The effect of exposure time and concentration of airborne PM$_{2.5}$ on lung injury in mice: A transcriptome analysis

Hongyun Wang$^1$, Xiyue Shen$^1$, Jingli Liu, Chunyan Wu, Junling Gao, Zezhong Zhang, Fang Zhang, Wenjun Ding **, Zhongbing Lu*

College of Life Science, University of Chinese Academy of Sciences, Beijing, 100049, China

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ABSTRACT

The association between airborne fine particulate matter (PM$_{2.5}$) concentration and the risk of respiratory diseases has been well documented by epidemiological studies. However, the mechanism underlying the harmful effect of PM$_{2.5}$ has not been fully understood. In this study, we exposed the C57BL/6J mice to airborne PM$_{2.5}$ for 3 months (mean daily concentration ~50 or ~110 μg/m$^3$, defined as PM$_{2.5}$–3L or PM$_{2.5}$–3H) or 6 months (mean daily concentration ~50 μg/m$^3$, defined as PM$_{2.5}$–6L) through a whole-body exposure system. Histological and biochemical analysis revealed that PM$_{2.5}$–3H exposure caused more severe lung injury than did PM$_{2.5}$–3L, and the difference was greater than that of PM$_{2.5}$–6L vs PM$_{2.5}$–3L exposure. With RNA-sequencing technique, we found that the lungs exposed with different concentration of PM$_{2.5}$ have distinct transcriptional profiles. PM$_{2.5}$–3H exposure caused more differentially expressed genes (DEGs) in lungs than did PM$_{2.5}$–3L or PM$_{2.5}$–6L. The DEGs induced by PM$_{2.5}$–3L or PM$_{2.5}$–6L exposure were mainly enriched in immune pathways, including Hematopoietic cell lineage and Cytokine-cytokine receptor interaction, while the DEGs induced by PM$_{2.5}$–3H exposure were mainly enriched in cardiovascular disease pathways, including Hypertrophic cardiomyopathy and Dilated cardiomyopathy. In addition, we found that upregulation of Cd5l and reduction of Hspa1 and peroxiredoxin-4 was associated with PM$_{2.5}$-induced pulmonary inflammation and oxidative stress. These results may provide new insight into the cytotoxicity mechanism of PM$_{2.5}$ and help to development of new strategies to attenuate air pollution associated respiratory disease.

1. Introduction

Currently, ambient air pollution has become a large threat to public health. Fine particulate matter (PM$_{2.5}$, aerodynamic diameter ≤ 2.5 μm) is one of the most important components of outdoor air pollution. High concentration of PM$_{2.5}$ increases the risk of respiratory diseases, including asthma [1], bronchitis [2], chronic obstructive pulmonary disease (COPD) [3] and lung cancer [4,5]. Using an analysis of daily time-series for the 20 largest US cities, the PM-mortality dose-response curves and threshold levels were firstly described in 2000 [6]. Then, multiple epidemiological studies have validated that there is a concentration-response relationship between airborne PM$_{2.5}$ and its harmful effects on respiratory system [5,7–9]. In mice models, a recent study found that the severity of lung injury caused by ambient PM exposure is associated with cumulative dose [10]. Acute exposure to low doses of fine PM by intranasal instillation also induced lung inflammation and oxidative stress in a dose-dependent manner [11]. It has been suggested that PM$_{2.5}$-induced inflammatory response was associated with Toll-like receptors (TLR2/TLR4) and PM$_{2.5}$ can drive a Th2-biased immune response in mice [12]. Notably, during inflammation induced by PM$_{2.5}$, enhanced production of reactive oxygen species (ROS) could result in DNA damage, lipid peroxidation and cell death [13–15]. However, the comprehensive mechanisms by which PM$_{2.5}$ causes lung injury have not been fully elucidated.

RNA-sequencing (RNA-seq) is a precise and sensitive tool for measuring global gene expression profiles expression. Recently, this technique has been used to investigate the mechanism of PM$_{2.5}$-induce cytotoxicity in cell models, including 16HBE [16], BEAS-2B [17], A549 [18] and human non-small-cell lung cancer (H1299) cells [19]. To better understand the harmful effects of PM$_{2.5}$ on respiratory system, we exposed mice to either airborne PM$_{2.5}$ or filtered air (FA) for 3–6 months through a whole-body exposure system and then obtained global gene expression profiles in lungs of FA or PM$_{2.5}$ exposed mice using RNA-seq.
Abbreviations

