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Original Research

Effects of Environmental $PM_{2.5}$ on Adult SD Rat Lung Transcriptional Profile

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Abstract

To study the effects of environmental Particulate Matter $(PM)_{2.5}$ on the transcriptional profiles of Sprague Dawley (SD) rats lung tissue, thus providing insights into the mechanisms through which $PM_{2.5}$ may exert on human beings. Environmental $PM_{2.5}$ was prepared with versatile aerosol concentration enrichment system. The healthy control rats and the chronic obstructive pulmonary disease (COPD) model rats were exposed to $PM_{2.5}$ and clean air, respectively. RNA-sequence technique was used for genetic changes analysis in lung tissue after exposure, and the data were analyzed with transcriptional profile and pathway analysis. The results showed that persistent exposure to $PM_{2.5}$ caused various transcriptional changes related to signal pathways mainly including reactive oxygen species (ROS), inflammation, cell proliferation and so on. Compared with the healthy rats, the COPD model rats showed significant changes on cell proliferation, inflammation, immune response and cell death. Furthermore, after exposure to $PM_{2.5}$, COPD rats showed more significant pathway changes in ROS, inflammation, immune response, cell proliferation and damage repair. Collectively, $PM_{2.5}$ exposure caused similar but more significant transcriptional changes in COPD rats than in health rats or clean-air raised COPD rats, suggesting that human with COPD is more sensitive to $PM_{2.5}$ and more severe impairments might be induced after exposure. Humans whom are suffering COPD needs more protection from $PM_{2.5}$ to avoid lung injury.

Keywords: environmental $PM_{2.5}$, transcriptional profile, lung injury, rat COPD model

Introduction

The development of modern industry causes increasing environmental pollutions, including air, water and soil pollution, in which air pollution is the main risk factor that related to human mortality and lung diseases [1]. Among all the air pollutants, $PM_{2.5}$, which was defined as particle matters (PMs) with an aerodynamic diameter less than 2.5 μm, is one of the most hazardous substrates that affecting human health and even shorten human length of life [2, 3]. Recent studies indicated the increased $PM_{2.5}$ concentration is closely associated with the prevalence of adverse birth outcomes including preterm birth and low birth weight [4], $PM_{2.5}$ also affected cardiovascular system and causes heart dysfunction and vascular damage [5-7], thus increasing the risk of cardiovascular system after they penetrate alveoli and enters blood [8]. Emerging

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studies indicated that $PM_{2.5}$ is associated with many other human diseases [9], while much common sense is that, $PM_{2.5}$ brought complex hazards to human lungs and could accelerate the process of respiratory disease [10, 11]. A number of studies had reported that long-term exposure to $PM_{2.5}$ could induce asthma, chronic obstructive pulmonary disease and even lung cancer [12, 13]. $PM_{2.5}$ in polluted air could decrease the value of lung function indicators of a healthy child and even affect the benefit of habitual physical activity of adult [14]. Though the effects of $PM_{2.5}$ on human lungs had been largely reported, most of the previous studies focused on their effects on healthy lungs, few of them care about the adverse effects of $PM_{2.5}$ on lungs with disease or pathological changes.

The toxicity of $PM_{2.5}$ was determined by many factors, including the concentrations, particle size, the absorptions and the meteorological conditions, therefore, the component of $PM_{2.5}$ differed greatly among different regions, thus contributing absolutely different effects after exposure. It has been reported that water soluble inorganic ions, especially secondary inorganic ions (SIAs: $SO4^{2-}$, NO^{3-}, NH^{4+}) are the main components of $PM_{2.5}$ in China [15]. Studies indicated that $PM_{2.5}$, which can easily enter depth respiratory tract than PM_{10}, would bring more adverse effects [16, 17]. In addition to the particle itself, the metal that bounded on PMs might mediate the toxicity and increased the healthy risk [18]. Meanwhile, SO_2, the recourse of $SO4^{2-}$, was also reported attribute to the increased toxicity of $PM_{2.5}$ [19]. Though studies had defined the risk factors of $PM_{2.5}$, how these factors work and what changes happened in the lung after exposure were largely unknown.

Several studies have suggested that the alternation of gene expression played a major role in the activation of pathways induced by toxicant exposure [20-24]. Recent studies indicated that $PM_{2.5}$ could enter cells via pinocytosis, and could alter the gene expression of lung epithelial and myocardial cells. These alterations then inhibited cell apoptosis, induced cell proliferation and promoted angiogenesis, which are considered as indicators of tumor genesis [21, 25]. Meanwhile, the chronic obstructive pulmonary disease (COPD) progress could also be accelerated, and even the mortality was increased by the short term $PM_{2.5}$ exposure [26].

The adverse effects of $PM_{2.5}$ on lungs, as well as the molecular changes in COPD progress, had been studied and the mechanisms had also been explored independently in many previous studies. However, the mechanism through which $PM_{2.5}$ affects human lung that suffering COPD, and the changes happened in COPD lungs after exposure are still unclear. To better understand the effects of environmental $PM_{2.5}$ on human lungs, especially in COPD lung, trying to providing potential intervention methods. We established a COPD rat model, and compared the transcriptional changes in lung tissues of healthy rats and COPD model rats after $PM_{2.5}$ exposure.

Material and Methods

Animals Grouping and Treatment

Animal experiment was conducted in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. Forty male adult Sprague Dawley (SD) rats, age between 8 to 10 weeks old, were purchased from the laboratory of Hubei medical university, with the average body weight of 338.35±50.18 g (mean±SD). The rats were randomly divided into four groups, and were raised in a controlled environment of 22±2ºC temperature and 40-60% humidity. Four groups were set up in this study: healthy rats raised in clean air (Group A), healthy rats exposed to $PM_{2.5}$ (Group B), the COPD model rats raised in clean air (Group C), and the COPD rats exposed to $PM_{2.5}$ (Group D).

COPD Model Establishment

COPD model rats were established according to a previously published document [27]. Briefly, the rats were treated by anesthesia, and tracheal was dripped with Lipopolysaccharides (LPS) solution (1 mg/kg, Beijing Huironghe Technology Co., Ltd., China). Then, the rats were put into a single channel smart smoking machine (HRH-SM-120) on the next day. The rats were administrated with a passive smoking treatment twice a day (five times a week), and each treatment lasted 60 min. The total exposure duration was 8 weeks. The tobacco used for model establishment was a Chinese cigarette band (Lushan, Jiangxi, China). The tar content was 10 mg; the nicotine content was 1 mg; the carbon monoxide content was 13 mg per cigarette according to the manufacturer's report. After an eight-week exposure, the rats were executed, and the lung tissues were collected for pathological changes and pathological scores analysis to confirm that the COPD model was successfully established. After finished hematoxyl in and eosin (HE) staining, the pathological changes were observed under 100× light microscope. Ten fields were chosen in each slice, capillary congestion, alveolar fibrin exudation, neutrophil exudation, airway epithelial cell exfoliation and alveolar septa widening was observed and recorded for the following score evaluation.

