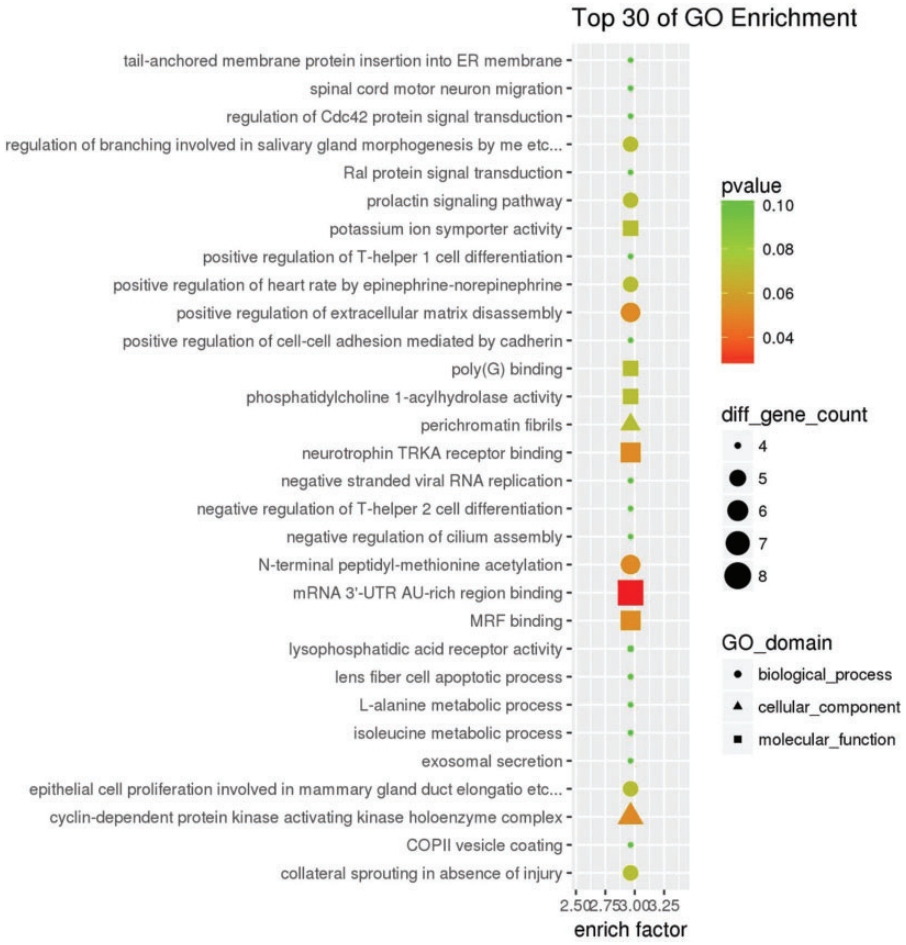

Figure 3. GO analysis of differentially expressed miRNA candidate target genes in PM2.5-affected AR rat nasal mucosa: ▲ Cellular component; ● Biological process; ■ Molecular function. AU, adenylate-uridylate; COPII, coat protein complex II; ER, endoplasmic reticulum; GO, gene ontology; MRF, myogenic regulatory factors; TRKA, tropomyosin receptor kinase A; UTR, untranslated region.

imbalance at protein level such as interleukin (IL)-4, IL-13, transforming growth factor (TGF)-β1, interferon-γ, eotaxin-1, etc, eosinophil infiltration, mitochondrial damage, and nasal mucosal remodeling.[2,8–10] The molecular regulatory mechanism of AR after PM2.5 exposure, especially the posttranscriptional regulation mechanism, is still unclear. Therefore, we further studied the miRNA expression profiles and functions of epigenetic posttranscriptional regulation mechanisms in AR incidence after PM2.5 exposure.

miRNAs are single-stranded small RNA molecules composed of about 21 to 23 bases, generated by Dicer enzyme from a hairpin-structured single-strain RNA precursor composed of about 70 to 90 bases, which is different from siRNA (double stranded), though closely related to siRNA. miRNAs pair with target gene mRNA bases to induce RISC-mediated mRNA degradation or suppression of mRNA translation, thus regulating protein expression at the posttranscriptional level (recently, microRNAs have been discovered to regulate gene expression at the transcriptional level as well). miRNAs are rather conserved in species evolution, which has been demonstrated in animals, plants, and fungi, showing strict histological specificity and sequence. miRNAs play various roles in cell growth and developmental processes, including regulation of development, differentiation, apoptosis, and proliferation.