3′-NT 3-nitrotyrosine
4-HNE 4-hydroxynonenal
BALF bronchoalveolar lavage fluid
CdSi CDS antigen-like
COPD chronic obstructive pulmonary disease
DCM dilated cardiomyopathy
DEGs differentially expressed genes
FA filtered air
Gpx glutathione peroxidase
GSH reduced glutathione
GSSG oxidized glutathione
H&E hematoxylin and eosin
HCM hypertrophic cardiomyopathy
Hspa1 heat shock 70 kDa protein 1
IL interleukin
ILr interleukin receptor
KEGG kyoto encyclopedia of genes and genomes
Ndufs NADH dehydrogenase iron-sulfur protein
PM particulate matter
Prdx4 peroxiredoxin-4
qPCR quantitative real-time polymerase chain reaction
RNA-seq RNA-sequencing
ROS reactive oxygen species
Sod superoxide dismutase
TLR Toll-like receptors
TNFα Tumor Necrosis Factor
TNFrsf TNF receptor superfamily member 14
Trx thioredoxin
Trxr2 thioredoxin reductase

2. Materials methods

2.1. Reagents and antibodies

BCA protein assay kit and reduced/oxidized glutathione (GSH and GSSG) kit were purchased from the Beyotime Institute of Biotechnology (#P0012, #S0053, Shanghai, China). Elisa kits for mouse tumor necrosis factor alpha (TNFα), 3′-nitrotyrosine (3′-NT) and 4-hydroxynonenal (4-HNE) were purchased from Sino Biological Inc (#SEK50349, Beijing, China), Abcam PLC (#ab116691, Cambridge, UK) and Donggeboye Biological Technology Co. LTD (#DG30947 M, Beijing, China), respectively. The Masson’s trichrome stain kit and su-peroxide dismutase 3 (SOD3) antibody were obtained from Solarbio Science &Technology Co. LTD (#G1340, #K006598P, Beijing, China). Primary antibodies against β-tubulin, SOD1, SOD2, peroxiredoxin 3 (PRDX3), PRDX4, PRDX5 and thioredoxin reductase 2 (TRXR2) were purchased from Signalway Antibody LLC (#38075, #32058, #32265, #38567, #43303, #38828, #32885, College Park, MD, USA). Anti-galectin 3 and anti-neutrophil monoclonal antibodies were purchased from Bios Biotechnology Co. LTD (#bs-20700R, #bs-6982R, Beijing, China).

2.2. Animal experiments

As described previously [20], male C57BL/6J mice (20–22 g, obtained from HFK Bioscience Co., Beijing, China) were exposed to either ambient PM2.5 or FA in a “real-world” exposure system for 12 h/day, 7 days/week, from HFK Bioscience Co., Beijing, China) were exposed to either ambient air (PM2.5 exposure chamber, PM with an aerodynamic diameter greater than 2.5 μm was removed by a swirler. The exposure system locates at Zhong-guanqian campus of the University of Chinese Academy of Sciences (N39°57′39.83″E116°20′10.97″), which is ~50 m away from a traffic main artery (Shihuan Road). During the whole exposure stage, the mice were fed commercial mouse chow and distilled water ad libitum, and were housed under a controlled temperature (22 ± 2 °C) and relative humidity (40–60%) with a 12 h light/dark cycle. Animal studies were performed in accordance with the principles of laboratory animal care (NIH publication no. 85–23, revised 1985) and with approval by the University Of Chinese Academy of Sciences Animal Care and Use Committee.

2.3. Bronchoalveolar lavage

The mice were anesthetized by pentobarbital sodium after exposure. Then, the whole lungs were lavaged 3 times with 1 ml phosphate buffer solution (PBS, pH = 7.4). The bronchoalveolar lavage fluid (BALF) was collected and centrifuged at 1000 rpm for 5–10 min. Total cell number and the protein content of the BALF were measured respectively.

2.4. Histologic assessment

Mouse lungs were harvested quickly, and then washed with ice-cold PBS for three times. After fixation with 4% paraformaldehyde for 48 h, the lungs were embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) or Masson trichrome staining kits. To identify macrophages and neutrophils, tissue sections were stained with anti-galectin 3 and anti-neutrophil monoclonal antibodies, respectively.

