The cytotoxicity of PM$_{2.5}$ and its effect on the secretome of normal human bronchial epithelial cells

Zhigang Sui$^1$ · Xiaoyao Song$^2$ · Yujie Wu$^{1,3}$ · Rui Hou$^1$ · Jianhui Liu$^1$ · Baofeng Zhao$^1$ · Zhen Liang$^1$ · Jiping Chen$^2$ · Lihua Zhang$^1$ · Yukui Zhang$^1$

Received: 19 November 2021 / Accepted: 5 May 2022 / Published online: 4 June 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
Exposure to airborne fine particulate matter (PM$_{2.5}$) induced various adverse health effects, such as metabolic syndrome, systemic inflammation, and respiratory disease. Many works have studied the effects of PM$_{2.5}$ exposure on cells through intracellular proteomics analyses. However, changes of the extracellular proteome under PM$_{2.5}$ exposure and its correlation with PM$_{2.5}$-induced cytotoxicity still remain unclear. Herein, the cytotoxicity of PM$_{2.5}$ on normal human bronchial epithelia cells (BEAS-2B cells) was evaluated, and the secretome profile of BEAS-2B cells before and after PM$_{2.5}$ exposure was investigated. A total of 83 proteins (58 upregulated and 25 downregulated) were differentially expressed in extracellular space after PM$_{2.5}$ treatment. Notably, we found that PM$_{2.5}$ promoted the release of several pro-apoptotic factors and induced dysregulated secretion of extracellular matrix (ECM) constituents, showing that the abnormal extracellular environment attributed to PM$_{2.5}$-induced cell damage. This study provided a secretome data for the deep understanding of the molecular mechanism underlying PM$_{2.5}$-caused human bronchial epithelia cell damage.

Keywords Secretome · Proteomic analysis · PM$_{2.5}$ · Cytotoxicity · Apoptosis · Extracellular matrix

Introduction
Air pollution, especially pollution of airborne fine particulate matter (PM$_{2.5}$), can cause adverse health effects and results in many diseases, such as lung disease, stroke, hypertension, and cardiovascular diseases (Yang et al. 2022; Xu et al. 2021; Duan et al. 2021). In the past decade, many time-series air pollution epidemiological studies have assumed that long-term exposure to PM$_{2.5}$ greatly increases the incidence of lung-related diseases (Adam et al. 2015; Kloog et al. 2013; Chen et al. 2021; Wu et al. 2021). It is also reported that PM$_{2.5}$ can trigger intracellular reactive oxygen species (ROS) overproduction, resulting in membrane injury and DNA damage on respiratory system and lung cells (Zhai et al. 2022; Niu et al. 2020; Bo et al. 2021). However, the mechanisms underlying respiratory cells injury caused by PM$_{2.5}$ are not fully elucidated.

In recent years, proteomics has been widely applied to the studies on toxicology of PM$_{2.5}$ exposure. Xue et al. found that MAPK and PI3K/AKT pathways might be involved in PM$_{2.5}$-induced lung injury (Xue et al. 2019). Zhang et al. employed strategies, combining proteome with transcriptome, to profile proteins and genes in the lung tissue. They found that the gradually upregulated proteins enriched in response to oxidative stress, phagosome, and the extracellular matrix (ECM)-receptor interaction pathway during the process of PM$_{2.5}$ exposure (Zhang et al. 2020). Furthermore, in our previous proteomics study, we found that PM$_{2.5}$ could affect the expression of integrin protein, which is closely
related to cell apoptosis (Song et al. 2022). In addition to intracellular proteins mentioned above, extracellular proteins, such as secretory proteins and exosomal proteins, have also been reported to be involved in the PM$_{2.5}$-induced diseases (Zhao et al. 2019; Xu et al. 2019a). However, few studies have explored the secretome changes of respiratory tract cells exposed to PM$_{2.5}$. The secretome is the set of proteins expressed by cells and secreted into the extracellular space, including secreted proteins and extracellular vesicles (EVs) proteins (Strack 2021; Forder et al. 2021; Kim et al. 2022). Secreted proteins and EVs are currently viewed as important functional mediators of intercellular communication, which is critical in various biological processes, such as proliferation, differentiation, and apoptosis (Stuehler 2020; Ortiz 2021; Nicin et al. 2022). Therefore, it is an innovative perspective to study the toxic effects of PM$_{2.5}$ on cells by secretome analysis. In this study, the cytotoxicity of PM$_{2.5}$ on BEAS-2B cells was evaluated according to cell viability and apoptosis rates. Then, a strategy-combining metabolic labeling, protein “equalization,” protein fractionation, and filter-aided sample preparation (FASP), called MLEFF, was employed to study secretome changes and the potential molecular mechanisms underlying cell damage caused by PM$_{2.5}$ exposure.

