Research Article

Fine Particulate Matter Leads to Unfolded Protein Response and Shortened Lifespan by Inducing Oxidative Stress in C. elegans

Yunli Zhao, Ling Jin, Yuxin Chi, Jing Yang, Quan Zhen, and Huazhang Wu

1Department of Preventive Medicine, Bengbu Medical College, Bengbu 233030, China
2School of Life Science, Anhui Province Key Laboratory of Translational Cancer Research, Bengbu Medical College, Bengbu, China

Correspondence should be addressed to Huazhang Wu; whzhang1025@163.com

Received 23 June 2019; Revised 27 August 2019; Accepted 12 September 2019; Published 7 December 2019

Academic Editor: Giuseppe Cirillo

Copyright © 2019 Yunli Zhao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Oxidative stress has been proven as one of the most critical regulatory mechanisms involved in fine Particulate Matter- (PM2.5-) mediated toxicity. For a better understanding of the underlying mechanisms that enable oxidative stress to participate in PM2.5-induced toxic effects, the current study explored the effects of oxidative stress induced by PM2.5 on UPR and lifespan in C. elegans. The results implicated that PM2.5 exposure induced oxidative stress response, enhanced metabolic enzyme activity, activated UPR, and shortened the lifespan of C. elegans. Antioxidant N-acetylcysteine (NAC) could suppress the UPR through reducing the oxidative stress; both the antioxidant NAC and UPR inhibitor 4-phenylbutyric acid (4-PBA) could rescue the lifespan attenuation caused by PM2.5, indicating that the antioxidant and moderate proteostasis contribute to the homeostasis and adaptation to oxidative stress induced by PM2.5.

1. Introduction

Oxidative stress has been described as the disturbances in the redox homeostasis resulting from increased reactive oxygen species (ROS) production or attenuated antioxidant defense systems [1, 2]. Oxidative stress usually leads to damage as free radicals can interact with cell components such as DNA, lipid, and protein [3, 4]. As a common denominator of toxicity, oxidative stress can be induced almost by all types of toxicants. The free radicals can be produced and aggregated in the endoplasmic reticulum (ER) and mitochondria, which may lead to the accumulation of unfolded/misfolded proteins and have extensive influence on homeostasis and function [5–9]. Organisms have developed excellent defense mechanisms to prevent damage caused by environmental stresses, such as signaling pathways that resolve aberrant proteins known as unfolded protein response (UPR) [10, 11]. Previous studies have shown that xenobiotics can activate UPR signaling network in vivo and in vitro [12–14].

The model system C. elegans was extensively used for toxicology researches because of its distinct advantages, such as short lifecycle and ease of culture [15, 16]. In C. elegans, hsp-4 encodes endoplasmic reticulum chaperone BiP homolog and hsp-6 encodes a mitochondrial-specific chaperone, which was believed to be involved in the endoplasmic reticulum (UPRER) and mitochondrial unfolded protein response (UPRmt), respectively [17, 18].

As a major compound of the ambient air, particulate matter (PM) has become a widespread environmental concern for its health threat [19, 20]. Fine particulate matter (PM2.5, particles with a diameter of 2.5 μm or less) can not only reach parts of the respiratory tract but also penetrate deeply into the lung alveoli and enter the bloodstream due to their small size [21, 22]. Numerous studies have demonstrated that long- and short-term exposures to PM2.5 are related to respiratory disease and cardiovascular morbidity [22–24].

Due to the adverse health effects of PM2.5, scientists have done extensive researches on the molecular mechanisms underlying the toxicity of PM2.5, and oxidative stress was considered as one of the primary mechanisms implicated in PM2.5-mediated toxicity [25, 26]. Previous studies have proven that PM2.5 exposure could generate excessive ROS and thus decrease antioxidant enzyme activities, resulting in...
oxidative stress in cells [27, 28]. Besides, chronic inhalation exposure to PM$_{2.5}$ triggered two distinct UPR signaling pathways in mice [21, 29]. We speculate that UPR may coordinate proteostasis responses in regulating oxidative stress tolerance following PM$_{2.5}$ exposure. But the association between UPR and oxidative stress still remains unclear, and the causal explanation for this interaction may require further investigation.