2.5. RNA isolate and RNA-sequencing

Total RNA was extracted from the lungs of FA- or PM2.5-exposed mice using TRizol reagent (Invitrogen, Carlsbad, CA, USA). RNA quality was measured by Agilent 2100 bioanalyzer (Thermo Fisher Scientific, MA, USA) and samples with an RNA integrity number over than 8 were used for subsequent experiments. The total RNA was further purified by digesting the double-stranded and single-stranded DNA with DNase I and remove of RNA using Ribo-Zero method (human, mouse, plants) (Illumina, USA). The library construction and RNA sequencing were performed on a BGISEQ500 platform (BGI-Shenzhen, China).

2.6. Read mapping and differentially expressed gene analysis

The raw data were firstly counted and cleaned using SOAPnuke (BGI-Shenzhen, China) and trimmomatic [21] software to remove ligation sequence, low quality sequence and repeats. The sequencing data for clean reads generated by this study have been deposited in the NCBI Sequence Read Archive (SRA) database (accession number: PRJNA540011). Then the clean reads were mapped to the reference genome (Mus_musculus, GCF_000001635.25_GRCm38.p5) using HISAT (Hierarchical Indexing for Spliced Alignment of Transcripts) [22] or Bowtie 2 [23] software. The matched reads were calculated and then normalized to RPKM value (reads per kilo base per million mapped reads) using RESEM software [24] to obtain the gene expression level. The differential expression of genes (DEGs) between two groups was screened by DEGseq [25] with the thresholds of fold change ≥ 2 and adjusted P value ≤ 0.001.

To further understand the biological functions of genes, the identified DEGs in each pair were mapped to terms in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html). In addition, we performed enrichment analysis using the phyper function of R software. The p-value was adjusted for
false discovery rate (FDR) to get q-value, and q-value ≤ 0.05 was considered as significant enrichment.

2.7. Quantitative real-time PCR analysis and western blot

The cDNA was synthesized using a PrimeScript RT Reagent Kit (#RR036B, TaKaRa, Otsu, Japan) and mRNA expression were measured by quantitative real-time polymerase chain reaction (qPCR) with the SYBR® Premix Ex Taq™ II Kit (#RR820A). The results were normalized to 18S ribosomal RNA. Primers used in this study are listed in Table S1.

Proteins were extracted from mouse lung using lysis buffer (150 mM NaCl, 100 μg/ml phenylmethylsulfonyl fluoride, 50 mM Tris-Cl and 1% Triton X-100) with protease and phosphatase inhibitor cocktail (#04693124001, #4906837001, Roche, Basel, Switzerland) on 4 °C for 20–30 min. After centrifugation at 12,000 g and 4 °C for 20 min, the supernatant was used for western blot analysis as reported previously [20].

2.8. Statistical analysis

All data were analyzed by StatView (SAS Institute Inc.) and expressed as mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's correction was used to make multiple comparisons among the groups. p < 0.05 was defined statistical significance.

3. Results

3.1. Effect of exposure time and concentration on PM2.5-induced lung inflammation & fibrosis

The average monthly concentration of PM2.5 during the exposed period was calculated based on the daily data from http://datacenter.mep.gov.cn/. The experiment groups were defined as PM2.5–3L group (exposed from July to September in 2017, average concentration ~50 μg/m³), PM2.5–6L group (exposed from July to December in 2017, average concentration ~50 μg/m³) and PM2.5–3H group (exposed from October to December in 2015, average concentration ~115 μg/m³) (Fig. 1A). As to FA-exposed mice, 3 months or 6 months exposure had no obvious difference in lung morphology, BALF cell flux and serum tumor necrosis factor (TNFα) levels. Therefore, the mice exposed to FA from July to September in 2017 were used as the control group.

To determine whether PM2.5 concentration or exposure time affects pulmonary alveoli injury, the cell flux and protein content in BALF were measured. As shown in Fig. 1B–C, PM2.5 exposure significantly increased the cell number and protein concentration of BALF. In addition, BALF from PM2.5–3H mice had significantly more cell number and higher protein concentration than that of PM2.5–3L mice, while the differences in cell number and protein concentration of BALF between PM2.5–3L and PM2.5–6L groups were not significant (Fig. 1B–C). Although serum TNFα levels were elevated in PM2.5-exposed mice, there was no obvious difference among PM2.5–3L, PM2.5–6L and PM2.5–3H groups (Fig. 1D).

As revealed by H&E and Masson staining, PM2.5 exposure resulted in obvious lung injury & fibrosis, as indicated by the collapse of alveoli, airway epithelial thickening and collagen deposition. Histopathological analysis of lung sections further demonstrated that lungs from PM2.5–6L or PM2.5–3H group developed significantly more severe injury and fibrosis than lungs from FA or PM2.5–3L group. Immunohistochemical staining by antibodies specific for macrophage marker (galectin-3) and neutrophil also revealed that lungs from PM2.5–3H group exhibited more infiltration of macrophages and neutrophils than lungs from FA or PM2.5–3L group (Fig. 2). Together, these results indicated that the degree of lung injury was associated with the PM2.5 concentration and exposure time.