**Materials and methods**

**Sample collection and component analysis of PM$_{2.5}$**

PM$_{2.5}$ samples were collected from November 2016 to March 2017 which was a whole winter coal heating period in Dalian, a city in northeast China. Samples were collected on quartz microfiber filter (20.3 × 25.4 cm$^2$, Whatman®, UK) at flow rate of 1 m$^3$/min with a high-volume sampler (AMS® 600/AFPM1001K, Pesaro, Italy). The daily average mass concentration of winter particulate matter was 110 μg/m$^3$. For the collection of inorganic components, each membrane was weighed and cut into 2×2 cm$^2$ squares. The scraps of membrane were submerged in ultrapure water (Millipore, Darmstadt, Germany) followed by sonication (20 min per period×6), and the sonicated PM$_{2.5}$ suspension was strained through 8 layers of sterile gauze. Then, the PM$_{2.5}$ suspension was processed with the vacuum freeze-drying. The dried PM$_{2.5}$ particles were blended in 1 ml ultrapure water followed by vortexing and sonicating for 5 min for stock solution. The vacuum dried powder were blended in ultrapure water to make stock solution (500 mg/mL), which was stored at −80 °C for further exposure.

**PM$_{2.5}$ samples were diluted with 1 ml ultrapure water to make stock solution (500 mg/mL), which was stored at −80 °C for further exposure.**

**Cell culture and PM$_{2.5}$ exposure**

Human lung-bronchial epithelium cells BEAS-2B (ATCC®, CRL-9609™) were cultured in BEBM medium (LONZA CC-3170, Basel, Switzerland) at 37 °C, supplied with 5% CO$_2$. For proteomic analysis, cells were labeled using stable isotope labeling with amino acids in cell culture (SILAC) medium (Thermo Fisher). For the “medium” labeling media, SILAC DMEM medium was supplemented with [4,4,5,5-D$_4$] L-lysine (100 μg/mL) and $[^{13}$C$_6$] L-arginine (100 μg/mL), 10% dialyzed fetal bovine serum (FBS), and 1% penicillin/streptomycin mixture. For the “heavy” labeling media, only [4,4,5,5-D$_4$] L-lysine and $[^{13}$C$_6$] L-arginine were replaced with $[^{13}$C$_6$, $^{15}$N$_2$] L-lysine and $[^{13}$C$_6$, $^{15}$N$_4$] L-arginine. The control group and exposure groups were grown in the “heavy” and “medium” media, respectively. After 24 h, the cells and conditioned media were both harvested and were mixed based on equal number of each cell type. For exposure treatment, the vacuum-dried PM$_{2.5}$ particles were diluted to 1000 μg/mL with medium. By using cell counting kit-8 (CCK-8) assay (Dojindo, Mashikimachi, Japan), the 24-h half-maximal inhibitory concentration (24 h-IC$_{50}$) of PM$_{2.5}$ was calculated with the dosage range of 5–500 μg/mL (9 dose groups) to ascertain the appropriate exposure dosage. Finally, BEAS-2B cells for in vitro exposure were treated with 10, 50, 100, and 250 μg/mL of PM$_{2.5}$ for 24 h base on the IC$_{50}$ value.

**Cell viability and apoptosis assay**

BEAS-2B cells were seeded in 6-well plates and exposed to different doses of PM$_{2.5}$ for 24 h. After exposure, calcine-acetyl methoxy methyl ester (AM)/propidium iodide (PI) double staining kit and Hoechst 33258 (Dojindo) were used to identify living cells, living cell nucleus, and dead cells. Concisely, the cells were collected from each well (1×10$^6$ cells/mL) and resuspended in 1 mL phosphate buffered saline (PBS) and stained with PI and AM, respectively, for 30 min in the dark. Then, the cells were washed with PBS three times and resuspended in PBS. Adding 100 μL of cell suspension to each well, the cell nuclei were observed by fluorescence microscope. The sample was double-stained with Hoechst 33258 and PI for DNA and cell membrane, respectively.
buffered solution (PBS), followed by adding 500 μL staining solution. After incubation for 15 min at 37 °C, live cells with green fluorescence and dead cells with red fluorescence were observed ($E_x = 490$ nm; $E_m = 545$ nm) by fluorescence microscope. Cell and nuclei were stained with Hoechst 33258 for 15 min, and stained nuclei were observed ($E_x = 350$ nm; $E_m = 460$ nm) by fluorescence microscope. For apoptosis assay, after 24-h exposure, the apoptotic rate of cells from different groups was determined with Annexin V-FITC/PI apoptosis kit (Liankebio, Hangzhou, China). Briefly, the cells were resuspended in the 500 μL 1x binding buffer. The 5 μL Annexin V- FITC (fluorescein isothiocyanate isomer) and 10 μL PI (propidium iodide) were added, respectively. After incubation for 5 min at room temperature in dark, the cells were examined by the Automatic Personal Cell Sorter SH800 (SONY, Tokyo, Japan). Annexin V-FITC was detected by FITC channel ($E_x = 488$ nm; $E_m = 530$ nm) and PI was detected by PI channel ($E_x = 535$ nm; $E_m = 615$ nm). Data were analyzed using the Cell Sorter Software (SONY).