Collection of Environmental $PM_{2.5}$

Environmental $PM_{2.5}$ was prepared with versatile aerosol concentration enrichment system (Beijing Huironghe Technology Co., Ltd., China). The device was placed at the top building of the Second Hospital of Hebei Medical University (Heping west road, Xinhua district, Shijiazhuang City, Hebei Province, 38°06′N, 114°48′E). The $PM_{2.5}$ was real time collected from December 2013 to January 2019. According to the monitoring data, $PM_{2.5}$ presented the highest average concentration from December 2016 to January 2017

(data not shown in this study), so we chose the $PM_{2.5}$ samples collected during this period for the following model establishment and exposure study.

Exposure of Rats to $PM_{2.5}$

The rats in group B and group D were exposed to $PM_{2.5}$, and the consecutive exposure to $PM_{2.5}$ was carried out with a commercial gas poisoning apparatus (HRH-CSED-K, Beijing Huironghe Technology Co., Ltd., China). The exposure dose of $PM_{2.5}$ was corresponding to the collected samples. The rats were exposed to $PM_{2.5}$ 8 hours a day for one month.

Sample Collection and RNA Sequencing Analysis

The rats were executed after intraperitoneal injection of 10% chloralhydrate, and then the lungs were collected and irrigated with physiological saline. 1-2 mg of the pulmonary tissue was homogenized in ice water, and the total RNA was extracted with TRIzol reagent. Total RNA integrity was detected by agarose gel electrophoresis, and NanoDrop ND-1000 was used for quantitative detection and purity inspection. The total RNA was enriched with NEB Next Poly mRNA Magnetic Isolation Module, and then carried out fragment treatment. Then the fragment was used for bank construction with KAPA Stranded RNA-Seq Library Prep Kit. The constructed gene bank was carried out quality check and quantitative analysis via Agilent 2100 Bioanalyzer and RT-PCR assay. The RNA sequencing analysis was carried out by KangChen Bio-tech (Shanghai, China). RNA sequencing was carried out with Illumina HiSeq 4000, and the raw sequencing data was used for following analysis after quality control.

Annotation of Sequencing Gene Results

The gene sequencing results was compared with the public reported gene bank, and the genes with similar functions were classified. The sequencing data was enriched by Gene Ontology (GO) and KEGG Pathway analysis. The GO analysis was used to describe the candidate genes coded proteins related pathways, functions and cellular environment. KEGG is a database to systematically analyze the metabolic pathways of gene products and various compounds in cells and the functions of these gene products.

Statistical Analysis

The transcriptional difference among four groups was analyzed by Hisat2 software with Balldown method. The transcriptional levels were calculated by FPKM, and the different expressed genes were then screened. StringTie software was employed to match the genes to the official data base, and rMATS software was used for the calculation and imaging of different expressed genes. The threshold of $P<0.01$ and the mean value of FPKM>0.5 were chosen to identify genes that differently expressed, respectively. Some important changes with the P value between 0.01-0.02 were also shown in the table.

Results and Discussion

COPD Model Establishment

Compared with control lungs, plenty of inflammatory cells were infiltrated around the mucosa and bronchi of treated lungs. The alveolar wall became thin, the alveolar cavity expanded obviously, and even formed a lung blister, the pathological score was significantly increased compared with control group, which indicated that the COPD model was successfully established (Fig. 1). As known, $PM_{2.5}$ was harmful to the lung of healthy humans, especially to the lung of COPD patients. In order to further explore the underlying mechanisms that $PM_{2.5}$ affecting the lung of COPD patients, and providing potential therapeutic methods according to the molecular mechanisms, the COPD rat

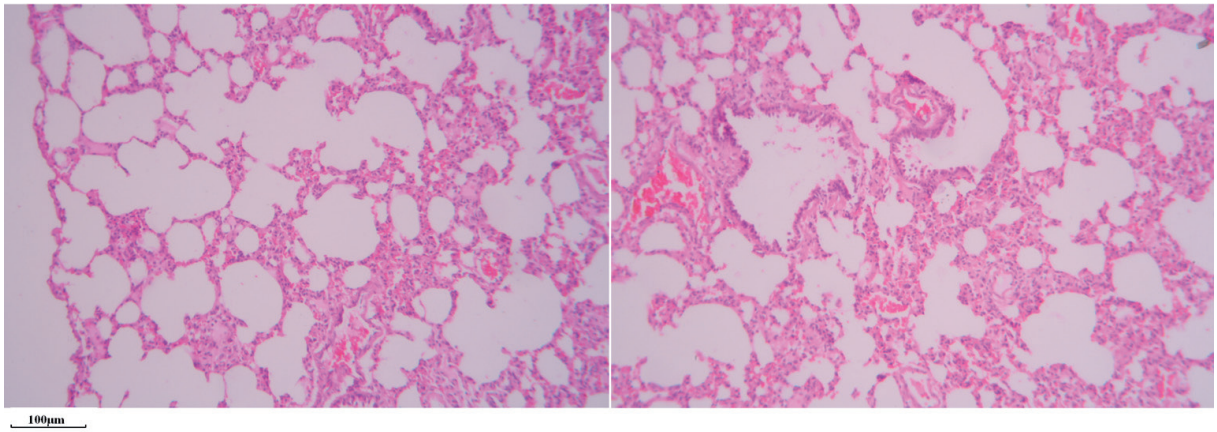

Fig. 1. The pathological changes of lung tissue after the rats finished COPD modeling.

Table 1. Monitoring of the exposure conditions.

Time	Inhaled $PM_{2.5}$ (mg/m^3) [a]	Atmospheric $PM_{2.5}$ ($\mu g/m^3$) [b]	Animal number	Exposure duration (min) [b]	Average temperature (ºC) [b]	Average humidity (%) [b]
2016-12-22	0.1-0.5	119	10	144.7	23.7	23.6
2016-12-23	0.1-3.2	99	10	231.6	24.5	28.9
2016-12-26	0.6-21.9	222	10	256.3	28.6	32.3
2016-12-27	0.1-1.4	122	10	302.6	29.1	32.0
2016-12-29	0.2-5.2	229	10	206.2	21.1	30.2
2017-01-03	0.4-37.5	267	10	247.4	21.9	36.0
2017-01-04	0.4-25.2	311	10	232.5	25.5	27.7
2017-01-05	0.4-16.5	289	10	219.1	28.0	26.9
2017-01-07	0.1-15.4	265	10	253.8	30.6	23.3
2017-01-09	0.1-12.2	137	10	252.4	23.6	32.2
2017-01-10	0.1-16.6	116	10	297.2	22.1	34.6
2017-01-13	0.3-3.9	126	10	211.5	20.7	43.4
2017-01-14	0.2-21.7	155	10	299.6	19.7	49.1
2017-01-15	0.1-13.0	229	10	298.1	18.7	32.0
2017-01-16	0.1-5.5	178	10	294.8	18.1	41.9
2017-01-17	0.1-18.3	222	10	295.7	18.7	36.7
2017-01-18	0.2-53.2	381	10	316.4	18.2	21.3
2017-01-19	0.1-3.4	149	10	297.0	17.8	57.0
2017-01-20	0.1-19.9	60	10	295.0	17.8	48.8
2017-01-21	0.1-23.4	79	10	301.8	18.0	56.5
2017-01-22	0.1-19.7	76	10	295.3	18.1	49.5

Note: [a] Data were presented by min-max; [b] Data were presented by mean.

model was established according to the previous study. COPD model could be established via LPS, tobacco, virus and so on [28-30]. In this study, we chose LPS dropping followed with positive smoking to better mimic the reality of human COPD happening.