3.2. PM2.5 exposure changed gene expression profile in mice lungs

To investigate the molecular mechanism by which PM2.5 exposure causes lung injury in mice, RNA-sequencing was performed to analysis the whole-genome expression profiling changes in PM2.5-exposed lungs. Boxplot showed the general distribution of genes expression of mice lungs from FA, PM2.5–3L, PM2.5–6L and PM2.5–3H groups (Fig. S1). A total of 455 DEGs (311 up-regulated and 144 down-regulated) were identified in comparison of FA and PM2.5–3L exposed lungs, and the fold change of these DEGs were visualized by Volcano plot (Fig. 3A-B). The top 10 up- and down-regulated genes were listed in Table S2. Among these genes, Prok2, Ccl19, Ucp1, Gm13277, Pet117, Tat, Nkx6-2 and Bnc1 are involved in inflammation and oxidative stress related biological process. We also identified 545 DEGs (376 up-regulated and 169 down-regulated) from FA vs PM2.5–6L group and 1133 DEGs (887 up-regulated and 246 down-regulated) from FA vs PM2.5–3H group (Fig. 3A). The fold change of these DEGs from FA vs PM2.5–6L and FA vs PM2.5–3H groups were not significant (Fig. 3A).
PM2.5–3H groups were presented in Supplemental Figs. 2–3 and the top 10 up- and down-regulated genes of each pair were listed in Supplemental Tables 3–4. Among these genes, Gm1987, Ccl19, LOC100043921, DXBay18, Pet117, 2310045n01rik-mef2b, Mpo, Hspa1b, Gm13305 (from FA vs PM 2.5–6L group), Sim2, Ifag, Spag11b, Lep, Lhx1 (from FA vs PM 2.5–3H group) and 2310045n01rik-mef2b (from both) may participate in regulation of oxidative stress and inflammation related pathway. Interestingly, the top 8 up-regulated genes, including Mybpc1, Myh8, Myh4, Tnnc2, Myot, Myh1, Acta1 and Jsrp1, are involved in muscle contraction regulation.

Next, we created a Venn diagram to visual depiction of the similarities and differences between the DEGs in each pair. As shown in Fig. 3C, there were 188 overlapped DEGs between FA vs PM2.5–3L and FA vs PM2.5–6L, 158 overlapped DEGs between FA vs PM2.5–3L and FA vs PM2.5–3H, and only 55 overlapped DEGs among the three groups. According to the KEGG annotation and official classification, we mapped the DEGs of each pair to KEGG pathways, and the results were presented in Fig. 3D and Figs. S2–S3. Among these perturbed pathways, signal transduction and immune system have the highest numbers of DEGs. We then performed KEGG pathway enrichment analysis and found that many DEGs from FA vs PM2.5–3L or FA vs PM2.5–6L were significantly enriched in immune pathways, including hematopoietic cell lineage, cytokine-cytokine receptor interaction and B cell receptor signaling pathway (Tables 1–2). We also found that the DEGs of FA vs PM2.5–3H group were significantly enriched in inflammation and immune pathways, including Malaria, and Amoebiasis. However, the top 3 most significantly enriched KEGG pathways (Hypertrophic cardiomyopathy (HCM), Dilated cardiomyopathy (DCM) and Cardiac muscle contraction) are associated with cardiovascular disease (Table 3). The DEGs were also significantly enriched in some metabolic pathways, including PPAR signaling pathway, Insulin secretion, Pancreatic secretion, Glycolysis/Gluconeogenesis and Adipocytokine signaling pathway.
3.3. Effect of exposure time and concentration on gene expression profile in PM$_{2.5}$-exposed lungs

To investigate the effect of exposure time and concentration on gene expression profile in PM$_{2.5}$-exposed lungs, we also identified 235 DEGs (113 up-regulated and 122 down-regulated) from PM$_{2.5}$–3L vs PM$_{2.5}$–6L group and 1201 DEGs (761 up-regulated and 440 down-regulated) from PM$_{2.5}$–3L vs PM$_{2.5}$–3H group, respectively (Fig. 4A). The fold change of differentially expressed genes (DEGs) of FA vs PM$_{2.5}$–3L were visualized by Volcano plot. (C) The number of different and overlapped DEGs from FA vs PM$_{2.5}$–3L group. The functions of genes identified cover six main categories: cellular processes, environmental information processing, genetic information processing, human disease, metabolism and organismal system.