**ROS assay**

The ability of PM$_{2.5}$ to generate intracellular ROS in BEAS-2B cells was measured by using the fluorescent indicator 2',7'-dichlorodihydrofluorescein diacetate (DCFH/DA), in which fluorescence appears if ROS are present, according to the instructions of the ROS assay kits (njjcbio, Nanjing, China).

**Secretome analysis**

The workflow of secretome analysis was illustrated in Fig. 1. BEAS-2B cells in control groups (heavy labeled) and exposure groups (medium labeled) were treated with 0 μg and 10 μg/mL of PM$_{2.5}$ for 24 h to explore the secretome changes caused by PM$_{2.5}$ exposure. Mixed conditioned media were processed according to our previously reported MLEFF strategy and one sample (Weng et al. 2016), by which eight fractions were collected from a single sample. All experiments were performed in three biological replicates, and digested peptides were stored at −80 °C for further analysis.
The peptides were analyzed with a nano reverse phase liquid chromatography mass spectrometry (nano-RPLC-MS/MS) on a Q-Exactive MS coupled with an Easynano LC system (Thermo Fisher). Buffer A is 2% acetonitrile, 98% water, and 0.1% formic acid whereas Buffer B is 98% acetonitrile, 2% water, and 0.1% formic acid. The gradient was comprised of 90 min of 6–22% buffer B, followed by a 20 min of 22–35% buffer B. The spray voltage was set to 2.5 kV, and temperature of the ion transfer capillary was set to 275 °C. The 10 most intense ions were subjected to high-energy collision dissociation (HCD) fragmentation with normalized collision energy at 28%. The MS scans were performed at a resolution of 70,000 from m/z 300 to 1800, and the data were acquired in profile mode. The MS/MS scans were performed at a resolution of 17,500, and the data were acquired in centroid mode using a 20-s exclusion window. The unassigned ions or those with a charge of +1 were matched by the matrisome database (http://matrixdb.org). Interaction network analysis was generated using STRING and visualized using the Cytoscape.

Western blotting analysis

Sample of BEAS-2B cells (5 × 10⁶/mL) was collected and extracted with 4% sodium dodecyl sulfate (SDS) containing 1% (v/v) protease inhibitor cocktail (Sigma-Aldrich, MA, USA). After centrifugation at 15,000 rpm for 20 min at 4 °C, the supernatant was collected, and the protein concentration was determined by bicinchoninic acid (BCA) assay (Beyotime, Shanhai, China). The 20 μg of proteins were separated on 12% SDS-PAGE, and then transferred onto polyvinylidene fluoride (PVDF) membrane (Bio-Rad, CA, USA). The PVDF membrane was blocked in Tris-buffered saline containing 5% milk for 1 h at room temperature, followed by incubation with the antibodies including desmoglein-2 (DSG2), extracellular matrix protein-1 (ECM1), and plasminogen activator inhibitor type-1 (PAI1) (Genetex, CA, USA) overnight at 4 °C. After incubation with anti-horseradish peroxidase (HRP) conjugate (Thermo), bands were visualized with a chemiluminescence reagent (Thermo Fisher) and detected using the ChemiDoc system (Bio-Rad). The intensity of the bands was measured using Quantity one software (Bio-Rad).

Data processing and statistical analysis

For cytotoxicity analysis, the GraphPad Prism 5.0 software (GraphPad Software, CA, US) was used to determine the cell viability and IC₅₀. The significance between the values obtained in different experimental treatments was determined by one-way analysis of variance (ANOVA) and repeated measures analysis of variance (PASW Statistics software 17.0, SPSS Inc., Chicago and GraphPad Prism5 Software, San Diego, CA), and the level of significance for all tests of effects was set at p<0.05. One-way ANOVA statistics analysis (p<0.05) was applied to analyze the data of the apoptosis test, intracellular ROS level, and western blotting.

For secretome, the data of all groups were analyzed with three replicates. Raw data were processed by Proteome Discoverer (PD, version 1.4.1.14) with Mascot (version 2.3.2) and searched against the UniProtKB Human proteome sequence database (release 2017_06, 24,148 entries). The reverse sequences were appended for a false discovery rate (FDR) evaluation. The mass tolerances were set at 0.5 Da for the parent ions and at 10 ppm for the fragments. The peptides were searched using tryptic cleavage constraints, and a maximum of two missed cleavages were allowed. The minimal peptide length was six amino acids. Carbamidomethylation (C, + 57.0215 Da) was used as the fixed modification. Oxidation (M, + 15.9949 Da) and acetylation (protein N-term., + 42.0106 Da) were searched as variable modifications. Two SILAC-based labels, (Lys 4, + 4.0251 Da) and (Lys 8, + 8.0142 Da), were used as variable modification. The peptide and protein identifications were filtered by PD to keep the FDR less than 1%. At least one unique peptide was required for each protein identification. The clustering strategy of extracellular proteins is as follows: a protein was defined as a classical secreted protein when it was annotated to contain a “signal peptide” or had the keyword “secreted” in UniProtKB (http://www.uniprot.org), or it was predicted by the Signal P 4.1 server to have a “signal peptide”; those proteins that did not contain a “signal peptide” and were predicted by Secretome P 2.0 with an NN score > 0.5, were defined as non-classical secreted proteins; the exosomal proteins were matched by the ExoCarta database (http://exocarta.org). The Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis were performed using DAVID 6.8 (https://david.ncifcrf.gov). ECM-related proteins were matched by the matrisome database (http://matrixdb.univ-lyon1.fr). Interaction network analysis was generated using STRING and visualized using the Cytoscape.