Currently, the methods used to study miRNA mainly include real-time PCR, biochip technology, and second-generation sequencing technologies. The Agilent Rat miRNA Chip V21.0 Database originates from the miRNA database (miRBase) version 21.0 and covers 758 rat-associated miRNAs. The chip design applies a detection technique unique to Agilent, which can detect matured miRNA specifically and can distinguish highly homologous miRNA molecules.

Based on gene chip studies on AR, to further specify the posttranscriptional regulation of AR, an miRNA expression profiles study of AR patient nasal mucosa revealed 421 differentially expressed miRNAs.[11] Based

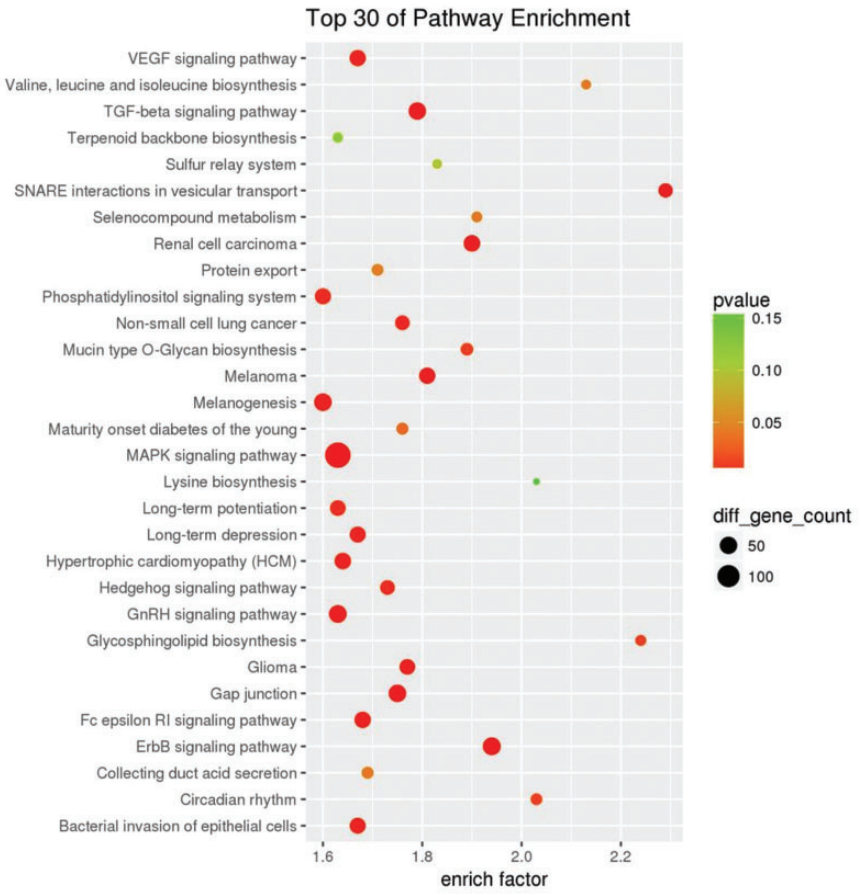

Figure 4. Pathway analysis of differentially expressed miRNA candidate target genes in PM2.5-affected AR rat nasal mucosa. Fc epsilon RI, high-affinity immunoglobulin E receptor; GnRH, gonadotropin-releasing hormone; MAPK, mitogen-activated protein kinase; TGF, transforming growth factor; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; VEGF, vascular endothelial growth factor.

on miRNA differential expression studies in the nasal mucosa, mir-143 lentivirus vector was stably transfected in to mast cell successfully to determine the action of mir-143 on the mast cell target gene IL-13Rα1; inhibition of IL-13Rα1 expression was observed, thus decreasing mast cell inflammation factors synthesis and release.[12] Mir-143 transfection in AR patient nasal mucosa epithelial cells could also inhibit IL-13Rα1 expression and the release of inflammatory factors and mucins; therefore, mir-143 is possibly a potential target of AR prevention and treatment.[13] A study found several miRNAs are negatively correlated with PM2.5 concentration in the population after PM2.5 exposure, whereas the expression of the semiRNAs and associated target mRNAs is decreased. This negative correlation is supposed to increase the inflammation reaction.[5,6] These findings suggest that PM2.5 possibly affects airway diseases through a complex immune regulation mechanism involving miRNA.