In the present study, we found that PM$_{2.5}$ exposure induced oxidative stress processes, enhanced metabolic enzyme activity, activated UPR, and shortened the lifespan of C. elegans. Our results not only reveal the role of UPR in response to oxidative stress following PM$_{2.5}$ exposure and the relationship between oxidative stress and UPR activation but also provide important information for protection against the toxic effect of PM$_{2.5}$.

2. Materials and Methods

2.1. PM$_{2.5}$ Sampling and Concentration Analysis. PM$_{2.5}$ was collected by medium-volume TSP samplers with PM$_{10}$ and PM$_{2.5}$ separators (TH-150 C; Wuhan Tianhong Environmental Protection Industry Co., Ltd., China). Samples of PM$_{2.5}$ were collected in the campus of Bengbu Medical College in Anhui Province of China, a place far away from the highway or manufacturing district. Continuous sampling was performed for 24 hours at a flow rate of 100 L/min on quartz microfiber filters for a toxicity assay and PTFE (polytetrafluoroethylene) microfiber filters for the determination of elemental composition (90 mm; Wuhan Tianhong Environmental Protection Industry Co., Ltd., China). The sampling was carried out from December 2018 to January 2019. To eliminate any adsorbed organic compounds, the filters were pretreated at 60°C for 2 h before collection. The PM$_{2.5}$ samples were extracted by cutting filters into small pieces and immersing into deionized water, followed by sonication for 40 min. Then, the sample was freeze dried and stored at -20°C. The particles were weighed and resuspended with K buffer or deionized water before use.

The concentrations of 16 elements were detected by inductively coupled plasma atomic emission spectroscopy (ICP-MS, GE Co., Ltd., USA). The PAHs (polycyclic aromatic hydrocarbons) were extracted with dichloromethane and were analyzed using gas chromatography coupled to an atomic emission spectrometer (GC-MS, Agilent, CA, USA). The PAHs (polycyclic aromatic hydrocarbons) were extracted with dichloromethane.

2.2. Strains and Maintenance. Strains used in this study were gifts from the Caenorhabditis Genetics Center (CGC) and maintained on standard nematode growth medium (NGM) seeded with Escherichia coli OP$_{50}$ as described in reference [30]. The transgenic strains CF1553 (muIs84 [pAD76] sod-3p::GFP+rol-6::[sa1006]) and CL2166 (dvIs19 [pAF15[gst-4::GFP::NLS]]) were used to visualize the expressions of the oxidative stress-resistance-related proteins SOD-3 and GST-4 illustrating the inducible oxidative stress in PM$_{2.5}$-exposed worms [31]. Transgenic strains SJ4005 (zcsIs4 [hsp-4::GFP] V) and SJ4100 (zcsIs13 [hsp-6::GFP]) were used as the indicators for the unfolded protein response in the endoplasmic reticulum UPR (UPR$_{ER}$) and mitochondrial UPR (UPR$_{mt}$) [32], respectively.

2.3. Exposure Methods. For exposure, PM$_{2.5}$ was diluted into different concentrations with K buffer (53 mmol/L NaCl and 32 mmol/L KCl) and added into a 96-well plate; then, L4 larvae worms were transferred with a platinum picker. Worms were treated from L4 larvae for 24 hours at 20°C in a 96-well plate with OP$_{50}$ as food, with 30 worms per well and 4 parallel wells for one concentration. Synchronized L4 larvae were obtained by culturing the synchronized eggs at 20°C for 36 hours fed with OP$_{50}$. To obtain synchronized eggs, gravid nematodes were washed by K buffer followed by a bleaching mixture (0.45 mol/L NaOH, 2% HOCI) [33].

