Mitochondrial damage mediated by ROS incurs bronchial epithelial cell apoptosis upon ambient PM$_{2.5}$ exposure

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**ABSTRACT** — Mitochondria can be used as important biomarkers of pollutants on human health, and fine particulate matter (PM$_{2.5}$) has been documented to cause respiratory damage. However, current studies about the relationship between PM$_{2.5}$ and mitochondria in respiratory tract are limited and warrant further detailed investigations. Hence, the study was aimed to evaluate effects of PM$_{2.5}$ on mitochondrial structure, investigate the link between PM$_{2.5}$-induced mitochondrial disorder and respiratory damage, and delineate the possible mechanisms using both *in vitro* and *in vivo* models. PM$_{2.5}$ exposure resulted in damage of mitochondrial structure, including mitochondrial dynamic, DNA biogenesis and morphological alteration 16HBE cells. Furthermore, PM$_{2.5}$ elevated ROS formation. However, DPI and NAC (inhibitor of ROS) in supplement restored PM$_{2.5}$-induced mitochondrial disorder. PM$_{2.5}$ also contributed to the 16HBE cells apoptosis via mitochondrial pathway. Additionally, the results coincided with the *in vivo* data which were obtained from bronchial tissues of SD rats exposed to PM$_{2.5}$ for 30 days. Collectively, this study uncovers that PM$_{2.5}$ leads to the disorder of mitochondrial structure via ROS generation, and then results in respiratory damage. It provides further understanding about the detrimental effect of PM$_{2.5}$ on respiratory damage, and reveals a mechanistic basis for preventing outcomes in polluted environments.

**Key words:** Particulate matter (PM$_{2.5}$), Mitochondria, ROS, Bronchial epithelial cells, Apoptosis

**INTRODUCTION**

Greater than three million deaths per year are attributable to ambient air pollution, which is the 9th leading factor contributing to worldwide burden of disease according to the 2010 Global Burden of Disease Study (Lim *et al*., 2012). Fine particulate matter (PM$_{2.5}$; aerodynamic diameter less than 2.5 μm) is well established as a key contributor of ambient air pollution, which can be readily inhaled by the human body and deposited in the respiratory system (Leung *et al*., 2014). Epidemiological and experimental investigations have linked PM$_{2.5}$ exposure to increased respiratory hospital admissions, mortality and morbidity due to the potential bio-accumulation (Stieb *et al*., 2009; Rückerl *et al*., 2011; Brunekreef and Holgate, 2002). However, the studies of mechanism on PM$_{2.5}$-induced respiratory damage are limited and warrant further detailed investigations.

In most mammalian cells, mitochondria are primarily considered the major supplier of ATP. In which, more than 95% of ATP is synthesized through oxidative reactions (Nunnari and Suomalainen, 2012). During oxidative phosphorylation, electrons are moved thorough the mitochondrial respiratory chain, and a proton gradient is established across the inner mitochondrial membrane as the energy source for ATP production (Prakash and Doublé, 2015). Mitochondria are morphologically dynamic organelles that continuously undergo two opposing events (fission and fusion), which have vital roles in cell metabolism and respiratory (Sheridan and Martin, 2010). Besides, they undergo prominent structural changes including fragmentation and cristae remodeling in response to intense stimulation (Arnoult, 2007). There are emerging data that mitochondrial disorders are
closely related to a variety of physiological and pathological processes, including aging, cardiovascular diseases and respiratory illness (Nunnari and Suomalainen, 2012). Therefore, mitochondria can be used as important biomarkers of pollutants on human health and it is imperative to understand the effect of PM$_{2.5}$ on mitochondria. However, current studies on the relationship between PM$_{2.5}$ and mitochondria are limited and need further assessment. In the present study, we carried out the comprehensive evaluation about the effect of PM$_{2.5}$ on structure and function of mitochondria. The structure involves in mitochondrial dynamic, DNA biogenesis and morphological alteration, and the function includes energy metabolism and respiratory function. Studying the effect of mitochondrial structure and function is of great significance for the evaluation of PM$_{2.5}$ toxicity on human health.