Monitoring of $PM_{2.5}$ Exposure

$PM_{2.5}$ was monitored and collected by versatile aerosol concentration enrichment system from December 22, 2016 to January 23, 2017, when the average $PM_{2.5}$ concentration was the highest from December 2013 to January 2019. The inhaled PM2.5, environmental $PM_{2.5}$, exposure duration, average temperature and average humidity were also recorded during the whole exposure period. All the records are presented in Table 1. As known, air temperature and humidification are major factors that affect environmental $PM_{2.5}$ concentrations, and further affecting their toxicity. The study indicated that $PM_{2.5}$ concentration showed a better correlation with humidification, while a poor correlation with temperatures, so these two factors were recorded for exposure condition control [31]. Our results indicated that the concentration of $PM_{2.5}$ in each day was relatively stable and well controlled. Previous studies showed that the cities in Hebei Province were the central of the unfairness of $PM_{2.5}$ pollution emissions across the Beijing-Tianjin-Hebei regions [32], so the air pollution monitoring and related studies are urgent.

RNA-Sequencing Quality Control

The integrity of total RNA was evaluated by agarose gel electrophoresis. The concentration and purity of total RNA were determined by NanoDrop2000/2000c Spectrophotometer. The results showed that the RNA samples were suitable for the following research. Quality control was used to evaluate the quality of original raw data, and the results are shown in Table 2. The original sequence of each sample was counted with Q30 when evaluating the quality of sequencing. If the value of Q30 was larger than 80%, indicating that the quality of sequencing was high, no sample was contaminated and the results was reliable for the following analysis.

Table 2. Quality of raw sequencing data used for the following analysis.

Group	Reads number	Total base count	Base count (Q ≥ 30)	Q30(%)
A (health control)	39594378	5939156700	5345885886	90.1
	40894604	6134190600	5569671025	90.8
	37645076	5646761400	512344513	90.7
	35605714	5340857100	4857126859	90.4
	50438650	7565797500	6917072965	91.4
B (health rats in $PM_{2.5}$)	35558244	5333736600	4860403732	91.1
	32667052	4900057800	4478519800	91.4
	46427414	6964112100	6347605872	91.2
	42816296	6422444400	5853137177	91.1
	42819482	6422922300	5872785397	91.4
C (COPD model)	59051520	8857728000	8062224676	91.0
	37824120	5673618000	5118936919	90.2
	50357052	7553557800	6951724393	92.0
	39618912	5942836800	5418779630	91.2
	49313818	7397072700	6762537285	91.4
D (COPD rats in $PM_{2.5}$)	41685878	6252881700	5738436185	91.8
	38338108	5750716200	5226708448	90.9
	36945206	5541780900	5041350840	90.9
	53263786	7989567900	7220060381	90.4
	45920500	6888075000	6246525524	90.7

Transcriptional Profile Changes after Healthy Rats Were Exposed to $PM_{2.5}$

Comparison of the transcriptional profiles between group A and group B showed that, 13299 genes showed no significant change, while 943 gene expressions were up regulated and 233 genes were down regulated. A volcano map showed the changes of these genes (Fig. 2). Then, the differently expressed genes were further analyzed with Gene Ontology (GO) analysis, and were enriched to cellular signal pathways. All the 34 significantly enriched pathways are indicated in Table 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway analysis showed that 60 pathways were enriched (Table 4).

Owing to their unique physical properties, $PM_{2.5}$ could reach deep respiratory tract, and cause not only physical effects, but also chemical and biological effects. The physical effect is reflected as mechanical damage. After $PM_{2.5}$ entered deep respiratory tract, they would injure bronchial tube and pulmonary alveoli. Our transcriptional profile (Table 3) showed that the damage repair and cell proliferation related pathways were altered after exposure. For instance, when $PM_{2.5}$ entered lungs or lung cells, the heavy metals absorbed on them may activate programmed cell death, as well

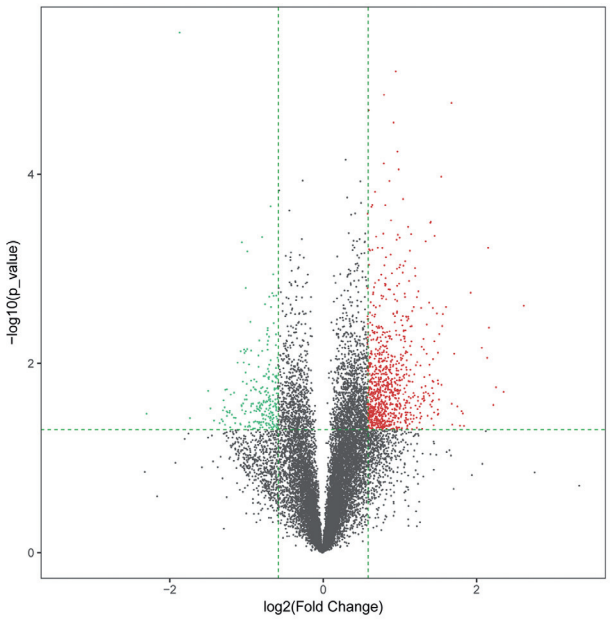

Fig. 2. A volcano map indicating the altered genes after rats finished $PM_{2.5}$ exposure (red dots are up regulated genes; gray dots are unchanged genes; green dots are down regulated genes).

Table 3. GO analysis of changed signals after healthy rats were exposed to $PM_{2.5}$.

ID	Term	*P* value	Genes
Damage repair and cell proliferation			
1990090	cellular_response_to_nerve_growth_factor_stimulus	0.000557017	Acap2//Cbl//Crk//Ep300//Fgfr1//Foxo3//Pten//Rapgef1//Stmn2
1902033	regulation_of_hematopoietic_stem_cell_proliferation	0.005710551	Ace//Eif2ak2
1901998	toxin_transport	0.001694312	Antxr2//Casp1//Cd274//Lrp6//Mtmr12//Nrp1//Slc22a3
Inflammation			
2000343	positive_regulation_of_chemokine_(C-X-C_motif)_ligand_2_production	0.000810573	Cd74//Tirap//Tnf
2001181	positive_regulation_of_interleukin-10_secretion	0.000810573	Cd274//Lgals9//Ptger4
Sprouting_angiogenesis and cancer genesis			
1903672	positive_regulation_of_sprouting_angiogenesis	0.004105933	Hmgb1//Itga5//Jak1
Immune response			
1904996	positive_regulation_of_leukocyte_adhesion_to_vascular_endothelial_cell	0.005710551	Ets1//Tnf
2000406	positive_regulation_of_T_cell_migration	0.003530332	Cxcl12//Itga4//Itgb3//Lgals9
2001200	positive_regulation_of_dendritic_cell_differentiation	0.004105933	Hmgb1//Lgals9//Zbtb46
1990268	response_to_gold_nanoparticle	0.005710551	Tlr4//Tnf
1904646	cellular_response_to_amyloid-beta	0.008232478	Casp4//Foxo3//Psen1
ROS			
1900407	regulation_of_cellular_response_to_oxidative_stress	0.011085795	Fut8//Met
Cell death			
1901300	positive_regulation_of_hydrogen_peroxide-mediated_programmed_cell_death	0.017935943	Abl1//Foxo3
Undetermined pathways			
1990851	Wnt-Frizzled-LRP5/6_complex	0.005668176	LOC100909849//Lrp6