2.4. ROS Induction Assessment. For ROS induction assessment, worms were washed into the centrifugal tube and CM-H$_2$DCFDA (C6827, Invitrogen/Molecular Probes) was added to a final concentration of 1 μM followed by incubating at 20°C for 5 hours in the dark. After treatment with CM-H$_2$DCFDA, worms were mounted onto agar pads for ROS production detection [34]. The fluorescence signal was observed under a fluorescence microscope (Zeiss, Axio Observer Z1, Germany) (excitation wavelength: 480 nm; emission wavelength: 510 nm), and the intensities of the relative fluorescent units (RFU) in the intestine were measured and quantified by the ImageJ program (NIH, Bethesda, MD). Three individual repetitions for each condition were performed, and at least 20 nematodes were measured per replication.

2.5. Measurement of Oxidative Stress Markers. After exposure, worms were collected and washed with K buffer, then precooled lysis buffer (pH 7.4, 0.01 mol/L Tris-HCl, 0.0001 mol/L EDTA-2Na, 0.01 mol/L saccharose, and 0.8% NaCl) was added and the mixture was transferred to a glass homogenizer for homogenizing. After homogenizing, the mixture was transferred to 1.5 mL Eppendorf tubes and centrifuged at 3000 × g for 10 min, and the supernatant was collected for the use of LDH and MDA measurement. The Bradford method was used for protein concentration with bovine serum albumin (BSA) as the standard.

The intracellular lactate dehydrogenase (LDH) release was used to reflect cell membrane integrity [35, 36]. LDH release was measured with a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, China) and monitored using the Multiskan Ascent (BioTek Instruments, Inc., USA) with the absorbance at the wavelength of 490 nm and normalized by protein concentrations. The LDH leakage (% of control) was presented as the percentage of control, with K buffer as blank control.

Malondialdehyde (MDA) as a unique end-product of lipid peroxidation was usually used to represent the extent of lipid peroxidation reactions because lipid peroxidation often occurs while animals were injured [37]. MDA was measured using the MDA assay kit (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's
instructions. The absorbance of the supernatant was detected by a microplate reader (BioTek Instruments, Inc., USA) at 532 nm and normalized by protein concentrations.

2.6. The Activities of Antioxidant Enzymes. After exposure, worms were collected and washed with K buffer. After that, precooled lysis buffer was added and transferred to a glass homogenizer for homogenizing. Then, the homogenate was transferred to 1.5 mL. Eppendorf tubes and centrifugated at 3000 × g for 10 min, and the supernatant was used to evaluate the activities of the antioxidant enzymes catalase (CAT) and glutathione peroxidase (GSH-Px) within 24 hours. The Bradford method was used for protein concentration with bovine serum albumin (BSA) as the standard.

The activities of CAT and GSH-Px were evaluated with a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, China) and the absorbance was monitored using a spectrophotometer (BioTek Instruments, Inc., USA) at the wavelengths of 450 nm and 405 nm, respectively. The activities of CAT and GSH-Px were normalized by protein concentrations and presented as the percentage of control.

2.7. Lifespan Measurement. After being exposed to PM2.5 in the 96-well plate for 24 hours, worms were transferred to 3.5 cm NGM plates with the E. coli OP50 for lifespan measurement. Worms were transferred to fresh plates daily for the first 5 days and every 2 days after that. Survival was monitored every day and worms with no response to touches with a platinum wire and no pharyngeal pumping were defined dead [16]. Survival curves were plotted by the Kaplan-Meier method using SPSS 23.0 (IBM, USA), and mean life-spans were calculated for statistical analyses. Experiments were performed in triplicate and more than 100 worms were scored for each experiment analysis.

2.8. Quantitative RT-PCR. RNA was isolated using Trizol (Invitrogen, Carlsbad, CA) and extracted with chloroform, and cDNA was synthesized by reverse transcription with random primers on total RNA (Takara, Japan). The expression of hsp-4 and hsp-6 was measured using SYBR® Green by Quantitative RT-PCR (qRT-PCR) and normalized with tba-1 and act-1 [38]. Primers used for qRT-PCR were listed in Table s3 in the Supplementary Materials.

2.9. Statistical Analysis. Statistical evaluation was conducted with the SPSS 23.0 (IBM, USA). One-way ANOVA was used to compare the differences among different exposure conditions and P values less than 0.05 or 0.01 were considered to be statistically significant.