Table 1

| Pathway ID | Pathway Name                          | Gene Number | Rich Ratio$^a$ | Q value   |
|------------|---------------------------------------|-------------|----------------|-----------|
| ko04640    | Hematopoietic cell lineage             | 14          | 0.11864407     | 4.91E-05  |
| ko04060    | Cytokine-cytokine receptor interaction | 20          | 0.05464481     | 0.01323701|
| ko04662    | B cell receptor signaling pathway      | 9           | 0.09           | 0.01786184|
| ko05150    | Staphylococcus aureus infection        | 8           | 0.10126582     | 0.01786184|
| ko04612    | Antigen processing and presentation    | 11          | 0.07142857     | 0.01786184|
| ko04672    | Intestinal immune network for IgA production | 6          | 0.12244898     | 0.02054528|
| ko04710    | Circadian rhythm                       | 5           | 0.15151515     | 0.00054528|
| ko05323    | Rheumatoid arthritis                   | 9           | 0.08181818     | 0.02054528|
| ko04711    | Circadian rhythm - fly                 | 3           | 0.33333333     | 0.02054528|
| ko04514    | Cell adhesion molecules (CAMs)         | 14          | 0.05555556     | 0.02559195|

$^a$ Rich ratio is defined as amount of differentially expressed genes enriched in the pathway/amount of all genes in background gene set.
S5 and S6, respectively. Although Venn diagram showed that there were 111 overlapped DEGs (Fig. 4B), the top 10 up-regulated of genes in PM2.5–3L-vs PM2.5–3H are totally different from those of PM2.5–3L vs PM2.5–6L. There are three overlapped top down-regulated genes, including Rps27r, GM40369 and Pcdha9. The functions of Rps27r, GM40369 remain unclear, while Pcdha9 mutation is associated with Hirschsprung’s disease [26].

We also mapped the DEGs of each pair to KEGG pathways, and most of DEGs belonged to signal transduction and immune pathways (Figs. S4–S5). KEGG pathway enrichment analysis demonstrated that the DEGs of PM2.5–3L vs PM2.5–6L were only significantly enriched in Cytokine-cytokine receptor interaction pathway, while the DEGs of PM2.5–3L vs PM2.5–3H were significantly enriched in inflammation and immune pathways (Hematopoietic cell lineage, Amoebiasis, Superslow-coccus aureus infection, Cytokine-cytokine receptor interaction, etc.) and cardiovascular disease pathways (Hypertrophic cardiomyopathy, Dilated cardiomyopathy and Cardiac muscle contraction) (Fig. 4C, Table S6).

3.4. Down-regulation of Hspa1 and up-regulation of Cd5l were associated with PM2.5-induced pulmonary inflammation

To explore the mechanism for the activated immune pathway in PM2.5-exposed lungs, the expression profile of some inflammatory response related genes, including chemokine (C–C motif) ligand (CCL), interleukin (IL), interleukin receptor (ILr), tumor necrosis factor receptor superfamily (TNfrsf), heat shock 70 kDa protein (Hspa) and cluster of differentiation (CD), were shown in the heat map (Fig. 5A). We also performed real-time qPCR to validate the changes of some genes, including heat shock protein family A member 1A (Hspa1a) and Hspa1b, Fis1 (mitochondrial fission), CD14 (endotoxin receptor), Tnfrsf4 and Cd5l antigen-like (Cd5l). We found that Hspa1a, Hspa1b, Fis1 and CD14 were significantly down-regulated in PM2.5-exposed lungs (Fig. 5B–E). In addition, the mRNA levels of Hspa1a and Hsap1b were further markedly decreased in PM2.5–6L and PM2.5–3H lungs (Fig. 5B–C). We also found that PM2.5 exposure significantly increased the mRNA levels of Cd5l via a concentration-dependent manner (Fig. 5F). Previous reports demonstrated that Hspa1 protects against TNFα-induced lethal inflammatory shock and cell death [27,28], while overexpression of Cd5l in alveolar type II epithelial cells induces spontaneous lung adenocarcinoma [29]. Thus, it is likely that the reduction of Hspa1a and Hsap1b expression, as well as upregulation of Cd5l, might be important factors for the lung injury and pulmonary inflammation induced by PM2.5–3H exposure. Although heat map indicated that the expression of Tnfrsf4 was increased in lungs of PM2.5-exposed mice, qPCR results demonstrated that the up-regulation of Tnfrsf4 was not statistic significant (Fig. 5G).

