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].

**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

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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: SO$_4^{2-}$, 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 recource of SO$_4^{2-}$, 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±2°C 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 hematoxylin 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 (Hebing 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.
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(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.](image-url)
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 1. Monitoring of the exposure conditions.

| Time       | Inhaled PM$_{2.5}$ (mg/m$^3$)$^a$ | Atmospheric PM$_{2.5}$ (μg/m$^3$)$^a$ | Animal number | Exposure duration (min)$^b$ | Average temperature ($^\circ$C)$^b$ | 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.
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

| 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       | 691702965           | 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$_{2.5}$

![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).](image-url)
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 genes.

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

| ID       | Term                                                                 | $P$ value          | Genes                                                                                   |
|----------|----------------------------------------------------------------------|--------------------|-----------------------------------------------------------------------------------------|
| 1990090  | cellular_response_to_nerve_growth_factor_stimulus                    | 0.000557017        | Acp2/Cbl/Crk/Ep300/Fgfr1/Foxo3/Pten/Rapgef1/Stmn2                                     |
| 1902033  | regulation_of_hematopoietic_stem_cell_proliferation                  | 0.005710551        | Acc/Eit2ak2                                                                             |
| 1901998  | toxin_transport                                                      | 0.001694312        | Antxr2//Casp1/Cd274/Lrp6//Mtmr12/Nrp1//Slc22a3                                          |
| 200343   | positive_regulation_of_chemokine_C-X-C_motif_2_production            | 0.000810573        | Cd74/Tirap/Tnf                                                                          |
| 2001181  | positive_regulation_of_interleukin-10_secretion                      | 0.000810573        | Cd274/Lgals9//Pger4                                                                    |
| 1903672  | positive_regulation_of_sprouting_angiogenesis                        | 0.004105933        | Hmgbl//Itga5//Jak1                                                                      |
| 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//Igfb3//Lgals9                                                            |
| 2001200  | positive_regulation_of_dendritic_cell_differentiation                | 0.004105933        | Hmgbl//Lgals9//Zbtb46                                                                   |
| 1990268  | response_to_gold_nanoparticle                                       | 0.005710551        | Tlr4/Tnf                                                                               |
| 1904646  | cellular_response_to_amyloid-beta                                    | 0.008232478        | Casp4//Foxo3//Psen1                                                                     |
| 1900407  | regulation_of_cellular_response_to_oxidative_stress                  | 0.011085795        | Fut8//Met                                                                              |
| 1901300  | positive_regulation_of_hydrogen_peroxide-mediated_programmed_cell_death | 0.017935943        | Ab1//Foxo3                                                                             |
| 1990851  | Wnt-Frizzled-LRP5/6_complex                                          | 0.005668176        | LOC100909849//Lrp6                                                                     |
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                  | 4.455E-07|                                   |
| rno05414 | Toll-like receptor signaling pathway                  | 4.3971E-05|                               |
| rno04550 | Pathways in cancer                                    | 1.336E-05|                               |
| rno04612 | Antigen processing and presentation                  | 4.3971E-05|                               |
| rno04062 | Chemokine signaling pathway                          | 0.000114566|                              |
| rno04658 | Th1 and Th2 cell differentiation                      | 0.000118083|                              |
| rno04151 | PI3K-Akt signaling pathway                            | 0.000159155|                              |
| rno05330 | Allograft rejection                                   | 0.000445146|                              |
| rno05167 | Kaposi's sarcoma-associated herpesvirus infection    | 0.000562415|                              |
| rno05150 | Staphylococcus aureus infection                       | 0.000656851|                              |
| rno05321 | Inflammatory bowel disease (IBD)                     | 0.000722022|                              |