as autophagy, which had been reported to trigger lung injury [33]. While cell apoptosis and autophagy keep the balance of cell death and survival [34] and their effects could not conclude in a word. Cell apoptosis might be an adverse effect caused by $PM_{2.5}$ exposure, but also could be a protective response to eliminate damaged cells and keep the cells from malignant transformation. After the lung damage occurred, damage repair program may begin. Nerve growth factors (NGFs), a species of cytokines, possess the ability to promote cell proliferation and wound tissues healing [35] were observed increased after exposure. B-cell lymphoma 2 (Bcl-2) is a prooncogene and is a catalyst for tumor formation and development. Bcl-2 gene expression is crucial in regulating Cyt C release, which leads to the reduction of caspase-3 and caspase-9 proteins, and eventually leads to apoptosis blockage. When the tissues were damaged by the PMs or bacteria, the stem cells and fibroblast begin to repair the damaged tissues. As known, higher numbers of repair means higher frequency of making mistakes, which was prone to generate tumors. In addition, tissue repair always could not recover itself to the origin condition, which may cause pulmonary fibrosis, and was observed in another study [36].

In addition to the activation of prooncogene, previous study showed that $PM_{2.5}$ exposure would induce genetic variation and DNA damage [37], which is also a potential risk for tumor genesis. Many studies reported that exposure to $PM_{2.5}$ not only caused damage of DNA, but also modified methylation or acetylation of DNA and histones, which may alter oncogene expression in turn [38, 39]. Indeed, our results showed that much tumor related genes were activated after exposure of $PM_{2.5}$. The Fibroblast growth factor (FGF) /FGFR pathway can also activate downstream

Table 4. KEGG analysis of the changed pathways after healthy rats were exposed to $PM_{2.5}$ ($P < 0.01$).

ID	Term	*P* value	Genes
rno04621	NOD-like_receptor_signaling_pathway	4.4556E-07	Antxr1//Antxr2//Casp1//Casp4//Gbp1//Gbp2//Gbp4//Gbp5//Ifnar1//Ikbkg//Irf7//Itpr2//Jak1//Mapk1//Mcu//Oas1a//Oas1b//Oas2//Stat1//Tlr4//Tnf//Tnfaip3//Trpm7//Xiap
rno04514	Cell_adhesion_molecules_(CAMs)	8.3800E-06	Cd274//Cd4//Cldn15//Cntn1//Itga4//Itga9//Itgal//Itgav//Jam2//Ptprm//RT1-Ba//RT1-Bb//RT1-CE3//RT1-CE5//RT1-CE7//RT1-Da//RT1-Ha//RT1-N2//RT1-T24-1//RT1-T24-3//Sdc3//Selplg//Vcam1
rno05200	Pathways_in_cancer	1.3364E-05	Abl1//Adcy7//Arnt//Cbl//Col4a3//Col4a5//Crk//Cxcl12//Ednrb//Ep300//Fgf7//Fgfr1//Foxo1//Fzd8//Gna11//Gnaq//Gnb4//Gnb5//Hhip//Ikbkg//Itgav//Jak1//Lama4//Lamc1//Mapk1//Met//Nras//Prkacb//Pten//Ptger4//Rb1//Smad2//Stat1//Stat5b//Tcf7//Tgfbr2//Wnt2//Wnt2b//Xiap
rno04620	Toll-like_receptor_signaling_pathway	3.3788E-05	Ifnar1//Ikbkg//Irf5//Irf7//Map2k4//Map2k7//Mapk1//Stat1//Tirap//Tlr3//Tlr4//Tlr5//Tlr7//Tlr8//Tnf
rno04612	Antigen_processing_and_presentation	4.3971E-05	Cd4//Cd74//Ciita//RT1-Ba//RT1-Bb//RT1-CE3//RT1-CE5//RT1-CE7//RT1-Da//RT1-Ha//RT1-N2//RT1-T24-1//RT1-T24-3//Tapbp//Tnf
rno04062	Chemokine_signaling_pathway	0.000114566	Adcy7//Arrb1//Ccl17//Ccr7//Crk//Cx3cr1//Cxcl12//Dock2//Elmo1//Foxo3//Gnb4//Gnb5//Grk5//Ikbkg//Mapk1//Nras//Prex1//Prkacb//Rap1b//Stat1//Stat5b
rno04658	Th1_and_Th2_cell_differentiation	0.000118083	Cd4//Ikbkg//Il12rb2//Jak1//Maml2//Mapk1//Notch3//RT1-Ba//RT1-Bb//RT1-Da//RT1-Ha//Runx3//Stat1//Stat5b
rno04151	PI3K-Akt_signaling_pathway	0.000159155	Angpt1//Atf2//Col4a3//Col4a5//Col6a1//Col6a5//Fgf7//Fgfr1//Foxo3//Gnb4//Gnb5//Ifnar1//Ikbkg//Il7r//Itga4//Itga5//Itga9//Itgav//Itgb3//Jak1//Lama4//Lamc1//Mapk1//Met//Nras//Osmr//Phlpp1//Pp-p2ca//Pten//Sgk3//Tlr4//Tsc1
rno04520	Adherens_junction	0.000160789	Ep300//Fgfr1//Iqgap1//Mapk1//Met//Ptprb//Ptprm//Smad2//Sorbs1//Tcf7//Tgfbr2//Yes1
rno04217	Necroptosis	0.000288279	Casp1//Cyld//Eif2ak2//Hist1h2an//Hmgb1//Ifnar1//Il33//Jak1//Spata2//Stat1//Stat5b//Tlr3//Tlr4//Tnf//Tnfaip3//Tnfsf10//Trpm7//Xiap
rno05416	Viral_myocarditis	0.000348025	Abl1//Cav1//Itgal//RT1-Ba//RT1-Bb//RT1-CE3//RT1-CE5//RT1-CE7//RT1-Da//RT1-Ha//RT1-N2//RT1-T24-1//RT1-T24-3
rno00533	Glycosaminoglycan_biosynthesis-keratan_sulfate	0.000395550	Chst2//Chst4//Fut8//St3gal1//St3gal3
rno05330	Allograft_rejection	0.000445146	RT1-Ba//RT1-Bb//RT1-CE3//RT1-CE5//RT1-CE7//RT1-Da//RT1-Ha//RT1-N2//RT1-T24-1//RT1-T24-3//Tnf
rno04659	Th17_cell_differentiation	0.000553621	Cd4//Ikbkg//Il1rap//Il6st//Jak1//Mapk1//RT1-Ba//RT1-Bb//RT1-Da//RT1-Ha//Smad2//Stat1//Stat5b//Tgfbr2
rno05167	Kaposi's_sarcoma-associated_herpesvirus_infection	0.000562415	Eif2ak2//Ep300//Gnb4//Gnb5//Ifnar1//Ikbkg//Il6st//Irf7//Itpr2//Jak1//Mapk1//Nras//Rb1//RT1-CE3//RT1-CE5//RT1-CE7//RT1-N2//RT1-T24-1//RT1-T24-3//Stat1//Tlr3
rno05150	Staphylococcus_aureus_infection	0.000656851	C4a//C5//Fgg//Itgal//RT1-Ba//RT1-Bb//RT1-Da//RT1-Ha//Selplg
rno04060	Cytokine-cytokine_receptor_interaction	0.000660033	Acvr2a//Bmpr1a//Bmpr2//Ccl17//Ccr7//Csf2rb//Cx3cr1//Cxcl12//Ifnar1//Il10ra//Il12rb2//Il1rap//Il6st//Il7r//Lepr//Lifr//Met//Osmr//Tgfbr2//Tnf//Tnfrsf17//Tnfrsf1b//Tnfsf10
rno05321	Inflammatory_bowel_disease_(IBD)	0.000722022	Il12rb2//RT1-Ba//RT1-Bb//RT1-Da//RT1-Ha//Smad2//Stat1//Tlr4//Tlr5//Tnf