Results

Component analysis of PM_{2.5}

We analyzed water-soluble ions, heavy metals, and organic components in PM_{2.5} particles (data not shown). The components and contents were complicated and abundant which is related to the difference toxic effects caused by PM_{2.5}. Medium molecular weight polycyclic aromatic hydrocarbons with 4-ring such as FLα, PYr, and CHR, as well as high molecular weight polycyclic aromatic hydrocarbons with 5- and 6-ring included BbF, BkF, IcbP, and BghiP, which had the
most contribution of total polycyclic aromatic hydrocarbons. The top five heavy metal elements with the highest contents in winter were Cu (22%), Ni (10%), As (8%), Cr (8%), and Ga (5%). Therefore, the heavy metals and macrocyclic polycyclic aromatic hydrocarbons as major components of PM$_{2.5}$ particles had strong correlation with intracellular ROS increase and could induce oxidative stress resulting in changes in the extracellular matrix.

**Cytotoxicity and oxidative stress induced by PM$_{2.5}$ exposure**

To determine the exposure dose of PM$_{2.5}$, we detected the cell viability via CCK-8 assay. Significant differences compared to control were observed starting at the concentration of 1 μg/mL and up to 500 μg/mL. The cell viability showed a decline along with the increase of the exposure doses (Fig. 2a) with IC$_{50}$ at 227.1 μg/mL. All of these data indicated that PM$_{2.5}$ induced more serious cytotoxicity of lung-bronchial epithelial cells. To study the mechanisms of the cytotoxicity induced by PM$_{2.5}$ exposure, the concentrations of PM$_{2.5}$ were set up at 10 μg/mL, 50 μg/mL, 100 μg/mL, and 250 μg/mL, fluctuated based on IC$_{50}$ value, as well as the time of exposure was 24 h.

To further explore PM$_{2.5}$-induced toxic effect in BEAS-2B cells, we measured the level of intracellular ROS. As shown in Fig. 2b, the intracellular ROS level in exposure groups increased significantly compared to the control group. The fluorescence intensity of PM$_{2.5}$ exposure groups was 1.08-fold, 1.16-fold, 1.29-fold, and 1.61-fold higher than that of control group, respectively. Intracellular ROS level was elevated along with the increase of PM$_{2.5}$ concentration in a dose-dependent manner. The variation in the intracellular ROS level revealed that PM$_{2.5}$ exacerbated the ROS generation and induced oxidative stress in BEAS-2B cell. In addition, research has shown that PM$_{2.5}$ can cause apoptosis. To investigate the apoptosis might be caused by PM$_{2.5}$ exposure in BEAS-2B cells, Annexin V-FITC/PI fluorescence double-staining was used to measure the apoptotic rate of cell via the flow cytometry. In comparison with the control group, the apoptotic rates were significantly increased in PM$_{2.5}$ exposure groups, showing 7.7-fold, 15.6-fold, 23.6-fold, and 28.4-fold higher than that of the control group respectively (Fig. 2c). The increase in apoptotic rate revealed that PM$_{2.5}$ exposure accelerated apoptosis in the BEAS-2B cells in a dose-dependent mode.

**Secretome analysis of conditioned media from BEAS-2B cells exposed to PM$_{2.5}$**

To investigate the secretome changes in BEAS-2B cells after PM$_{2.5}$ exposure, a LC-MS/MS-based quantitative proteomic analysis was performed. The SILAC-labeled control cells (medium labeled) and treated cells (heavy labeled) were used to ensure the quantitative accuracy. The conditioned media were subjected to our recently developed MLEFF strategy (Weng et al. 2016), overcoming the challenge to proteomic analysis of serum-containing conditioned media, where the low-abundant secreted proteins were seriously masked by high-abundant proteins derived from FBS.

In total, 481 proteins were quantified in all three replicates (Table S-1), including 8 growth factors, 37 proteases, 15 protease inhibitors, and 18 ECM structural proteins (Table S-2). Meanwhile, we analyzed the subcellular location of 184 proteins (Table S-3), which were identified in all three replicates and with $t$ test $p$ value less than 0.05 ($n=3$). Among them, 89 proteins (48.6%) contained signal peptide and were identified as classical secreted proteins, 16 proteins were predicted as non-classical secreted proteins by Secretome P 2.0, and 47 proteins were identified as extracellular exosome based on uniprot database (Fig. 3). Collectively, 83.1% of these quantified proteins were extracellular-region located proteins, showing this extracellular proteome profiling a real profile of cell secretion in the presence of even 10% (v/v) FBS in cell culture media.