3.5. Down-regulation of Prdx4 was associated with PM2.5-induced pulmonary oxidative stress

PM2.5–3L exposure resulted in slightly decrease in the GSH/GSSG ratio and moderately increase of 3′-NT and 4-HNE levels, and the changes in GSH/GSSG ratio and 4-HNE levels were not significant. However, compared with FA-exposed lungs, PM2.5–6L- and PM2.5–3H-exposed lungs exhibited significantly lower GSH/GSSG ratio and higher 3′-NT and 4-HNE levels (Fig. 6A–C). We also compared the oxidative stress degree in lung of PM2.5-exposed mice. There was no significantly difference between PM2.5–6L and PM2.5–3L groups. However, PM2.5–3H lungs exhibited significantly lower GSH/GSSG ratio and higher levels of 3′-NT and 4-HNE than those of PM2.5–3L lungs (Fig. 6A–C), indicating that PM2.5 exposure causes pulmonary oxidative stress via a concentration-dependent manner.

Table 2

Significantly enriched KEGG pathway of DEGs in FA vs PM2.5–6L group.

| Pathway ID | Pathway Name | Gene Number | Rich Ratio | Q value |
|------------|--------------|-------------|------------|---------|
| ko04640    | Hematoipoietic cell lineage | 16 | 0.13559322 | 1.05E-05 |
| ko04060    | Cytokine-cytokine receptor interaction | 27 | 0.07370492 | 7.06E-05 |
| ko05310    | Asthma       | 9 | 0.2 | 0.00127211 |
| ko04662    | B cell receptor signaling pathway | 11 | 0.1 | 0.0029461 |
| ko04711    | Gricadian rhythm - fy | 14 | 0.484444444 | 0.0029461 |
| ko04062    | Chemokine signaling pathway | 18 | 0.07142857 | 0.003007857 |
| ko03320    | PPAR signaling pathway | 11 | 0.094017094 | 0.006579429 |
| ko05134    | Legionellosis | 8 | 0.103896104 | 0.02277741 |
| ko04672    | Intestinal immune network for IgA production | 6 | 0.1224998 | 0.03628709 |
| ko04710    | Gricadian rhythm | 5 | 0.151515152 | 0.04275478 |
| ko04612    | Antigen processing and presentation | 11 | 0.07142857 | 0.04275478 |

Table 3

Significantly enriched KEGG pathway of DEGs in FA-vs-PM2.5–3H group.
To investigate the underlying mechanism for the increased oxidative stress in PM$_{2.5}$–3H lungs, some oxidative-related genes (Gpx, Prdx, Sod, Txn, Txnrd, and Ndufs) expression profile were demonstrated as the heat map (Fig. 6D). From the heat map, we found that only Prdx4 and Sod3 were down-regulated, while other anti-oxidative genes were up regulated in PM$_{2.5}$–3H lungs. To confirm the changes in these anti-oxidant genes, we also examined the protein expression of PRDX (as PRDX3, PRDX4, and PRDX5), SOD (SOD1, SOD2, and SOD3) and TRXR2 in lung lysates by western blot. As shown in Fig. 6E, PM$_{2.5}$ exposure significantly decreased SOD2, SOD3 and PRDX4 expression in all groups, whereas had no obvious effect on PRDX5 and TRXR2 expression. PRDX3 expression was slightly increased in PM$_{2.5}$–3L lungs, and was significantly elevated in PM$_{2.5}$–3L vs PM$_{2.5}$–6L and PM$_{2.5}$–3L vs PM$_{2.5}$–3H group. SOD1 expression was reduced in PM$_{2.5}$–3L lungs, while the reduction was diminished in PM$_{2.5}$–6L and PM$_{2.5}$–3H lungs. Compared to PM$_{2.5}$–3L lungs, PM$_{2.5}$–6L lungs exhibited higher levels of PRDX3 and similar levels of other antioxidant enzymes, while PM$_{2.5}$–3H lungs exhibited significantly higher levels of PRDX1 and SOD1, and lower levels of PRDX4. Considering that PRDX4 was the only identified antioxidant enzyme that was reduced by PM$_{2.5}$ exposure in a concentration-dependent manner, we postulated that PRDX4 reduction may play an important role in PM$_{2.5}$-induced pulmonary oxidative stress.