Table 4. Continued.

rno04510	Focal_adhesion	0.001677715	Cav1//Col4a3//Col4a5//Col6a1//Col6a5//Crk//Itga4//Itga5//Itga9//Itgav//Itgb3//Lama4//Lamc1//Mapk1//Met//Pten//Rap1b//Rapgef1//Tln1//Xiap
rno05310	Asthma	0.001704295	Fcer1a//RT1-Ba//RT1-Bb//RT1-Da//RT1-Ha//Tnf
rno04919	Thyroid_hormone_signaling_pathway	0.001788120	Ep300//Foxo1//Gata4//Itgav//Itgb3//Mapk1//Med13//Ncoa3//Notch3//Nras//Plce1//Prkacb//Slc16a2//Stat1
rno05203	Viral_carcinogenesis	0.002098630	Atf2//Eif2ak2//Ep300//Hist2h2be//Ikbkg//Il6st//Irf7//Jak1//Mad1l1//Mapk1//Nras//Prkacb//Rb1//RT1-CE3//RT1-CE5//RT1-CE7//RT1-N2//RT1-T24-1//RT1-T24-3//Sp100//Stat5b
rno04512	ECM-receptor_interaction	0.002415143	Col4a3//Col4a5//Col6a1//Col6a5//Itga4//Itga5//Itga9//Itgav//Itgb3//Lama4//Lamc1
rno05220	Chronic_myeloid_leukemia	0.002649554	Abl1//Cbl//Crk//Gab2//Ikbkg//Mapk1//Nras//Rb1//Stat5b//Tgfbr2
rno05320	Autoimmune_thyroid_disease	0.003592344	RT1-Ba//RT1-Bb//RT1-CE3//RT1-CE5//RT1-CE7//RT1-Da//RT1-Ha//RT1-N2//RT1-T24-1//RT1-T24-3
rno05211	Renal_cell_carcinoma	0.003927206	Arnt//Crk//Ep300//Ets1//Mapk1//Met//Nras//Rap1b//Rapgef1
rno04064	NF-kappa_B_signaling_pathway	0.004980054	Btk//Card10//Cxcl12//Ddx58//Ikbkg//Tirap//Tlr4//Tnf//Tnfaip3//Vcam1//Xiap
rno05205	Proteoglycans_in_cancer	0.005367242	Cav1//Cbl//Fgfr1//Fzd8//Iqgap1//Itga5//Itgav//Itgb3//Itpr2//Mapk1//Met//Nras//Plce1//Prkacb//Smad2//Tlr4//Tnf//Wnt2//Wnt2b
rno04068	FoxO_signaling_pathway	0.005450996	Ccng2//Ep300//Foxo1//Foxo3//Il7r//Klf2//Mapk1//Nras//Pten//Setd7//Sgk3//Smad2//Tgfbr2//Tnfsf10
rno04360	Axon_guidance	0.005709911	Abl1//Bmpr2//Cxcl12//Efnb2//Mapk1//Met//Nras//Nrp1//Ntn1//Plxnc1//Sema3d//Sema3g//Sema4d//Sema6a//Slit3//Ssh1//Ssh2
rno04672	Intestinal_immune_network_for_IgA_production	0.006056501	Cxcl12//Itga4//RT1-Ba//RT1-Bb//RT1-Da//RT1-Ha//Tnfrsf17
rno04550	Signaling_pathways_regulating_pluripotency_of_stem_cells	0.007081355	Acvr2a//Bmpr1a//Bmpr2//Fgfr1//Fzd8//Il6st//Jak1//Lifr//Mapk1//Nras//Skil//Smad2//Wnt2//Wnt2b
rno04350	TGF-beta_signaling_pathway	0.008082143	Acvr2a//Bmpr1a//Bmpr2//Ep300//Mapk1//Ppp2ca//Smad2//Smad6//Tgfbr2//Tnf
rno05412	Arrhythmogenic_right_ventricular_cardiomyopathy_(ARVC)	0.008589086	Cacna2d2//Gja1//Itga4//Itga5//Itga9//Itgav//Itgb3//Slc8a1//Tcf7
rno04145	Phagosome	0.009803893	Colec12//Itga5//Itgav//Itgb3//M6pr//RT1-Ba//RT1-Bb//RT1-CE3//RT1-CE5//RT1-CE7//RT1-Da//RT1-Ha//RT1-N2//RT1-T24-1//RT1-T24-3//Scarb1//Tlr4
rno05224	Breast_cancer	0.010850132	Fgf7//Fgfr1//Fzd8//Hey1//Lrp6//Mapk1//Ncoa3//Notch3//Nras//Pten//Rb1//Tcf7//Wnt2//Wnt2b

mitogen-activated protein kinases (MAPK) signaling pathways, which can induce the activation of JNKs [40], JNKs is reported playing important roles in the progress of cancer development. Meanwhile, JAK-STAT pathways, NF-κB family genes, Nrf2/ARE pathway and MAPK pathway, which were closely related to tumor growth and developing, were observed activated when health rats were exposed to $PM_{2.5}$ [41-44]. Sprouting angiogenesis was an important character for tumor genesis, the new generated blood vessels could supply more nutrition for tumor cells which would accelerate cancer development. FGF family and MAPK pathways were reported related to angiogenesis, FGF family is also a signal that promotes angiogenesis, and MAPK pathway was reported to change the expression of vascular endothelial growth factor thus participated in new blood vessels formation. When they are abnormally expressed, more blood vessels continuously exist to nourish tumor tissue, which is consistent with the fact that $PM_{2.5}$ exposure is associated with lung cancer [45].