---

**Fig. 2** a The cell viability in BEAS-2B cells exposed to PM$_{2.5}$. b The ROS level in BEAS-2B cells exposed to PM$_{2.5}$. c The apoptosis rate of BEAS-2B cells exposed to PM$_{2.5}$. (*, $p<0.05$; **, $p<0.01$)
For further analysis, proteins with ratios more than 1.5 or less than 0.67 (Log₂ ratio (exposure/control) > 0.585 or < −0.585) and p value of <0.05 were considered as differentially expressed proteins. Based on this criterion, 58 proteins were identified upregulated in the conditioned media of BEAS-2B cell in exposure to PM₂.₅, whereas 25 proteins were downregulated (Fig. 4).

By using the software DAVID, the GO analysis of these differentially expressed proteins was performed (p value < 0.01), as shown in Fig. 5. As expected, the top 4 ranked GO terms of cellular component categories were extracellular-related (Fig. 5a). The highest ranked term was extracellular exosome, indicating a huge variation in secretion of exosomal proteins. Among the enriched 9 GO terms of the molecular function category (Fig. 5b), the mostly enriched terms were heparin binding and receptor binding, which play important roles in the ECM organization. Biological process clustering revealed 12 GO terms, showing the most abundant terms related to cell adhesion and ECM organization (Fig. 5c). Other terms were also enriched, such as negative regulation of endopeptidase activity, ECM disassembly, and proteolysis. Among KEGG enriched from the differentially expressed proteins using DAVID software (Fig. 5d), the most significant pathways included ECM-receptor interaction, spliceosome, focal adhesion, and complement and coagulation cascades.

Differentially expressed proteins were mapped onto STRING to build a protein–protein interaction networks (Fig. 6). Proteins involved in some pathways such as regulation of complement activation and ribonucleic acid (RNA) splicing were all upregulated, indicating these pathways were promoted by PM₂.₅ stimulation. However, some intercellular communication-related pathways, such as ECM organization, response to stimulus, and secretion by cell, contained many up/downregulated proteins, which suggested that the extracellular environment was greatly dysregulated under PM₂.₅ exposure.

Western blotting analysis results

To validate the expression changes of DSG2, ECM1, and PAI1 before and after PM₂.₅ exposure, their expression levels were analyzed via western blotting. Significant upregulated expression of DSG2 and downregulated expression of ECM1 and PAI1 were detected in cells exposed to PM₂.₅ (Fig. 7a), which was consistent with the MS quantification. Compared with the control group, the DSG2 level of the PM₂.₅ exposure group was 3.84-fold higher; and the level of ECM1 and PAI1 were 2.62-fold and 3.01-fold lower respectively (Fig. 7b).

Discussion

As shown in our study, the viability of BEAS-2B cells decreased with increasing exposure doses, which was in a dose-response mode. This result was coincident with other studies using different cell lines such as endothelial cells and placenta cells, that the cell proliferation was inhibited by PM₂.₅, indicated the PM₂.₅ could have adverse effects on living cells (Rui et al. 2016; Wang et al. 2017; Kim et al. 2018). A growing number of evidence showed that the generation of the ROS and oxidative stress played a critical character in the process of cytotoxicity of PM₂.₅ (Hong et al. 2016; Deng et al. 2013). Therefore, we detected the ROS...
generation by PM$_{2.5}$, and our results showed that the PM$_{2.5}$ aggravated the level of intracellular ROS. Similar to our results, it was reported that exposure of PM$_{2.5}$ led to an upregulated-ROS production and oxidative stress in fatty cells, red blood cell, and airway cells (Xu et al. 2019b; Torres-Ramos et al. 2011; Yan et al. 2016). It could be seen that PM$_{2.5}$ could directly stimulate the increase of intracellular ROS level and induce oxidative damage in cells. Therefore, it is necessary to study the relationship between the composition of PM$_{2.5}$ particles versus the increased intracellular ROS level and oxidative damage. In our another study (data not shown), we found the positive correlation between heavy metals (Mn, Cd, Cr et al.) and PAHs in PM$_{2.5}$ and the intracellular ROS level, but not water-soluble ions. Previous research found that PAHs toxicity mainly depends on high ring number (4–6 rings) PAHs (Liu et al. 2015), which was...
coincident with our results. Similarly, previous study of our team also revealed that toxicity of PM2.5 was mostly correlated with PAHs, N-PAHs, and OH-PAHs (Song et al. 2022). N-PAHs induced lipid metabolic perturbation of zebrafish and membrane phospholipid skeleton (Kiedrzyńska et al. 2014; Ning et al. 2020). Moreover, it was found that polycyclic aromatic hydrocarbons and PCDD of PM2.5 could induce oxidative stress (Bae et al. 2010; Zhang et al. 2019). Studies have shown that PCBs ingested through the respiratory system could inhibit the activity of lung cytochrome P450-dependent mono-oxygenase and could be closely related to the incidence of lung cancer (Fouchecourt et al. 1998; Ćwieląg-Drabek et al. 2020). Therefore, the chemical composition might be part of the cause of oxidative stress induced by PM2.5. Our results indicated that PM2.5 had complex constitute and was a harmful mixture that could induce adverse effects on lung-bronchial epithelial cells, which major components such as heavy metals and macrocyclic PAHs could induce cellular oxidative damage and increase intracellular ROS content; and further interfered with integrin, which are important regulators of cell survival, proliferation, adhesion, and migration (Hynes 2002) and also exists in exosomes (Cheng and Hill 2022); so it may also be one of the components of a series of changes in the exosomal proteome of cells induced by particulate matter.