4. Discussion

Nowadays, air pollution or PM$_{2.5}$ has become a big threat to the respiratory and cardiovascular system [30–32]. Studies by others and ourselves have suggested that immune and inflammatory response, oxidative stress and DNA damage are potential mechanisms responsible for the adverse health effect of PM$_{2.5}$ [20,33–35]. In addition, transcriptomic analyses of PM$_{2.5}$-exposed 16HBE cells demonstrated that PM$_{2.5}$-induced DEGs were involved in inflammatory and immune response pathways, response to xenobiotic stimuli and metabolic response [16]. Another study revealed that PM$_{2.5}$ triggers infectious disease, cancers, cardiovascular diseases, and immune pathways in A549 cells [18]. It is therefore no strange that most of DEGs in PM$_{2.5}$–3L vs FA group were enriched in immune system and infectious disease pathways, such as Cytokine-cytokine receptor interaction, Antigen processing and presentation and Intestinal immune network for IgA production in the present study. Surprisingly, we found that the most significantly enriched KEGG pathway of DEGs in PM$_{2.5}$–3L vs FA group were enriched in immune system and infectious disease pathways, such as Cytokine-cytokine receptor interaction, Antigen processing and presentation and Intestinal immune network for IgA production in the present study. Surprisingly, we found that the most significantly enriched KEGG pathway of DEGs in PM$_{2.5}$–3L vs FA group were enriched in immune system and infectious disease pathways, such as Cytokine-cytokine receptor interaction, Antigen processing and presentation and Intestinal immune network for IgA production in the present study. Surprisingly, we found that the most significantly enriched KEGG pathway of DEGs in PM$_{2.5}$–3L vs FA group were enriched in immune system and infectious disease pathways, such as Cytokine-cytokine receptor interaction, Antigen processing and presentation and Intestinal immune network for IgA production in the present study. Surprisingly, we found that the most significantly enriched KEGG pathway of DEGs in PM$_{2.5}$–3L vs FA group were enriched in immune system and infectious disease pathways, such as Cytokine-cytokine receptor interaction, Antigen processing and presentation and Intestinal immune network for IgA production in the present study. Surprisingly, we found that the most significantly enriched KEGG pathway of DEGs in PM$_{2.5}$–3L vs FA group were enriched in immune system and infectious disease pathways, such as Cytokine-cytokine receptor interaction, Antigen processing and presentation and Intestinal immune network for IgA production in the present study.
exposure may include hematopoiesis dysregulation.

It is well documented that the cytotoxicity of PM2.5 is dependent on its concentration. Epidemiological studies provide evidence that each 10 μg/m³ elevation in PM2.5 concentration was associated with significantly increased risk of all-cause mortality, COPD, asthma and cardiovascular disease [40]. In vitro experiments showed that PM2.5 dose-dependently decreases cell viability in BEAS-2B [41], A549 [42], 16HBE [16] and macrophages [43]. In the present study, we also demonstrated that PM2.5–3H exposure caused more severe lung injury and higher number of DEGs than did PM2.5–3L. Interestingly, the top 2 most significantly enriched KEGG pathways of DEGs in FA vs PM2.5–3H group were HCM and DCM, which are two common clinical subtypes of cardiomyopathy. Such pathway enrichment is in agreement with previous epidemiological studies, which confirmed that high PM2.5 concentration is significantly associated with increased cardiovascular risk, including ischemic heart disease, heart failure, arrhythmias, and
cardiac death [44,45]. Using mouse model, we recently found that short-term PM2.5 exposure is sufficient to induce a robust lung inflammation, vascular remodeling, and promote transition from left ventricular failure to right ventricular hypertrophy [34]. In addition, as the DEGs (Mybpc1, Myh8/4/1, Myot, and etc; Table S4) enriched in HCM and DCM pathways were mainly related to myopathy, we speculated that the activation of HCM and DCM pathways in PM2.5-exposed lungs might be associated with pulmonary vessel remodeling and muscularization. Hspa1 encodes a 70 kDa heat shock protein, which exhibits a broad protective role in multiple diseases, including sepsis, insulin resistance and liver injury [46]. The anti-inflammatory mechanisms of Hspa1 involves inhibition of NF-κB activation [47] and high-mobility group box 1 (HMGB1) release [48]. Furthermore, Hspa1 facilitates DNA repair in Benzo[a]pyrene exposed 16HBE cells [49]. Cd5l, also called apoptosis inhibitor of macrophage (AIM), plays an important role in the control of immune homeostasis and inflammatory disease [50]. The finding shows that Cd5l is concentration-dependently upregulated in PM2.5-exposed lung which is in agreement with previous study which demonstrated Cd5l was one of the significantly upregulated genes in welding fumes exposed rat lungs [51]. It has been
reported that deletion or blockade of CdSl attenuates the inflammatory response in acute myocardial infarction [52] and experimental sepsis [53]. By contrast, overexpression of CdSl in myeloid or alveolar type II epithelial cells induces systemic inflammation and adenocarcinoma in the lung [29,54]. Therefore, the significantly repressed Hspa1 expression and dramatically up-regulated CdSl may contribute to the enhanced pulmonary inflammation and fibrosis in PM2.5-3H-exposed lungs.