It has been documented that reactive oxygen species (ROS) captured the attention of biologists and physicians due to their important function in the regulation of multiple cellular and extracellular processes such as activation of stress-induced mitochondrial biogenesis (Murphy et al., 2011). Thus, mitochondria were the target of ROS, which could affect the mitochondrial structure and function in turn (Scherz-Shouval and Elazar, 2007). Besides, mitochondria are also accountable for the generation of ROS, which is generated with the leak of electrons mainly from mitochondrial respiratory chain complexes I and III (Szewczyk et al., 2015). Impairment of mitochondrial dynamics and pathological changes in metabolic properties leading to unnecessarily high ROS formation must result in diverse abnormalities. Notably, PM$_{2.5}$-induced excessive formation of ROS could trigger cell damage by oxidizing macromolecular structures and modifying their biological functions, ultimately leading to cell apoptosis, suggesting the pivotal roles of ROS on PM$_{2.5}$-mediated adverse health on human body (Kampa and Castanas, 2008).

Due to the extensive vehicle exhaust emissions and coal combustions in residential stoves for cooking and heating, northern Chinese cities still face serious problems of PM$_{2.5}$ pollution, especially in winter (Shen et al., 2010). This situation is worsening with the urbanization and industrialization of Taiyuan, a northern city of China and a center of coal-based electricity production and many chemicals industries (Li et al., 2014). Therefore, the current study was focused on winter PM$_{2.5}$ from North China and designed to address three goals: (1) to investigate effects of PM$_{2.5}$ on mitochondrial structure in respiratory tract; (2) to explore the link between PM$_{2.5}$-induced mitochondrial disorder and respiratory damage; (3) to delineate the possible mechanisms using both in vitro and in vivo models.

**MATERIALS AND METHODS**

**PM$_{2.5}$ collection and preparation**

As described by Li et al. (2014), PM$_{2.5}$ samples were collected during winter 2012/2013 at Taiyuan, a site of Shanxi urban background for atmospheric pollution. Briefly, PM$_{2.5}$ high volume air sampler (Thermon Anderson, New York, NY, USA) was placed on the rooftop of a building about 25 meters tall. PM$_{2.5}$ concentrations were measured using DustTrakTM II Aerosol Monitor (TSI Inc., Knoxville, TN, USA), and daily samples were collected on quartz fiber filters (QFFs) with a pump flow rate of 1.13 m$^3$/min. These filters loading samples were cut and surged in Milli-Q water with sonication. To obtain PM$_{2.5}$ suspensions, the above suspensions were freeze-dried in vacuum and weighed. Then the samples were dried and stored at -20°C. The 20 mg PM$_{2.5}$ particles supplemented with 1 mL 0.9% physiological saline were blended, treated with ultrasonic oscillation for 30 min. Particles were UV-irradiated overnight to inactivate possible contaminating endotoxin and to be germicidal, as indicated in the paper of Peeters et al. (2014). Then 20 mg/mL PM$_{2.5}$ was stored at 4°C, with gentle oscillation before usage. Prior to use, PM$_{2.5}$ suspension was diluted with sterilized 0.9% physiological saline, surged for 20 min and mixed fully.

**Cells and culture conditions**

16HBE cell line (human bronchial epithelial cells), obtained from the Institute of Biochemistry and Cell Biology (SIBS, CAS, Shanghai, China), was maintained in DMEM medium (HyClone, New York, NY, USA) supplemented with 10% FBS (Boster, Wuhan, China) and 1% penicillin/streptomycin (Solarbio, Beijing, China). Cells were kept at 37°C in a 5% CO$_2$ humidified cell culture incubator. 16HBE cells were treated with different doses of PM$_{2.5}$ (0, 10, 50 and 100 μg/mL) for 48 hr, based on the doses used in other in vitro research papers (Gualtieri et al., 2011; Risom et al., 2005). In the experiments involving pharmacological inhibitors, 16HBE cells were pre-treated with fresh media containing DPI (3 μM) or NAC (1 mM) for 24 hr and then treated with PM$_{2.5}$ suspensions in the presence of inhibitors for 48 hr.