3. Results and Discussion

3.1. PM2.5 Induced Oxidative Stress in C. elegans. To explore the potential role of PM2.5 in oxidative stress, three oxidative stress markers (reactive oxygen species (ROS), lactate dehydrogenase (LDH), and malondialdehyde (MDA)) were used to assess oxidative stress in response to PM2.5 in C. elegans. As shown in Figure 1, signs of oxidative stress in C. elegans were observed when exposed to PM2.5 at the concentrations of 10 mg/L and 100 mg/L (Figures 1(a) and 1(b)). PM2.5 exposure induced more intestine ROS production, elevated LDH release, and MDA generation, and these inductions were proportional to the concentration of PM2.5 when the exposure concentrations were higher than 10 mg/L (Figure 1). These results suggested that PM2.5 was able to increase oxidative stress in C. elegans.

3.2. PM2.5 Altered the Activities of Antioxidant Enzymes in C. elegans. Under oxidative conditions, animals would activate some oxidative stress response enzymes to defend against oxidative stress and maintain the balance of the oxidative-antioxidant system. The transgenic nematodes CL2166 and CF1553 were treated as a transgenic reporter to monitor the inducible glutathione S-transferase (GST-4) and superoxide dismutase (SOD-3) expression. As shown in Figure 2, the expressions of GST-4 and SOD-3 were both significantly elevated with the increasing concentration of PM2.5 (Figure 2). The activities of antioxidant enzymes were also observed by determining the activities of glutathione peroxidase (GSH-Px) and catalase (CAT) levels. Results demonstrated that C. elegans enhanced the antioxidant defenses by increasing the activities of antioxidant enzymes, as the activities of GSH-Px and CAT were both evaluated in PM2.5-exposed C. elegans (Figure 3). As shown in Figure 3(a), the activities of GSH-Px were significantly enhanced in worms exposed with PM2.5 at 100 mg/L compared to the untreated ones (P < 0.01). In addition, the activities of CAT also increased after having been exposed to PM2.5 at 1 mg/L (P < 0.05), 10 mg/L, and 100 mg/L (P < 0.01). Taken all together, these data suggested that the expressions of antioxidant enzymes were induced or activities were enhanced to eliminate the excessive oxidation generated in PM2.5-exposed nematodes.

3.3. PM2.5 Activated Unfolded Protein Response in C. elegans. The unfolded protein response (UPR) induced by PM2.5 was detected using the following transgenic strains: SJ4005 (zcs14 [hsp-4::GFP]) for UPRER and SJ4100 (zcs13 [hsp-6::GFP]) for UPRER. As shown in Figure 4, after having been exposed to PM2.5 at different concentrations, the GFP fluorescence intensities in hsp-4::GFP and hsp-6::GFP were both enhanced (Figure 4), indicating that both ER and mitochondrial UPR were activated in PM2.5-exposed animals. The hsp-4 and hsp-6 mRNA expressions were significantly elevated in PM2.5-exposed animals compared with control (Figure s1). These findings suggested that PM2.5 could induce both UPRER and UPRER in C. elegans.

3.4. ROS Scavenger NAC Could Suppress the UPRER and UPRER Activated by PM2.5 through Reducing the Oxidative Stress in C. elegans. To investigate whether the UPRER and UPRER activated by PM2.5 was the consequence of oxidative stress, we then used ROS scavenger NAC to ameliorate the oxidative state in PM2.5-exposed worms. According to the results above, 100 mg/L was chosen for exposure concentration of PM2.5 and 2.5 mM NAC was added at the same time. Results indicated that less GFP fluorescence was induced in worms exposed to PM2.5 and NAC simultaneously than those merely exposed to PM2.5, but this was still slightly higher than in those under normal conditions (Figure 5),
indicating that the UPR\textsuperscript{mt} and UPR\textsuperscript{ER} induced by PM\textsubscript{2.5} was not merely through oxidative damage. Analysis of mRNA expression for hsp-4 and hsp-6 received the same results (Figure s1). These findings supported the hypothesis that the UPR induced by PM\textsubscript{2.5} may be the direct consequence of oxidative stress.