Primary research on differential gene expression in AR patient nasal mucosa tissue was performed using an expression profiling chip containing 12,800 human complementary DNA. There are 734 differentially expressed genes in the Chinese AR gene expression profiles, among which there are 430 upregulated genes and 304 downregulated genes. These differentially expressed genes are mainly associated with inflammation reactions, immune response, immune regulation, and the neuroendocrine network system and signaling. It can be seen that AR is a multigene-regulated immune and inflammatory disease. Many genes are involved in the pathogenesis of AR.[14] In this study, miRNA gene chip detection revealed 20 significant differentially expressed miRNAs in the PM2.5 exposure group of the AR model, including 4 upregulated miRNAs and 16 downregulated miRNAs. Many target genes among these differentially expressed miRNAs are functionally enriched in the FCεRI signaling pathway, response to stimulus, and exocytosis. Nine miRNAs were selected from these 20 differentially expressed miRNAs screened by miRNA chip. Their expression in the nasal mucosa of the ARE group and the ARC group was confirmed by qRT-PCR to verify

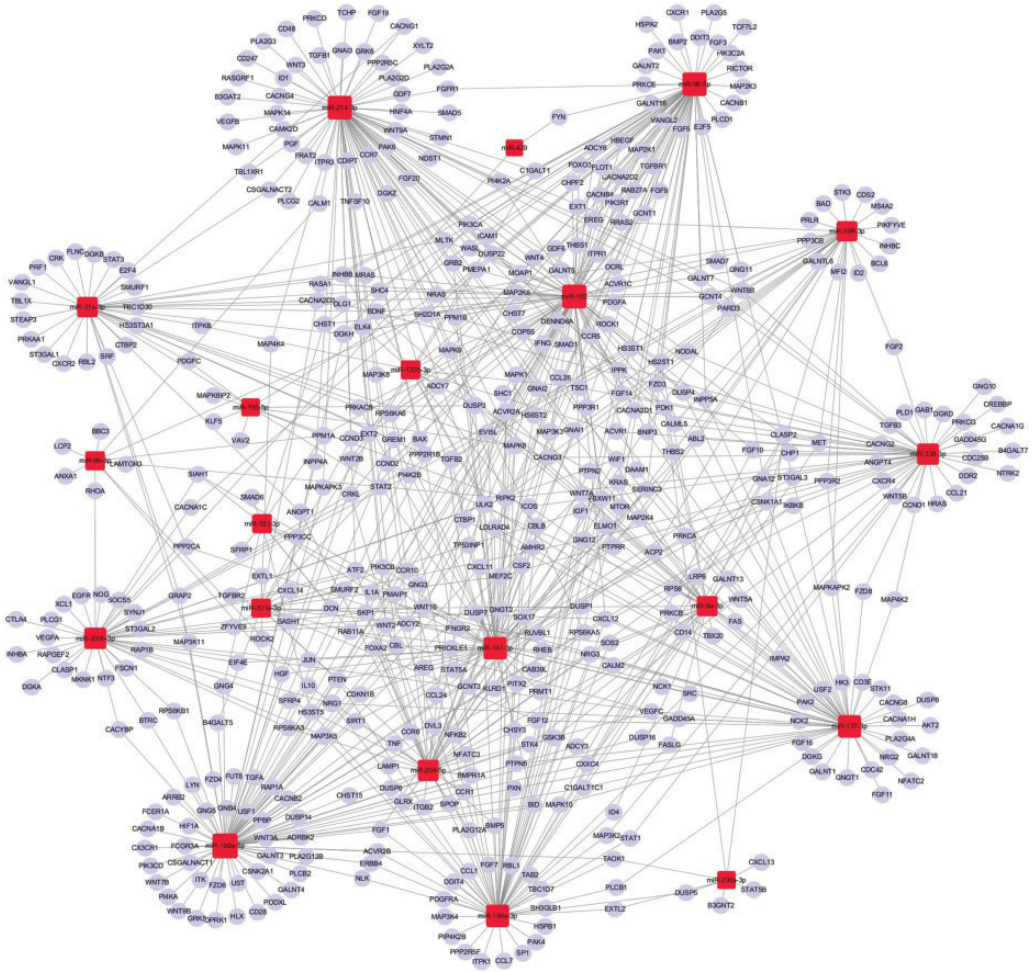

Figure 5. Regulatory network relationship among miRNAs and their candidate target genes in PM2.5-affected AR rat nasal mucosa.

the reliability of miRNA chip results. The qRT-PCR results of 8 miRNAs were in accordance with the miRNA gene chip detection. The coincidence rate of the 2 results was 88.9% (8/9). The above results indicate that the miRNA chip results had good reliability. Rno-mir-199a-5p was upregulated in miRNA chip detection while downregulated in qRT-PCR verification. Therefore, further research is needed to determine whether rno-mir-199a-5p plays a role in PM2.5 induced and aggravated AR and whether it is upregulated or downregulated actually.