**Quantitative real time PCR**

Quantitative real time PCR (qRT-PCR) was employed using an Applied Bio-systems platform, according to our previous methods (Jin et al., 2014a). Human or rat prim-
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Mitochondrial membrane potential (Δψm) assay
Mitochondrial membrane potential (Δψm) was analyzed in 16HBE cells, according to the manufacturer’s protocol for a commercial kit (KeyGEN BioTECH, Nanjing, China). The details were as described for our previous methods (Jin et al., 2014b).

Determination of ROS generation
Generation of ROS was determined by fluorometric analysis, using 2,7-dichlorofluorescin diacetate (DCFH-DA), which was obtained from Beyotime Institute of Biotechnology (Nan tong, China) as described for our previous methods (Jin et al., 2014b).

Flow cytometric analysis of apoptosis
Apoptosis was examined by using the Annexin V-FITC and propidium iodide (PI) staining method. In brief, 5 × 10$^5$ cells were collected and incubated in the buffer containing 200 μL Annexin V solutions (10 μL AnnexinV + 200 μL binding buffer) and 300 μL PI (5 μL PI + 300 μL binding buffer) in the dark (15 min at RT). Untreated cells were used as the control. Then the samples were examined using a FAC Sort Flow Cytometer within 45 min after staining.

Trypan blue exclusion counts and LDH release for toxicity estimation
After 16HBE cells were treated by PM$_{2.5}$ for 48 hr with or without NAC or DPI pre-treatment for 24 hr, the culture medium was collected and assayed for LDH activity. It was investigated by the linear region of a pyruvate standard graph using regression analysis and expressed as % release, according to a commercial determination kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Total number of viable cells was counted by trypan blue exclusion method using a hemocytometer. Cell viability (% of control) = (Number of control cells – Number of treated cells) / Number of control cells × 100.

Western blotting assay
Proteins extracted from cells and tissues were resolved by 10% SDS-PAGE and transferred onto nitrocellulose membranes for Western blotting, according to our previous methods (Jin et al., 2014a). The antibodies used in this study were as follows: antibodies for Bax, Bcl-2, and Cyt-c were obtained from Bioworld Technology (Minneapolis, MN, USA); antibodies for caspase-3, and caspase-9 were from Beyotime Institute of Biotechnology (Haimen, China); α-tubulin was purchased from Sigma (St. Louis, MO, USA).

Animal experiment
As indicated in our previous study (Li et al., 2015), healthy adult, clean-grade male Sprague-Dawley rats (weighing 200 ± 20 g) were commercially obtained from Experimental Animal Center of the Chinese Military Medical Science Academy (Beijing, China). The animals were kept in standard animal houses under a 12 hr light/dark cycle (lights on at 8:00 a.m.) at a constant temperature of 24 ± 2°C and relative humidity of 50 ± 5%. After 7 days of habitation, the rats were randomly divided into the following five groups (n = 6); (1) control group, (2) 0.3 mg/kg body weight (b.w.) PM$_{2.5}$ group, (3) 0.9 mg/kg b.w. PM$_{2.5}$ group, (4) 1.8 mg/kg b.w. PM$_{2.5}$ group and (5) 2.7 mg/kg b.w. PM$_{2.5}$ group. The treatment groups were instilled with a 0.5 mL PM$_{2.5}$ suspension, and the final exposure concentrations reached 0.3, 0.9, 1.8, 2.7 mg/kg b.w., while the control group was instilled with physiological saline at the same volume as that was used for the treatment group. The instillation was conducted using a nonsurgical intratracheal instillation method (Bai et al., 2010), and performed one time every 3 days. When not being treated, the rats had free access to food and water. All of these rats were maintained under standard nutritional and environmental conditions throughout the experiment according to the requirement of the National Act on the Use of Experimental Animals (China). Maximal effort was made to minimize animal suffering and the number of animals necessary for the acquisition of reliable data.