3.5. Fine Particle Matter Reduced the Lifespan of C. elegans.
As organisms are usually short-lived under an oxidative state [39], the lifespans of worms under normal and PM\textsubscript{2.5}-exposed conditions were tested. No significant influences on the median lifespan were observed in worms exposed to the low dose of 0.1 mg/L, but nematodes exposed to 10 mg/L and 100 mg/L PM\textsubscript{2.5} had significantly shorter lifespans compared with untreated worms (Figure 6), which was consistent with previous results of oxidative stress. These results indicated that PM\textsubscript{2.5} exposure reduced the lifespan of C. elegans.

3.6. Antioxidant NAC and UPR Inhibitor 4-PBA Could Attenuate the Lifespan Reduction Phenotype Caused by PM\textsubscript{2.5} in C. elegans.
Previous studies demonstrated that resistance to oxidative damage increased the life of C. elegans [39], so we reasoned that if nematodes exposed to PM\textsubscript{2.5} lived shorter lifespans because of the oxidative stress, then supplementation with antioxidant NAC could alleviate the short-lifespan phenotype in PM\textsubscript{2.5}-exposed worms. As expected, when worms were exposed to the antioxidant NAC (2.5 mM) and PM\textsubscript{2.5} (100 mg/L) simultaneously, the mean lifespans were significantly extended compared to those exposed only to PM\textsubscript{2.5} (Figure 7(a)), suggesting that the short-lifespan phenotype was a consequence of increased oxidative stress induced by PM\textsubscript{2.5}. Similar phenomena were found when 5 mM 4-phenylbutyric acid was used to attenuate the UPR response in PM\textsubscript{2.5}-exposed nematodes (Figure 7(b)), which means that the short-lifespan phenotype was correlated with UPR response induced by PM\textsubscript{2.5}. As the NAC has a slight effect of extending the lifespan of worms.

![Figure 1: Signs of oxidative stress in C. elegans induced by PM\textsubscript{2.5}. (a) Image shows the fluorescence in the intestine of C. elegans. (b) Comparison of the reactive oxygen species (ROS) induced by PM\textsubscript{2.5}. (c) Effects of PM\textsubscript{2.5} exposure on the production of malondialdehyde (MDA). (d) Effects of PM\textsubscript{2.5} exposure on lactate dehydrogenase (LDH) release. The error bars represent the standard deviation of measurements. *P < 0.05 vs. control; **P < 0.01 vs. control.](image-url)
Figure 2: PM$_{2.5}$ treatment enhanced the activity of the antioxidant enzyme in C. elegans. (a) Image of the expression of gst-4P::GFP in CL2166 worms and quantification of GFP fluorescence. (b) Image of the expression of sod-3P::GFP in CF1553 worms and quantification of GFP fluorescence. The error bars represent the standard deviation of measurements. $^*$ $P < 0.05$ vs. control; $^{**} P < 0.01$ vs. control.

Figure 3: PM$_{2.5}$ treatment increased the activities of GSH-Px (a) and CAT (b) levels in C. elegans. The error bars represent the standard deviation of measurements. $^*$ $P < 0.05$ vs. control; $^{**} P < 0.01$ vs. control.
and 4-PBA has no effects on the lifespan (Figure 7), these results thus indicate that lifespan reduction in PM$_{2.5}$-exposed nematodes was mediated by oxidative stress and UPR.