Corresponding to the cell survival rate analysis, we also found that PM2.5 could trigger apoptosis in BEAS-2B cells. The apoptosis rate increased significantly with increasing exposure dose of PM2.5. Similarly, a recent study showed PM2.5 induced higher apoptosis rate than PM10 in
macrophages (Reyes-Zárate et al. 2016). It is reported that the increased intracellular ROS could induce apoptosis by participating in signal pathways such as P53 and Caspase (Circu and Aw 2010; Johnson et al. 1996). Another research showed that PM$_{2.5}$ induce skin cells apoptosis by elevating intracellular ROS level and mitochondrial damage (Piao et al. 2018). Previous study also demonstrated that PM$_{2.5}$ could increase ROS production in keratinocytes and inhibit the intracellular antioxidant system, resulting in cell viability decreased (Hu et al. 2017). Herein, in the current study, our results have revealed that PM$_{2.5}$ could increase intracellular ROS levels that played an important role in induction of cell apoptosis.

Toxicological results showed that PM$_{2.5}$ could cause oxidative damage and apoptosis in BEAS-2B cells. It is coincident with a previous report, which observed that some intracellular cascades, such as integrin signaling pathway, can be affected and might ultimately induce apoptosis. Similarly, in our secretome data, expression of six secreted proteins involved in integrin signaling pathway was changed, including PAI1 with 0.2-fold downregulation, and vitronectin (VTN) with 1.9-fold upregulation. The changes of these integrin's ligands in extracellular space provided new evidence for the hypothesis of integrin signaling pathway is disordered after PM$_{2.5}$ exposure.

In addition, some pro-apoptosis proteins, such as growth arrest-specific protein 1 (GAS1), DSG2, and macrophage migration inhibitory factor (MIF), were observed upregulated in secretome of migration inhibitory factor (MIF), DSG2, and macrophage regulation at the protein level after PM$_{2.5}$ exposure. The ECM

is a highly dynamic structural network and plays an essential role in cell behavior (Popova and Juecker 2022). Among the 83 significantly up/downregulated proteins, we found 36 ECM-related proteins (Table S-4) which could be divided into three subtypes, including constituent proteins (22 proteins), ECM regulators (12 proteins), and secreted factors (2 proteins). A notable finding was that 14 constituent proteins were upregulated and only two constituent proteins were downregulated in secretome of PM$_{2.5}$-exposed BEAS-2B cell, including laminin all subunit alpha-4 (LAMA4) with the highest upregulation ratio (9.7-fold). LAMA4 has been shown to promote migration, proliferation, and survival of various cell types (Shan et al. 2015). In contrast, two inhibitors of matrix metalloproteinases (MMPs), ECM1 and PAI1, were downregulated. ECM1 and PAI1 reduce the proteolytic activity of MMPs, which are key regulators of the ECM organization and involve in the degradation of various ECM proteins. (Freeberg et al. 2018; Heinbockel et al. 2018). Taken together, under PM$_{2.5}$ stimulation, increased secretion of ECM constituent proteins promoted the ECM organization, as well as upregulation of MMPs activities accelerated ECM degradation. These seemingly contradictory results reflected abnormal ECM remodeling, which impairs cell plasticity required for diverse cellular functions and even leads to cell death (Lu et al. 2011; Xia et al. 2020).

Epidemiological studies have shown the strong correlation between PM$_{2.5}$ and the risk of pulmonary inflammation, which was actively regulated by complement system (Pei et al. 2019; Kang et al. 2010). Interestingly, the results of interaction network analysis revealed that all proteins involved in complement activation were upregulated, with complement C3 showed a very high change rate (5.0-fold). In an investigation based on a panel of 175 older adults, short-term exposure to PM$_{2.5}$ could result in inflammatory reaction with a significant increase in serum level of C3 (Pei et al. 2019). In comparison, the proteins clustered into pathway of response to stimulus included a great amount of up/downregulated proteins, indicating that this pathway was seriously affected by PM$_{2.5}$. For example, the expression of Dickkopf1 (DKK1) showed the lowest change rate (0.15-fold). DKK1 is a potent antagonist of Wnt signaling, and the inhibition of it could activate Wnt/beta-catenin signaling and attenuated PM$_{2.5}$-induced pulmonary fibrosis in mice (Niida et al. 2004; Yang et al. 2020). Therefore, the significant decrease of DKK1 in our study indicated abnormal activation of Wnt signaling in BEAS-2B cells under PM$_{2.5}$ exposure. More research is needed to investigate the role of C3 and DKK1 in evaluation of air pollution injury caused by PM$_{2.5}$.