As has been reported, the respiratory volume of an adult rat was 200 mL/min, and the respiratory volume for 3 days reached 0.864 m$^3$. According to the China National Ambient Quality Standard (NAAQS, 2012) for PM$_{2.5}$ (0.075 mg/m$^3$), the amount of PM$_{2.5}$ inhalation over 3 days is 0.0648 mg and the concentration of PM$_{2.5}$ exposure for each rat every 3 days is estimated to be 0.324 mg/kg b.w. (rat 200 ± 20 g). PM$_{2.5}$ mean mass concentration determined in our samples was 0.161 ± 0.060 mg/m$^3$ on non-haze weather in Taiyuan (Li et al., 2014), and the higher concentrations corresponded to the haze weather reached 0.692 ± 0.272 mg/m$^3$ (Cao et al., 2014). Taken together, the average concentrations of PM$_{2.5}$ exposure for each rat every 3 days were estimated in the range from 0.324 to 2.989 mg/kg b.w.. Therefore, we selected 0.3, 0.9, 1.8, 2.7 mg/kg b.w. PM$_{2.5}$ as exposure doses in our SD rat experiment.
Statistical analysis

Statistical analysis was carried out using the SPSS 17.0 software program. Data, derived from at least three independent experiments, were presented as the mean ± standard deviation (S.D.). Differences among groups were tested by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. P-values lower than 0.05 were considered statistically significant.

RESULTS

PM$_{2.5}$ causes disorders of mitochondrial structure

Since mitochondria are direct stress organelles upon foreign pollutant exposure (Adam-Vizi and Chinopoulos, 2006) and important biomarkers of pollutant on human health, we carried out the comprehensive evaluation about the impact of PM$_{2.5}$ on mitochondrial structure and function. The structure of mitochondria is involved in mitochondrial dynamic, DNA biogenesis and morphological alteration. As shown in Fig. 1A and B, expressions of profusion factors (Mfn1 and OPA1) were notably suppressed by 100 μg/mL PM$_{2.5}$ exposure, and there was significant alteration in profusion factors (Drp1) compared with non-exposed cells. In addition, PM$_{2.5}$ induced a significant decrease in mRNA levels of SIRT1 and p53R2, which are related to mitochondrial DNA biogenesis in 16HBE cells (Fig. 1C). Based on Fig. 1D-F, a bright green fluorescence, showing the decrease of mitochondrial membrane potential, was observed in PM$_{2.5}$-treated cells, while control cells displayed a broad yellow-green fluorescence. The data indicated that PM$_{2.5}$ treatment caused the damage of mitochondrial structure.

PM$_{2.5}$-induced ROS promotes mitochondrial damage in 16HBE cells

Given that ROS are accountable for mitochondria disorder and are the main target of PM$_{2.5}$-caused toxicity on human health (Kampa and Castanas, 2008), their generation was examined. Fluorometric analysis revealed that PM$_{2.5}$ induced significant dose-dependent production of ROS (Fig. 2A). In order to further confirm the possible role of ROS on mitochondria disorder, DPI and NAC, inhibitors of ROS, were applied. After pre-treatment with
DPI or NAC for 24 hr, ROS levels were reduced compared to PM2.5 treatment alone, indicating that ROS were induced by PM2.5 (Fig. 2B). As shown in Fig. 3A and B, DPI or NAC induced a marked increase in mRNA levels of fusion protein Mfn1 and OPA1 decreased by PM2.5. Additionally, elevated mRNA expressions of mitochondrial biogenesis markers SIRT1 and p53R2 were observed in PM2.5 + DPI or NAC group compared to PM2.5 group (Fig. 3C and D). Moreover, based on Fig. 3E and F, the expression of NDUFS2 and UQCR11 was successfully attenuated by pre-treatment with DPI or NAC for 24 hr. Likewise, mitochondrial membrane potential attenuated by PM2.5 treatment was alleviated by pre-treatment with DPI or NAC (Fig. 3G and H). The data indicated that enhanced ROS generation promoted the damage of mitochondrial structure and function.