The miRNA target gene prediction software was utilized to analyze differentially expressed miRNA candidate target genes in the AR rat nasal mucosa. GO and Pathway function enrichment and annotation were performed for the associated candidate target genes to analyze the function of these differentially expressed miRNAs and their target genes in AR rats mucosa.

GO is a widely used ontology tool in bioinformatics and is based on layering the gene function expression of different categories. GO-related gene and gene product vocabulary is divided into 3 categories covering 3 aspects of biology: (1) cellular component, referring to every component of the cell and extracellular environment; (2) molecular function, described as activity at the molecular level such as catalysis activity or binding activity; and (3) biological process, indicating serial events involving single molecules or multiple molecules combined sequentially. By applying GO enrichment analysis to differentially expressed genes, the genes could be categorized by different functions, thus achieving the purpose of annotating and categorizing genes. In this study, GO analysis was processed to analyze the associated target genes and revealed that the differentially expressed miRNAs and their target genes might participate in oxidative stress, inflammatory reaction, and so on. These findings suggest that PM2.5 exposure may activate oxidative stress and inflammatory reactions in nasal epithelial cells to participate in AR morbidity, which is consistent with our previous findings in vitro.[2]

Kyoto Encyclopedia of Genes and Genomes (KEGG) analyzes gene functions systematically and helps

researchers to study genes and their expression information as an integral network. Genome information is stored in the GENES database, including completely and partly sequenced genome sequences. Advanced functional information is stored in the PATHWAY database, including graphic cellular biochemical processes such as metabolism, membrane transport, signal transmission, cell cycle, and homologous conservative subpathway information. KEGG is a powerful tool for the research of metabolism analysis and metabolic network study in organisms. Processing KEGG enrichment analysis for differentially expressed genes could significantly enrich differential pathways, which helps to find significant differentially changed biological regulatory pathways. In this study, inhalation of PM2.5 in AR rats was found to induce differential miRNA variation in rat nasal mucosa. The biological regulatory pathways include the FCεRI signaling pathway, ErbB signaling pathway, biosynthesis of mucin O-glycans, TGF-β signaling pathway, MAPK signal transduction pathway, phosphatidylinositol signal system, biosynthesis of mucopolysaccharide, mammalian target of rapamycin signaling pathway, T cell receptor signaling pathway, Wnt signaling pathway, chemokine signal transduction pathway, natural killer cell-mediated cytotoxicity, and so on. These results suggest that PM2.5 exposure may activate oxidative stress and inflammatory reaction in the nasal epithelium cell through the above signaling pathways, thus inducing and aggravating AR.

Based on the combination of target gene prediction and function enrichment analysis results, related differentially expressed miRNAs and their candidate target genes were selected, and the targeting regulatory network of miRNA and associated candidate target genes was constructed. We found that there is a complex regulatory network among the differentially expressed miRNAs and candidate target genes, wherein each miRNA regulates multiple genes, and each target gene is regulated by multiple miRNAs. Target gene functions of differentially expressed miRNAs include participating in oxidative stress reactions, release of inflammatory mediators, and immunocellular function and reaction regulation. These findings suggest that PM2.5 exposure induces and aggravates AR through the above pathways.

Conclusion

The effect of PM2.5 on AR gene regulation and its molecular mechanism was preliminarily explored in this study. PM2.5 plays an important role in gene regulation of AR nasal mucosa cells, affecting the AR oxidative stress pathway, synthesis of inflammatory factors, and release of inflammatory mediators through regulating the differential expression of miRNAs. The study of miRNA target genes and their related signaling pathways may provide a new way to mitigate and prevent the harm caused by PM2.5 in AR. The results are of great significance to reveal the role of PM2.5 in the pathogenesis of AR and for further study of AR pathogenesis at the gene level. Although we have proved that PM2.5 can cause the regulation of related genes to induce AR in animal models, we have not proved that it has the same effect on humans. We will conduct clinical genetic research on different PM2.5-exposed AR populations and further reveal the mechanism of PM2.5 in inducing and aggravating AR.

Declaration of Conflicting Interests

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