The limitations of this study also need to be mentioned. Due to the complexity of the composition of fine atmospheric particles, we measured the composition of PM$_{2.5}$ according to recognized component classifications.
Therefore, some potentially important components of PM<sub>2.5</sub> and their toxicity are inevitably not investigated. Meanwhile, we only performed extracellular proteomic analysis to explore the changes in the extracellular environment of bronchial epithelial cells exposed to PM<sub>2.5</sub>. In future multiomics analyses, such as intracellular proteomics, transcriptome, and metabolism are needed for further understanding of the mechanisms underlying PM<sub>2.5</sub>-caused cell damages.

In conclusion, we evaluated the cytotoxicity of PM<sub>2.5</sub> exposure on BEAS-2B cells and investigated the corresponding variation in secretome. A total of 83 proteins (58 upregulated and 25 downregulated) were differentially expressed in extracellular space after PM<sub>2.5</sub> treatment. We observed promoted release of several pro-apoptotic factors, indicating intercellular communication might attribute to PM<sub>2.5</sub>-induced apoptosis of bronchial epithelial cells. Meanwhile, we found dysregulated secretion of many ECM constituents mediated abnormal ECM remodeling, which might impair cell plasticity and even lead to apoptosis. Taken together, these results provide an extracellular proteomic analysis to further understanding of the molecular mechanisms underlying PM<sub>2.5</sub>-caused cell damages.

Abbreviation AM: acetyl methoxy methyl ester; ANOVA: one-way analysis of variance; BCA: bicinchoninic acid; BEAS-2B cells: bronchial epithelia cells; BEBM: stable isotope labeling with amino acids in cell culture; CCK-8: cell counting kit-8; DCFH/DA: 2,7'-dichlorodihydrofluorescein diacetate; DKK1: dickkopf1; DSG2: Desmoglein-2; ECM: extracellular matrix; ECM1: extracellular matrix protein-1; EVs: extracellular vesicles; FASP: filter-aided sample preparation; FBS: fetal bovine serum; FDR: false discovery rate; FHRP: horseradish peroxidase; IC50: half-maximal inhibitory concentration; ICP-MS: inductively coupled plasma mass spectrometry; KEGG: Kyoto encyclopedia of genes and genomes; LAMA4: laminin subunit alpha-4; MIEF: migration inhibitory factor; MLEFF: a strategy combining metabolic labeling, protein “equalization,” protein fractionation, and filter-aided sample preparation; MMPs: matrix metalloproteinases; nano-RPLC-MS/MS: nano reverse phase liquid chromatography mass spectrometry; PARs: polymeric aromatic hydrocarbons; PAI1: plasminogen activator inhibitor type-1; PBS: phosphate buffered solution; PCDD/Fs: polychlorinated dibenzo-p-dioxins and dibenzofurans; PD: proteome discover; PI: propidium iodide; PI3K/AKT pathway: phosphatidylinositol-3-kinase/AKT serine/threonine kinase pathway; PVDF: polyvinylidene fluoride; RNA: ribonucleic acid; ROS: reactive oxygen species; SDS: sodium dodecyl sulfate; TFs: transcription factors; TNF-alpha: tumor necrosis factor alpha; VTN: vitronectin

Supplementary Information The online version contains supplementary material available at https://10.1007/s11356-022-20726-9.

Author contribution Conceptualization: SZ, CJ, and ZL; methodology: SZ, SX, and WY; writing—original draft preparation: SZ, SX, and WY; writing—review and editing: LJ, ZB, and LZ; supervision: CJ, ZL, and ZY; funding acquisition: ZL, SZ, and SX. All authors read and approved the final manuscript.

Funding This work was supported by National Key Research and Development Program of China (Grant 2017YFA0505003) and National Natural Science Foundation (Grants 21775149, 91543201, 21806165, and 21725506).