**PM2.5 contributes to the cell death of 16HBE cells**

In order to explore the link between PM2.5-induced mitochondrial disorder and respiratory damage, we next determined the cell viability of human bronchial epithelial cells upon PM2.5 exposure. Flow cytometric analysis was performed to determine the effective dose of PM2.5 on 16HBE cells for 24 hr and 48 hr exposure. As shown in Fig. 4A, PM2.5 exposure at 10, 50 and 100 μg/mL induced considerable cell death in a dose-dependent manner after 48 hr of exposure. Especially, 100 μg/mL PM2.5 exposure induced 48.93% apoptosis compared to control cells with 6.15% apoptosis. Trypan Blue Exclusion counts was adopted to confirm the cell damage incurred by PM2.5. Cells with PM2.5 exposure presenting shrinkage shape representing apoptotic cells, which were elevated in a PM2.5 dose-dependent manner (Fig. 4B and C). Similar findings were observed in the LDH assay, displaying a decline of cell viability in response to PM2.5 (Fig. 4D). These data clarify that PM2.5 led to the cell death of human bronchial epithelial cells.

**PM2.5 leads to the cell death of 16HBE cells via mitochondrial pathway mediated by ROS**

We further assessed the association between PM2.5-induced respiratory damage and mitochondrial disorder, as well as the possible mechanism, adopting the DPI or NAC addition. As revealed in Fig. 5A and B, the reduction of cell viability by PM2.5 was ameliorated by the addition of DPI or NAC, indicating that the cell death of 16HBE cells was mediated by PM2.5-induced ROS generation. Due to the major role of mitochondrial pathway in cell death, a further step was taken to determine the pathway. The results from Western blotting indicated that the levels of Bax, cytochrome c, active-caspase3 and active-caspase9 were up-regulated in response to PM2.5 in a dose-dependent manner. In addition, the protein level of anti-apoptotic gene Bcl-2 was down-regulated in PM2.5-treated cells as compared to control (Fig. 5C). Moreover, these alterations were significantly reversed by the DPI or NAC addition (Fig. 5D). All these results suggested that PM2.5 possibly caused the induction of permeability transition in mitochondria, which was mediated by ROS generation, and played a key role in cell death of 16HBE cells.

**Fig. 2.** PM2.5 elevates the ROS production in 16HBE cells. 16HBE cells were exposed to PM2.5, and ROS generation was determined. (A) Alterations in the ROS generation of 16HBE cells relative to control. (B) After pre-treated with fresh media containing DPI (3 μM) or NAC (1 mM) for 24 hr and then treated with 100 μg/mL PM2.5 in the presence of inhibitors for 48 hr, the ROS generation was investigated in 16HBE cells. Results were reported as average ± S.D. for at least three independent experiments. Statistically significant different from PM2.5-treated cells: *p < 0.05, **p < 0.01.
PM$_{2.5}$ induces mitochondrial damage and apoptosis pathway in SD rats

The above results uncovered that PM$_{2.5}$ contributed to mitochondrial disorder and cell apoptosis of human bronchial epithelial cells, which were mediated by ROS. To validate our findings, in vivo SD rat assay was performed to further determine the toxicity induced by PM$_{2.5}$. After 0.3, 0.9, 1.8, 2.7 mg/kg b.w. PM$_{2.5}$ exposure of SD rats for 30 days with intratracheal instillation, we separated bronchial tissues of SD rats and determined the alteration of mitochondria and relevant apoptosis pathway. The result from qRT-PCR showed that levels of Mfn1, OPA1, SIRT1 and p53R2 in bronchial tissues were dose-dependently reduced in response to PM$_{2.5}$ (Fig. 6A and B). A pronounced dose-related attenuation in NDUFS2 and UQCR11 expressions was also was observed (Fig. 6C). Furthermore, the data from Western blotting indicated that 2.7 mg/kg b.w. PM$_{2.5}$ contributed to up-regulations of apoptotic proteins and down-regulations of anti-apoptotic protein Bcl-2 in bronchial tissues (Fig. 6D).