Another important change after $PM_{2.5}$ exposure was ROS generation. Oxidation stress can be defined as the damage caused by the imbalance of the individual's redox state. Excessive ROS have multiple adverse effects on cells including mitochondrial damage [46] and cell death [33], and can also induce a variety of diseases. Furthermore, excessive ROS in the lung tissue would activate neutrophils, which resulted in continuous airway inflammatory reactions. Studies indicated that $PM_{2.5}$ would cause cell infiltration and structural remodeling [20]. And in this study, we observed the cells response to amyloid-beta after $PM_{2.5}$ exposure, which was reported to promote fiber deposition [47].

Transcriptional Profile Changes after COPD Modeling

The comparison between group A and group C showed that, 14202 genes showed no difference between two groups, 128 genes were up regulated and 191 genes were down regulated. The results are shown with the volcano map (Fig. 3). The following Go analysis showed that 34 pathways were enriched, and the KEGG Pathway analysis showed that 25 pathways were enriched in COPD group (Table 5 and Table 6). Abnormal inflammatory reactions are usually considered to be the cause and contributing factor for the progression of COPD. Airway inflammation response is the main alteration in COPD model, which leads to the remodeling of airway, and finally exudates blocked the airway lumen [48]. And our results showed that the signal pathways mainly focused on inflammatory cell chemotaxis, inflammatory factor release, inflammatory medium and kinase cascade reaction activation. Current study clearly indicated that proteinase-antiproteinase imbalance, chronic inflammatory reaction, apoptosis and oxidative stress reactions played important roles in the development of COPD [49]. Meanwhile, the inflammation related pathways, including NF-κB, peroxidase in prostaglandins, and multiple inflammatory factors including tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8, IL-1A, IL-1B, IL-10 were all up-regulated in COPD model, which was consistent with our knowledge [49, 50].

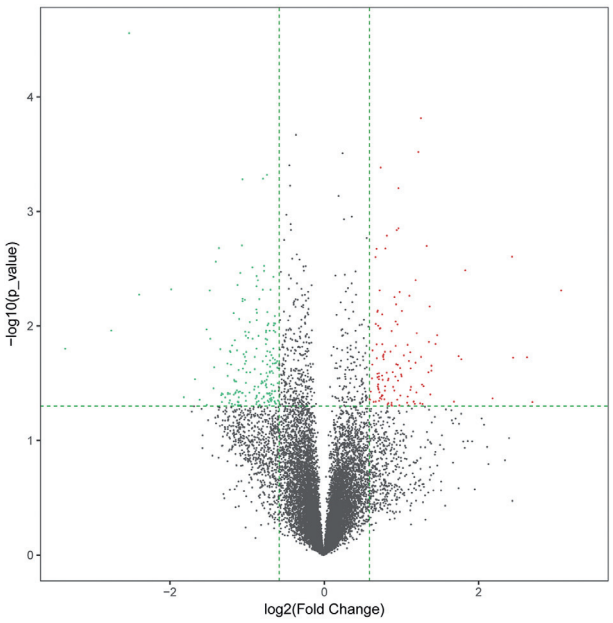

Fig. 3. A volcano map indicating the altered genes after rats finished COPD modeling (red dots are up regulated genes; gray dots are unchanged genes; green dots are down regulated genes).

Programmed cell death was also observed in COPD models. Given the fact that COPD models were established by passive smoking, the cell death might be induced by toxins in cigarette smog, which was consistent with our finding that the toxin transport pathway was activated in the current study. In addition, immune responses were activated, and the phagocytosis might be enhanced to clean out the dead, damaged or malignant cells. Previous study showed that, immune imbalance was a character of COPD lungs, immune imbalance joined in the development and progression of COPD, so the over activated immune response might be a rapier in COPD. In addition, our results showed that dendritic cell differentiation was inhibited, which may antigen presenting and infections resistant ability.

Transcriptional Profile Changes after COPD Rats Were Exposed to $PM_{2.5}$

Transcriptional profiles were compared between group C and group D, and the volcano map showed that 14130 genes showed no difference between two groups. 328 genes were increased, and 81 genes were decreased (Fig. 4). The GO analysis, as well as the KEGG Pathway analysis showed 84 and 35 enriched pathways, respectively (Table 7 and Table 8). COPD patients are more sensitive to air quality, and $PM_{2.5}$ in

Table 5. GO analysis of the signals that were significantly changed after COPD modeling.

ID	Term	*P* value	Genes
Damage repair and cell proliferation			
1901998	toxin_transport	4.5755E-05	Antxr2//Casp1//Cd274//Rab43
0050766	positive_regulation_of_phagocytosis	0.003545833	C4a//C4b//Fcgr2b
0042127	regulation_of_cell_proliferation	0.011127629	Fcgr2b//Muc16//Ptger4
Immune response			
2001199	negative_regulation_of_dendritic_cell_differentiation	7.3353E-05	Tmem176a//Tmem176b
0002381	immunoglobulin_production_involved_in_immunoglobulin_mediated_immune_response	0.008026889	RT1-Bb//RT1-Db1
0045582	positive_regulation_of_T_cell_differentiation	0.009218099	Cd74//Gimap5//RT1-Ba
0010803	regulation_of_tumor_necrosis_factor-mediated_signaling_pathway	0.010497037	Casp1//Casp4
0042613	MHC_class_II_protein_complex	3.1400E-11	Cd74//RT1-Ba//RT1-Bb
Inflammation			
2001181	positive_regulation_of_interleukin-10_secretion	0.000242928	Cd274//Ptger4
0007263	nitric_oxide_mediated_signal_transduction	0.002844055	Apoe//Mt1
0032611	interleukin-1_beta_production	0.002844055	Casp1//Gbp5
0071380	cellular_response_to_prostaglandin_E_stimulus	0.004896830	Ptger4//Sfrp1
0032652	regulation_of_interleukin-1_production	0.005432363	Casp1//Casp4//Ptger4
0071379	cellular_response_to_prostaglandin_stimulus	0.007456438	Ptger4//Sfrp1
0050718	positive_regulation_of_interleukin-1_beta_secretion	0.008616374	Casp1//Casp4
0050716	positive_regulation_of_interleukin-1_secretion	0.009224693	Casp1//Casp4
0072557	IPAF_inflammasome_complex	0.000228869	Casp1//Casp4
0097169	AIM2_inflammasome_complex	0.000228869	Casp1//Casp4
0072559	NLRP3_inflammasome_complex	0.000634802	Casp1//Casp4
0034695	response_to_prostaglandin_E	0.011160668	Ptger4//Sfrp1
Cell death and survival			
0043067	regulation_of_programmed_cell_death	0.005735530	Apoe//Casp1//Casp4//Ccnd2

the atmosphere may accelerate the disease progression, while the underlying mechanisms keep largely unknown. Our results showed that 328 genes were up regulated and 81 were decreased. The pathways showed similarity profile with both $PM_{2.5}$ solitary exposure and COPD model, and mainly focused on ROS, inflammation, cell proliferation, immune response, vasculogenesis and tumor development related signals, but more significant than solitary exposure.