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

References

Adam M, Schikowski T, Carsin AE, Cai Y, Jacquemin B et al (2015) Adult lung function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-analysis. Eur Respir J 45:38–50. https://doi.org/10.1183/09031936.00130014

Bae S, Pan X-C, Kim S-Y, Park K, Kim Y-H et al (2010) Exposures to particulate matter and polycyclic aromatic hydrocarbons and oxidative stress in schoolchildren. Environ Health Perspect 118:579–583. https://doi.org/10.1289/ehp.0901077

Bo Y, Chang L-Y, Guo C, Lin C, Lau AKH et al (2021) Reduced ambient PM<sub>2.5</sub>, better lung function, and decreased risk of chronic obstructive pulmonary disease. Environ Int 156:106706. https://doi.org/10.1016/j.envint.2021.106706

Chen H, Liu X, Gao X, Lv Y, Zhou L et al (2021) Epidemiological evidence relating risk factors to chronic obstructive pulmonary disease in China: a systematic review and meta-analysis. PLoS One 16:e0261692. https://doi.org/10.1371/journal.pone.0261692

Cheng L, Hill AF (2022) Therapeutically harnessing extracellular vesicles. Nat Rev Drug Discov. https://doi.org/10.1038/s41573-022-00410-w

Circu ML, Aw TY (2010) Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 48:749–762. https://doi.org/10.1016/j.freeradbiomed.2009.12.022

Cwielag-Drabek M, Parascandola M, Piekut A, Spychała A, Dziubanek G et al (2020) Non-dioxin-like PCBs – the key air pollutant associated with lung cancer in 15 cities in Silesia. Pol J Environ Stud 29:1111–1117. https://doi.org/10.15244/pjoes.102786

Dagher Z, Garcon G, Billet S, Gosset P, Ledoux F et al (2006) Activation of different pathways of apoptosis by air pollution particulate matter (PM<sub>2.5</sub>) in human epithelial lung cells (L132) in culture. Toxicology 225:12–24. https://doi.org/10.1016/j.tox.2006.04.038

Deng X, Zhang F, Rui W, Long F, Wang L et al (2013) PM<sub>2.5</sub>-induced oxidative stress triggers autophagy in human lung epithelial A549 cells. Toxicol in Vitro 27:1762–1770. https://doi.org/10.1016/j.tiv.2013.05.004

Duan R, Niu H, Yu T, Huang K, Cui H et al (2021) Adverse effects of short-term personal exposure to fine particulate matter on the lung function of patients with chronic obstructive pulmonary disease and asthma: a longitudinal panel study in Beijing, China. Environ Sci Pollut Res 28:47463–47473. https://doi.org/10.1007/s11356-021-13811-y

Forder A, Hsing C-Y, Trejo Vazquez J, Garnis C (2021) Emerging role of extracellular vesicles and cellular communication in metastasis. Cells 10:3429. https://doi.org/10.3390/cells10123429
Torres-Ramos Y, Montoya-Estrada A, Guzman-Grenfell A, Mancilla J, Cardenas B et al (2011) Urban PM2.5 induces ROS generation and RBC damage in COPD patients. Front Biosci (Elite edition) 3:808–817. https://doi.org/10.2741/e288

Wang W, Deng Z, Feng Y, Liao F, Zhou F et al (2017) PM2.5 induced apoptosis in endothelial cell through the activation of the p53-bax-caspase pathway. Chemosphere 177:135–143. https://doi.org/10.1016/j.chemosphere.2017.02.144

Weng Y, Sui Z, Shan Y, Jiang H, Zhou Y et al (2016) In-depth proteomic quantification of cell secretome in serum-containing conditioned medium. Anal Chem 88:4971–4978. https://doi.org/10.1021/acs.analchem.6b00910

Wu X, Zhu B, Zhou J, Bi Y, Xu S et al (2021) The epidemiological trends in the burden of lung cancer attributable to PM2.5 exposure in China. BMC Public Health 21:737. https://doi.org/10.1186/s12889-021-10765-1

Xia T, Shen Z, Cai J, Pan M, Sun C (2020) ColXV aggravates adipocyte apoptosis by facilitating abnormal extracellular matrix remodeling in mice. Int J Mol Sci 21:959. https://doi.org/10.3390/ijms21030959

Xu H, Jiao X, Wu Y, Li S, Cao L et al (2019a) Exosomes derived from PM2.5-treated lung cancer cells promote the growth of lung cancer via the Wnt3a/-catenin pathway. Oncol Rep 41:1180–1188. https://doi.org/10.3892/or.2018.6868

Xu M-X, Ge C-X, Qin Y-T, Gu T-T, Lou D-S et al (2019b) Prolonged PM2.5 exposure elevates risk of oxidative stress-driven nonalcoholic fatty liver disease by triggering increase of dyslipidemia. Free Radic Biol Med 130:542–556. https://doi.org/10.1016/j.freeradbiomed.2018.11.016

Xu D, Zhang Y, Sun Q, Wang X, Li T (2021) Long-term PM(2.5) exposure and survival among cardiovascular disease patients in Beijing, China. Environ Sci Pollut Res Int 28:47367–47374. https://doi.org/10.1007/s11356-021-14043-w

Xue Z, Li A, Zhang X, Yu W, Wang J et al (2019) iTRAQ based proteomic analysis of PM2.5 induced lung damage. RSC Adv 9:11707–11717. https://doi.org/10.1039/C9RA00252A

Yan Z, Wang J, Li J, Jiang N, Zhang R et al (2016) Oxidative stress and endocytosis are involved in upregulation of interleukin-8 expression in airway cells exposed to PM2.5. Environ Toxicol 31:1869–1878. https://doi.org/10.1002/tox.22188

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.