**DISCUSSION**

There are emerging data documenting that PM$_{2.5}$ expo-

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**Fig. 3.** The effect of PM$_{2.5}$-induced ROS on mitochondrial structure and function in 16HBE cells. After pre-treatment with DPI (3 μM) or NAC (1 mM) for 24 hr, inhibiting ROS from NOX or total ROS, and 100 μg/mL PM$_{2.5}$ exposure for 48 hr, the mitochondrial structure and function were investigated. (A-B) Mfn1 and OPA1. (C-D) SIRT1 and p53R2. (E-F) NDUFS2 and UQCR11. (G) JC-1 was carried out to assess the alteration in mitochondrial structure. Scale bar is 10 μm. (H) The JC-1 green fluorescence intensities were analyzed using the Image J (NIH) software. The values were showed as means ± S.D. of triplicate independent determinations. *$p < 0.05$, **$p < 0.01$, compared to PM$_{2.5}$-treated cells.
PM$_{2.5}$ induces mitochondrial disorder and respiratory damage

PM$_{2.5}$ is closely associated with the increased respiratory hospital admissions, mortality and morbidity, and mitochondria are important markers of evaluating pollutant’s toxicity. However, current researches about the relationship between PM$_{2.5}$ and mitochondria in respiratory tract are limited and warrant further detailed investigations. In this report, we identify the effects of PM$_{2.5}$ on respiratory damage and mitochondrial disorder, as well as classify the possible mechanism involved involved the induction of ROS using both \emph{in vitro} and \emph{in vivo} models.

The pollutant and chemical characteristics of PM$_{2.5}$ in our study have been reported by Li \textit{et al.} (2014). Briefly, the measured daily average PM$_{2.5}$ mass concentration ($0.161 \pm 0.060 \text{ mg/m}^3$) was much higher than those of the Chinese national recommended safety standards ($0.075 \text{ mg/m}^3$). The levels of 16 polycyclic aromatic hydrocarbons (PAHs) in PM$_{2.5}$ were obviously higher than those of the Chinese national standard for PAHs ($10 \text{ ng/m}^3$). The daily mean levels of SO$_4^{2-}$ and NO$_3^-$ ions in the PM$_{2.5}$ samples reached 5.87 and 1.71 $\mu$g/m$^3$, respectively. These data indicated that PM$_{2.5}$ pollution in Taiyuan might be hazardous to human health.

Mitochondria are essential intracellular energy-generating organelles with various cellular functions. Fusion and fission of mitochondria are important for many biological processes, including homeostasis of mitochondrial bio-
genesis, turnover, subcellular distribution, cell division, and apoptosis. Mitochondrial dynamics play a crucial role in mitochondrial quality control. Through proper fusion/fission dynamics coordinated with contents amplification, new daughter mitochondria are formed (Chan, 2006). Mitochondrial biogenesis is a complex process involving the replication of mitochondrial DNA (mtDNA) and the expression of mitochondrial proteins encoded by both nuclear and mitochondrial genomes (Higashida et al., 2013). In addition, up-regulation of other mitochondrial contents including respiratory chain complexes and their assembly proteins, is important for preventing dilution of the contents for a successful mitochondrial proliferation (Muster et al., 2010). Consequently, mitochondria are considered as a new paradigm for the research of PM$_{2.5}$ toxicity. During apoptosis, mitochondria undergo prominent structural changes including fragmentation and cristae remodelling (Arnoult, 2007). This study has demonstrated that PM$_{2.5}$ exposure damaged the structure and function of 16HBE cells. Our previous results also implicated that PM$_{2.5}$-induced mitochondrial damage was potentially important mechanisms for the heart injury (Li et al., 2015). Consistently, several studies have implicated the aberrant mitochondrial alteration upon PM$_{2.5}$ exposure. Li et al. (2003) have reported that ultrafine particulate matter collected in Los Angeles induced mitochondrial structural damage, which was in accordance with the redox cycling potential of the particles. Xu et al. also have document-
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ed that long-term ambient PM$_{2.5}$ exposure impaired mitochondriald alteration, which was a risk factor for the development of type 2 diabetes (Gualtieri et al., 2011).