Notably, significant immune related changes were observed in COPD rats after $PM_{2.5}$ exposure. $PM_{2.5}$ is a component of multiple substrates, and microorganisms (bacteria, virus and fungus) were involved [51], the microorganisms and their endotoxins are considered to play important roles in activating inflammatory and immune response [52]. The important features of immune activation are immune cells activation and immunization factors release. Our results showed that, the dendritic cell differentiated for antigen presentation and leukocyte migrated to the intrusive site to kill the bacteria or fungus. In addition, the immunization factors related pathways and gene expressions were also observed up-regulated. Studies indicated that immune imbalance plays crucial roles in COPD progression, immunization factors usually play positive roles in protecting lungs, but studies also indicated that abnormal immunization factors could also destroy lungs and accelerate the process of COPD [53], so if the immunology response is out of control, it would damage the lung on the contrary.

Inflammation factors, most are immunization factors, mediated the most inflammation response just as in COPD model. A large number of studies had shown that, exposure to $PM_{2.5}$ induced excessive inflammatory

Table 6. KEGG pathway analysis of the changed pathways after COPD modeling **($P < 0.01$).**

ID	Term	*P* value	Genes
rno05150	Staphylococcus_aureus_infection	7.2056E-13	C1qb//C1qc//C1s//C4a//Fcgr2b
rno04612	Antigen_processing_and_presentation	1.7264E-07	Cd74//Ciita//Klrc1//RT1-Ba//RT1-Bb
rno05310	Asthma	8.6315E-07	RT1-Ba//RT1-Bb//RT1-Da
rno05321	Inflammatory_bowel_disease_(IBD)	3.0797E-06	RT1-Ba//RT1-Bb//RT1-Da//RT1-Db1//RT1-DMb//Smad2
rno05133	Pertussis	8.1852E-06	C1qb//C1qc
rno05164	Influenza_A	1.0685E-05	Casp1//Ciita//Ivns1abp
rno04610	Complement_and_coagulation_cascades	1.6227E-05	C1qb//C1qc//C1s//C4a
rno05152	Tuberculosis	1.9724E-05	Cd74//Ciita//Fcgr2b//RT1-Ba
rno04659	Th17_cell_differentiation	6.7343E-05	RT1-Ba//RT1-Bb
rno05320	Autoimmune_thyroid_disease	0.000144207	RT1-Ba//RT1-Bb//RT1-Da
rno04145	Phagosome	0.000231684	Fcgr2b//Rab7b//RT1-Ba
rno05416	Viral_myocarditis	0.000306209	RT1-Ba//RT1-Bb
rno04658	Th1_and_Th2_cell_differentiation	0.000340016	RT1-Ba//RT1-Bb
rno04640	Hematopoietic_cell_lineage	0.000357944	RT1-Ba//RT1-Bb
rno05168	Herpes_simplex_infection	0.000420609	Cd74//RT1-Ba//RT1-Bb
rno05166	HTLV-I_infection	0.000597795	Ccnd2//RT1-Ba//RT1-Bb//RT1-Da
rno04514	Cell_adhesion_molecules_(CAMs)	0.000937492	Cd274//RT1-Ba//RT1-Bb//RT1-Da//RT1-Db1//RT1-DMb

reactions in the body [54]. The immunization response participated in the lung protection, damage repair, but also may accelerate disease progress. So whether the inflammation is more a protective factors or risk factors for COPD needs further study, previous study indicated that antagonism of inflammatory factors presented protective effects of the COPD lungs from deterioration, while whether this treatment would induce potential hazard to lungs or increase the risk of tumor genesis needs long term observation.

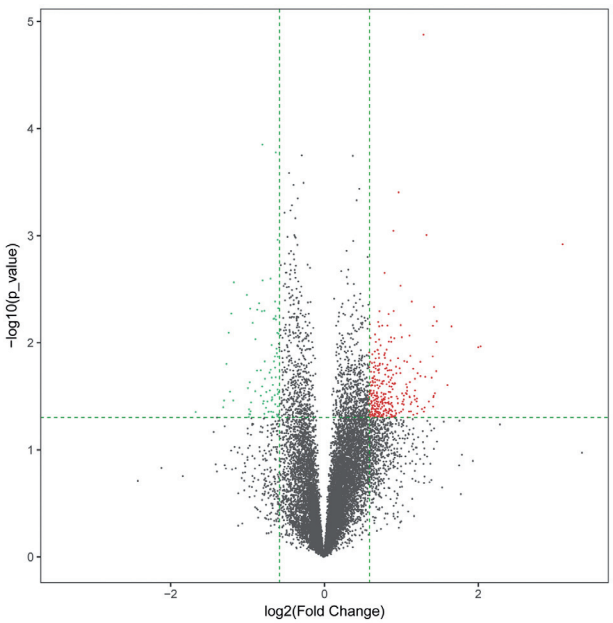

Fig. 4. A volcano map indicating the altered genes after rats finished COPD modeling and PM2.5 exposure (red dots are up regulated genes; gray dots are unchanged genes; green dots are down regulated genes).

Different from the $PM_{2.5}$ solitary exposure, cell apoptosis was inhibited after COPD rats exposed to $PM_{2.5}$. Cell apoptosis is a duplex response, proper apoptosis helps the body to defend the happening of tumor, while abnormal apoptosis could damage tissues [46]. The inhibition of cell apoptosis may cause the accumulation of cancerous cells and increase the risk of tumor. In addition to the inhibited cell apoptosis, we observed that the angiogenesis related pathways were enhanced, which suggested that it is easier for the patients to develop lung tumors after $PM_{2.5}$ exposure. In this condition, the activated cell proliferation related pathways after COPD rats exposed to $PM_{2.5}$, may not help for damage repair but was an increased risk of tumor development [55].

Study Limitations

In this study, we not only explored the adverse effects of $PM_{2.5}$ on healthy lung, but explored their effects on COPD model rats, whose lung functions had been destroyed. But it is worth noting that, the pathways activated after $PM_{2.5}$ exposure were complex and work in a network, we cannot easily drew the conclusion

Table 7. GO analysis of the changed signals after COPD rats exposed to $PM_{2.5}$.