Other pathways and their respective genes and P values are also listed in the table, but the table is truncated for brevity.
| rno04510     | Focal_adhesion   | 0.001677715 | Cav1//Col4a3//Col4a5//Col6a1//Col6a5//Crk//Itga4//Itga5//Itga9//Itgb3//Lama4//Lamc1//Mapk1//Mert//Pen//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//Itgb3//Mapk1//Med13//Ncoa3//Notch3//Nras//Pcle1//Prkaeb//Sle16a2//Stat1 |
| rno05203     | Viral_carcinogenesis | 0.002098630 | Atf2//Eif2ak2//Ep300//Hist2h2be//Ikkbg//Il6st//Irf7//Jak1//Mad11//Mapk1//Nras//Prkaeb//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//Itgb3//Lama4//Lamc1 |
| rno05220     | Chronic_myeloid_leukemia | 0.002649554 | Abl1//Cbl//Crk//Gab2//Ikkbg//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 | Amt//Crl//Ep300//Ets1//Mapk1//Met//Nras//Pral1b//Rapgef1 |
| rno04064     | NF-kappa_B_signaling_pathway | 0.004980054 | Btk//Card10//Cxl12//Ddc58//Ikkbg//Tirap//Tlr4//Tnf//Tnfaj3//Vcam1//Xiap |
| rno05205     | Proteoglycans_in_cancer | 0.005367242 | Cav1//Cbl//Fgfr1//Fzd8//Igagp1//Itga5//Itgb3//Itpr2//Mapk1//Met//Nras//Pcle1//Prkaeb//Smad2//Tlr4//Tnf//Wnt2//Wnt2b |
| rno04068     | FoxO_signaling_pathway | 0.005450996 | Ccng2//Ep300//Foxo1//Foxo3//Il7r//Klf2//Mapk1//Nms//Pten//Sdet7//Sgk3//Smad2//Tgfbr2//Tnfstf10 |
| rno04360     | Axon_guidance     | 0.005709911 | Abl1//Bmpr2//Cxl12//Efnb2//Mapk1//Met//Nras//Nrp1//Ntn1//Plxnc1//Sema3d//Sema3g//Sema4d//Sema6a//Slit3//Ssh1//Ssh2 |
| rno04672     | Intestinal_immune_network_for_IgA_production | 0.006056501 | Cxl12//Itga4//RT1-Ba//RT1-Bb//RT1-Da//RT1-Ha//Tnfsf17 |
| 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//Gia1//Itga4//Itga5//Itga9//Itgb3//Sle16a1//Tc7 |
| rno04145     | Phagosome         | 0.009803893 | Colec12//Itga5//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//Lp6//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].

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
the atmosphere may accelerate the disease progression, while the underlying mechanisms keep largely unknown. Our results showed that 328 genes were upregulated 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

| ID      | Term                                           | P value       | Genes                                      |
|---------|------------------------------------------------|---------------|---------------------------------------------|
| 1901998 | toxin_transport                                 | 4.5755E-05    | Antrx2/Casp1/Cd274/Rab43                   |
| 0050766 | positive_regulation_of_phagocytosis            | 0.003545833   | C4a/C4b/Fcg2b                              |
| 0042127 | regulation_of_cell_proliferation               | 0.011127629   | Fcg2b/Muc16/Ptg4                          |
| 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                       |

| ID      | Term                                           | P value       | Genes                                      |
|---------|------------------------------------------------|---------------|---------------------------------------------|
| 2001181 | positive_regulation_of_interleukin-10_secretion| 0.000242928   | Cd274/Ptg4                                 |
| 0007263 | nitric_oxide_mediated_signal_transduction      | 0.002844055   | Apoe/Mt1                                   |
| 0032611 | interleukin-1-beta_production                  | 0.002844055   | Casp1/Gbp5                                 |
| 0071380 | cellular_response_to_prostaglandin_F_stimulus  | 0.004896830   | Ptg4/Sfrp1                                 |
| 0032652 | regulation_of_interleukin-1_production         | 0.005432363   | Casp1/Casp4/Ptg4                          |
| 0071379 | cellular_response_to_prostaglandin_stimulus    | 0.007456438   | Ptg4/Sfrp1                                 |
| 0050718 | positive_regulation_of_interleukin-1-beta_secretion | 0.009224693 | Casp1/Casp4                               |
| 0050716 | positive_regulation_of_interleukin-1_secretion | 0.011120668   | Ptg4/Sfrp1                                 |
| 0072557 | IPAF_inflammasome_complex                      | 0.000228699   | Casp1/Casp4                               |
| 0097169 | AIM2_inflammasome_complex                      | 0.000228699   | Casp1/Casp4                               |
| 0072559 | NLRP3_inflammasome_complex                     | 0.000634802   | Casp1/Casp4                               |
| 0034695 | response_to_prostaglandin_E                    | 0.011120668   | Ptg4/Sfrp1                                 |