The present results indicate PM$_{2.5}$ promoted the damage of mitochondrial structure via the ROS generation. Similarly, mitochondria have been regarded as both target and source of ROS, whose generation may induce the oxidative damage to mitochondrial proteins (Scherz-Shouval and Elazar, 2007). Oxidation of the mitochondrial by ROS may contribute to cytochrome c release due to disruption of the mitochondrial membrane potential (Zamzami et al., 1995). ROS are also one of the important mechanisms of cell damage induced by PM$_{2.5}$ (Bräuner et al., 2007; Risom et al., 2005). Under normal conditions, the production and elimination of ROS should be in balance, while sustained ROS production is often responsible for oxidative cell damage through lipid peroxidation, DNA breakdown, and protein damage (Finkel and Holbrook, 2000). When mitochondria are damaged by pollutants, ROS can be generated from mitochondrial respiratory chain (Adam-Vizi and Chinoopoulos, 2006). The main sources of cellular ROS are mitochondria and NADPH oxidases (NOXs), which are cell membrane-bound proteins (Lambeth, 2004). As shown in Fig. 2B, the reversed effect of NAC (inhibitor of ROS) on ROS levels was more prominent than DPI (inhibitor of ROS from NOXs), suggesting that ROS induced by PM$_{2.5}$ could generate from mitochondria and NOXs. The elevated expression of DUOX1 and DUOX1 in bronchial tissues of SD rats exposed to PM$_{2.5}$ confirmed our speculation (Fig. S1).

In the present study, we found that PM$_{2.5}$ contributed

Fig. 6. Mitochondrial alteration and cell death caused by PM$_{2.5}$ in bronchial tissues. After 0.3, 0.9, 1.8, 2.7 mg/kg body weight (b.w.) PM$_{2.5}$ exposed to SD rats for 30 days with the intratracheal instillation, bronchial tissues of SD rats were separated and homogenized with the liquid nitrogen for qRT-PCR and Western blotting. qRT-PCR assay was applied to analyze the expressions of mitochondrial relevant genes, inducing (A) Mfn1, OPA1, (B) SIRT1, p53R2, (C) NDUFS2 and UQCRI1 in bronchial tissues. (D) Western blotting was performed as mentioned above to examine apoptosis protein expression in control and 2.7 mg/kg b.w. PM$_{2.5}$-treated bronchial tissues. Values shown were given as the mean ± S.D. of 6 animals in each group. *$p<0.05$, **$p<0.01$, compared to control mice.
to respiratory damage, which was consistent with some studies at home and abroad. Guo et al. (2014) indicated that PM\textsubscript{2.5} concentration was closely correlated with the increase of patients with bronchitis. Annesi-Maesano et al. (2007) found that the morbidity of asthma and bronchitis induced by exercises was apparently connected with PM\textsubscript{2.5} concentration, which was evidently higher in high-concentration regions than that in low-concentration regions. In our previous study, we observed that exposure to Taiyuan winter PM\textsubscript{2.5} induced cellular oxidative heart damage, and filter-derived quartz debris did not cause bias in our experimental outcomes (Li et al., 2015). PAHs, as an important part of organic compounds toxic substances in PM\textsubscript{2.5}, occupy a large proportion. Many studies also have shown that the biological effects of PM\textsubscript{2.5} are directly related to PAHs (Abbas et al., 2013; Billet et al., 2008). Therefore, we speculated that PAHs in PM\textsubscript{2.5} were important for the mitochondrial damage.

To the best of our knowledge, this is the first report to examine the relationship between PM\textsubscript{2.5}-induced mitochondrial disorder and respiratory damage. The data implicated that PM\textsubscript{2.5} exposure elevated the ROS generation via binding to cell membrane-bound NOXs, which further provoked the disorder of mitochondrial structure and function in respiratory tract. Moreover, the damaged mitochondria induced the mitochondrial apoptosis pathway, and then resulted in cell apoptosis of respiratory tract. Accordingly, this project contributes to explaining the damage effects of PM\textsubscript{2.5} on human respiratory tract and provides a kind of mechanistic basis for the development of preventive treatments for urban dust-haze.

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Conflict of interest——The authors declare that there is no conflict of interest.

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