ID	Term	*P* value	Genes
Cell death			
1902043	Positive regulation of extrinsic apoptotic signaling pathway via death domain receptors	0.001635598	Mal//Pidd1//Thbs1
0012501	Programmed cell death	0.000165895	Adrb1//Ano6//Arrb1//Atp7a
Cell proliferation and differentiation			
2000648	Positive regulation of stem cell proliferation	0.018250241	Nfya//Tbx3
1903078	Positive regulation of protein localization to plasma membrane	0.013500723	Myo5b//Sptbn1
0030335	Positive regulation of cell migration	2.65264E-09	Adam9//Amotl1//Ano6//Atp7a cl1//Cxcl3//Dock5
0043408	Regulation of MAPK cascade	0.000149908	Adam9//Arrb1//Ash1l//C5ar1// C5ar2//Crk//Csf1r/
0043410	Positive regulation of MAPK cascade	0.001123941	Crk//Csf1r//Fgfr1//Fgg
0000165	MAPK cascade	0.000711382	Brap//Crk//Csf1r//Dok4//Fgfr1
0070374	positive_regulation_of_ERK1_and_ERK2_cascade	0.003992620	Arrb1//C5ar1//C5ar2//Csf1r
1902459	Positive regulation of stem cell population maintenance	0.005959473	Tead1//Yap1
0070372	Regulation of ERK1_and_ERK2 cascade	0.010805871	Arrb1//C5ar1//C5ar2//Csf1r
Inflammation			
0002526	Acute inflammatory response	0.002542938	Ano6//Ass1//Cxcl1//Il1a
0032623	interleukin-2_production	0.009020635	Gpam//Il1rap
0090197	Positive regulation of chemokine secretion	0.012975861	Csf1r//Lpl
0071731	Response to nitric oxide	0.000621656	Crk//Il1r1//Sftpa1//Thbs1
0004908	interleukin-1 receptor activity	0.003300900	Il1r1//Il1rap
0050715	Positive regulation of cytokine secretion	0.013160049	Clec5a//Csf1r//Il1a//Il1rap
Immune response			
2000391	Positive regulation of neutrophil extravasation	0.004514314	Il1a//Il1r1
0010759	Positive regulation of macrophage chemotaxis	0.001851254	C5ar1//Ptprj//Thbs1
0090023	Positive regulation of neutrophil chemotaxis	0.009599903	C5ar1//Cxcl1//Cxcl3
0010758	Regulation of macrophage chemotaxis	0.000621656	C5ar1//Mmp28//Ptprj//Thbs1
0009617	Response to bacterium	0.014556731	Adam9//Ass1//Bcr//C5ar1//Cfb
0050766	Positive regulation of phagocytosis	0.012513735	Ano6//Bcr//Ptk2//Sftpa1
0071622	Regulation of granulocyte chemotaxis	0.000124986	C5ar1//C5ar2//Cxcl1//Cxcl3
Vasculogenesis and tumor development			
0001944	Vasculature development	0.001664899	Adipor2//Amotl1//Atp7a//Clic4
1904754	Positive regulation of vascular associated smooth muscle cell migration	0.001851254	Atp7a//Dock5//Iqgap1
0014910	Regulation of smooth muscle cell migration	0.001812485	Atp7a//Crk//Dock5//Iqgap1
0001568	Blood vessel development	0.002224112	Adipor2//Fgfr1//Heg1//Itgav
0014911	Positive regulation of smooth muscle cell migration	0.001895475	Atp7a//Crk//Dock5//Iqgap1
0097755	Positive regulation of blood vessel diameter	0.006241572	Adrb1//Dock5//Gch1//Ptk2
0048514	Blood vessel morphogenesis	0.010665994	Adipor2//Amotl1//Clic4//Fgfr1
0010574	Regulation of vascular endothelial growth factor production	0.012490316	Aqp4//C5ar1//Il1a
0001570	vasculogenesis	0.000897526	Heg1//Itgav//Ptk2

Table 7. Continued.

ROS			
0006801	Superoxide metabolic process	0.011477775	Atp7a//Sh3pxd2b//Sod2
0000302	Response to reactive oxygen species	0.012157422	Adam9//Atp7a//Crk//Il1a//Il1r1
0034599	Cellular response to oxidative stress	0.014669887	Atp7a//Crk//Kdm6b//Oxr1
Undefined pathways			
0042325	Regulation of phosphorylation	9.4079E-05	Adam9//Akap5//Arrb1//Ash1l rd10//Cntn1
0051592	Response to calcium ion	0.00092769	Adam9//Clic4//Cpne2//Cyp2b2
0045309	Protein phosphorylated amino acid binding	0.000145070	Arrb1//Crk//Hck//Pik3r3//She//Yes1
1904264	Ubiquitin protein ligase activity involved in ERAD pathway	0.007923703	Amfr//March6
0060921	Sinoatrial node cell differentiation	0.001275254	Popdc2//Tbx3
0006639	Acylglycerol metabolic process	0.001422515	Abhd2//Apobr//Gk//Gpam//Lpl
0042327	Positive regulation of phosphorylation	0.001631022	Adam9//C5ar2//Card10//Cntn1
0045859	Regulation of protein kinase activity	0.006756971	Adam9//Arrb1//Aspn//Card10
0038178	Complement component C5a signaling pathway	0.000216750	C5ar1//C5ar2
0007169	transmembrane_receptor_protein_tyrosine_kinase_signaling_pathway	0.001282903	Bcr//Crk//Csf1r//Dok4//Fgfr1

Table 8. KEGG pathway analysis of the changed pathways after COPD rats exposed to $PM_{2.5}$ ($P < 0.01$).

ID	Term	*P* value	Genes
rno04810	Regulation_of_actin_cytoskeleton	0.000430804	Abi2//Arhgef12//Crk
rno04520	Adherens_junction	0.000776523	Fgfr1//Iqgap1//Lmo7//Ptprj//Sorbs1//Yes1
rno04610	Complement_and_coagulation_cascades	0.001551763	C4bpa//C5ar1
rno04071	Sphingolipid_signaling_pathway	0.002433225	Cers6//Gnai1//Gnaq
rno05332	Graft-versus-host_disease	0.003258649	Gzmb//Il1a//Prf1//RT1-T24-1//RT1-T24-4
rno05205	Proteoglycans_in_cancer	0.004206284	Arhgef12//Fgfr1//Iqgap1
rno04978	Mineral_absorption	0.004642774	Atp7a//Slc30a1//Tf//Trpm6
rno04062	Chemokine_signaling_pathway	0.005360074	Arrb1//Crk//Cxcl1//Cxcl3
rno00600	Sphingolipid_metabolism	0.006828952	Cers6//Sgms2//Sgpp2//Sptlc2
rno04923	Regulation_of_lipolysis_in_adipocytes	0.010239165	Adrb1//Gnai1//Mgll//Pik3r3

that the changes are risks or protective factors, and we cannot determine the causal relationship. In addition, our findings were not further proved in rat models or COPD patients, which limited the application of these results to human beings. It is essential for us to verify the candidate pathways and related biomarkers in rat model and COPD patients in the future.

Conclusions

We found that inhalation of $PM_{2.5}$ could change the transcriptional level of multiple genes mainly on damage repair, inflammation, immune response, ROS, cell death and proliferation, vasculogenesis, and tumor development in rat lung tissues. For rats with COPD, inhalation of $PM_{2.5}$ induced similar pathway profiles changes. In addition, more cell proliferation related pathways, which also proved to be activated in tumor development, were observed changed, indicating higher risks of tumor genesis in COPD patients. Our study provided insights into mechanisms underlying $PM_{2.5}$ caused lung injury in humans, especially in COPD patients, which helps to better understand the adverse effects caused by $PM_{2.5}$ and provided potential intervention methods.

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Conflicts of Interest

The authors declare no conflict of interest.

Disclosures and Declarations

All animal studies were approved by the appropriate ethics committee of The Second Hospital of Hebei Medical University.

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