Previous studies have demonstrated that PM2.5 exposure could promote ROS production, which causes inflammatory cytokines release and DNA damage, and then leads to cell death and lung injury [1,11,55]. In the present study, we found that GSH/GSSG ratio and expression of some antioxidants enzymes (SOD2, SOD3 and PRDX4) were decreased, whereas 3′-NT and 4-HNE levels were increased in PM2.5-exposed lungs. Consistent with our previous finding that PM2.5 dose-dependently increases intracellular ROS in A549 cells [56], here we found that PM2.5-3H caused greater pulmonary oxidative stress than did PM2.5-3L. PRDX4 is an antioxidant enzyme located in the endoplasmic reticulum (ER) and plays an important role in H2O2 scavenging and protein folding in the ER [57]. PRDX4 deficiency has been found to exacerbate diethylnitrosamine-induced hepatic oxidative stress [58] and dextran sulfate sodium-induced intestinal inflammation [59]. Thus, the reduction of PRDX4 expression might be an important contributor for the elevated oxidative stress in PM2.5-3H-exposed lungs.

In vitro experiments consistently demonstrated that PM2.5 can affect cell viability, inflammation response and intracellular ROS in a time-dependent manner [55,60]. However, we found that there were no significant difference in system inflammation, lung injury, pulmonary GSH/GSSH ratio, 3′-NT and 4-HNE levels and antioxidant enzymes (PRDX4, PRDX5, SOD1, SOD2, SOD3 and TRXR2) expression between PM2.5-3L and PM2.5-6L groups, indicating that exposure time had no obvious effect on PM2.5-induced inflammation and oxidative stress. This may due to the adaptive response triggered by PM2.5 exposure. First, cytokines induced by early PM2.5 exposure may contribute to mount an adaptive immune response to affect cytokines expression at late stage. For example, Ccl17 expression was lower in PM2.5-6L lungs than that of PM2.5-3L lungs (Tables S2–S3). The finding that the DEGs were only significantly enriched in cytokine-cytokine receptor interaction pathways between PM2.5-3L vs PM2.5-6L, suggesting that the interaction among different cytokines may contribute to the similarity of lung injury. Second, PM2.5 exposure could promote nuclear factor erythroid-2-related factor 2 (NRF2) nuclear translocation to orchestrate the antioxidant and detoxification genes [42,61]. The activation of NRF2 then protects cell against PM2.5-induced cytotoxicity [42]. This kind of adaptive response may also explain why some antioxidant enzymes were upregulated in PM2.5-exposed lungs, especially in lungs of PM2.5-6L.

The present study has several limitations. It is well acknowledged that there are discrepancies between the mRNA and protein levels for some genes [62]. In this study, the profile of RNA-seq only provides a comprehensive overview of the entire transcriptome. However, the expression and activity levels of protein obtained from the study should be further examined. Moreover, additional analysis should be carried out to identify all the genes which have a trend for dose- or time-dependent changes in expression profile. These genes/pathways may be important for elucidating the harmful effect of PM2.5 in respiratory system. Further studies are necessary in order to clarify the underlying mechanism whether the changes in gene expression profile are due to the direct or indirect/systematic effects of PM2.5 on lung tissue.

In summary, our study indicated a strong concentration-response relationship between airborne PM2.5 and lung injury. The effect of exposure time and concentration of airborne PM2.5 on whole transcriptome profiling of mice lungs were illuminated by RNA-seq. As revealed by KEGG enrichment analysis, PM2.5 mainly induced immune pathways, especially hematopoietic cell lineage pathway. Furthermore, our results suggest that Hspa1, CdSl and Prdx4 may play important roles in PM2.5-induced pulmonary inflammation and oxidative stress. These data may provide deeper insight into the molecular mechanism for the harmful effect of PM2.5.

Author disclosure statement

No competing financial interests exist.

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Appendix A. Supplementary data

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