| ID      | Term                                           | P value       | Genes                                      |
|---------|------------------------------------------------|---------------|---------------------------------------------|
| 0043067 | regulation_of_programmed_cell_death            | 0.005735530   | Apoe/Casp1/Casp4/Ccnd2                      |
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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.

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

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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/Krcl1/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-Dbl/RT1-DMb/Smad2 |
| rno05133 | Pertussis                        | 8.1852E-06 | C1qb/C1qc                                 |
| rno05164 | Influenza_A                      | 1.0685E-05 | Casp1/Ciita/Invs1apb                      |
| 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-Dbl/RT1-DMb |

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

| ID     | Term                                                                 | P value          | Genes                      |
|--------|----------------------------------------------------------------------|------------------|----------------------------|
| 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 death**

| ID     | Term                                                                 | P value          | Genes                      |
|--------|----------------------------------------------------------------------|------------------|----------------------------|
| 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//Amot1//Ano6//Atp7a    |
| 0043408| Regulation of MAPK cascade                                            | 0.000149908      | Adam9//Arrb1//Ash1//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**

| ID     | Term                                                                 | P value          | Genes                      |
|--------|----------------------------------------------------------------------|------------------|----------------------------|
| 0002526| Acute inflammatory response                                          | 0.002542938      | Ano6//Ass1//Cxc11//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   |
| 004908  | interleukin-1 receptor activity                                       | 0.003300900      | Il1r1//Il1rap              |
| 0050715| Positive regulation of cytokine secretion                             | 0.013160049      | Clec5a//Csf1r//Il1a//Il1rap |

**Immune response**

| ID     | Term                                                                 | P value          | Genes                      |
|--------|----------------------------------------------------------------------|------------------|----------------------------|
| 2000391| Positive regulation of neutrophil extravasation                      | 0.004514314      | Il1a//Il1r1               |
| 0010759| Positive regulation of macrophage chemotaxis                          | 0.001851254      | C5ar1//Pprj//Thbs1          |
| 0090023| Positive regulation of neutrophil chemotaxis                          | 0.009599903      | C5ar1//Cxc11//Cxc13        |
| 0010758| Regulation of macrophage chemotaxis                                   | 0.000621656      | C5ar1//Mmp28//Pprj//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//Cxc11//Cxc13  |

**Vasculogenesis and tumor development**

| ID     | Term                                                                 | P value          | Genes                      |
|--------|----------------------------------------------------------------------|------------------|----------------------------|
| 0001944| Vasculature development                                               | 0.001664899      | Adipor2//Amot1//Atp7a//Clec4 |
| 1904754| Positive regulation of vascular associated smooth muscle cell migration| 0.001851254      | Atp7a//Dock5//Iqgap1        |
| 0041910| Regulation of smooth muscle cell migration                             | 0.0001812485     | Atp7a//Crk//Dock5//Iqgap1   |
| 0001568| Blood vessel development                                              | 0.0002224112     | 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//Amot1//Clec4//Fgfr1 |
| 0010574| Regulation of vascular endothelial growth factor production           | 0.012490316      | Aqp4//C5ar1//Il1a          |
| 0001570| vasculogenesis                                                        | 0.000897526      | Heg1//Itgav//Ptk2          |
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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