4. Conclusion

Oxidative stress acts as a response to the unfriendly environment and numerous toxicants in most organisms. In this study, our results were consistent with this viewpoint as ROS was produced in a dose-dependent manner in PM$_{2.5}$-exposed worms, and LDH and MDA also increased by the same way (Figure 1). Organisms also have a defense mechanism against the adverse environment, and the detoxification response to xenobiotics was the vital one. SOD, CAT, GSH-Px, and glutathione S-transferase are important antioxidants that diminish oxidative stress through destroying the superoxide radical (O$_2^-$) or detoxifying H$_2$O$_2$ [40]. These antioxidative enzymes usually were induced in response to oxidative stress when organisms were under unfriendly conditions. In *C. elegans*, SOD-3, CAT, GSH-Px, and GST-4 act as antioxidative enzymes in diminishing the overproduced oxidative stress. The analytic results of the transgenic strains containing SOD-3::GFP and GST-4::GFP showed that SOD-3 and GST-3 were induced after exposure to PM$_{2.5}$ (Figure 2) and the activities of CAT and GSH-Px were also increased after PM$_{2.5}$ exposure, suggesting more antioxidants were induced to eliminate oxidative damage in *C. elegans* under PM$_{2.5}$-exposed conditions. But this result does not agree with the study *in vitro*, as the LDH release is an indicator of the loss of cell membrane integrity, and an increase of LDH release usually leads to cell lysis and cell death [27, 36]; however, we still found enhanced CAT and GSH-Px activities in worms as LDH increased (Figure 3). We speculate that this may be due to organisms having a complex antioxidant defensive system in responding to oxidative stress. When the body deals with oxidative damage, the organism

---

**Figure 4:** PM$_{2.5}$ activated unfolded protein response in *C. elegans*. (a) Image of the expression of *hsp-6::GFP* in SJ4100 (zcIs13 [hsp-6::GFP]) worms and quantification of GFP fluorescence. (b) Image of the expression of *hsp-4::GFP* in SJ4005 (zcIs4 [hsp-4::GFP] V) worms and quantification of GFP fluorescence. The error bars represent the standard deviation of measurements. *P < 0.05 vs. control; **P < 0.01 vs. control.
attempted to clean excess free radicals through increasing the activities of antioxidants in order to maintain a new balance of oxidation and antioxidation, hence the LDH and antioxidants were all increasing in PM2.5-treated worms. The responses to oxidative stress were inconsistent between cells and worms—maybe worms respond to stress as a whole with complex mechanisms, but the cells just act as an individual.
Oxidants can be generated and restored in the ER and mitochondria, which may lead to the accumulation of unfolded/misfolded proteins and vice versa [5]. Signaling pathways that resolve unfolded/misfolded proteins are called unfolded protein response (UPR) [11]. Based on this theory, we assume that PM2.5 may induce UPR in the ER and mitochondria. Transgenic strains SJ4005 (zcls4 [hsp-4::GFP] V) and SJ4100 (zcls13 [hsp-6::GFP]) were used as indicators for the unfolded protein response in the ER (UPR ER) and mitochondria (UPR mt). Our results showed that PM 2.5 could induce the expression of hsp-4::GFP and hsp-6::GFP indicating that UPR ER and UPR mt were activated by PM 2.5. In order to clarify whether UPR ER and UPR mt alleviate with the decrease of oxidative stress level, antioxidant NAC [41] was used to reduce oxidants induced by PM2.5. When worms were exposed to PM2.5 and NAC simultaneously, the fluorescence intensity in SJ4005 (zcls4 [hsp-4::GFP] V) and SJ4100 (zcls13 [hsp-6::GFP]) were both decreased compared to those in PM2.5-exposed ones (Figure 4), which suggest that the UPR ER and UPR mt activated by PM2.5 were associated with oxidative stress.

In biota, the rate of aging correlates with environmental factors and the lifespan usually declines under various stressors [42, 43]. C. elegans was a fit animal model for many stress analyses as its lifespan was short [43, 44]. In previous studies, environmental stresses including temperature, quality of nutrients, and toxicants could influence the lifespan of C. elegans [45, 46]. The same results were found with traffic-related fine particulate matter: both acute and prolonged exposure to PM2.5 could decline the lifespan and mean lifespan of C. elegans [16]. As for airborne PM2.5, we have found a similar tendency in the decline of lifespan, since worms lived a much shorter lifespan under PM2.5-exposed conditions (Figure 5). This is in accordance with the results showing that elevated total mortality and morbidity were associated with long-term exposure against PM [47]. As oxidative stress and ER stress are found associated with lifespan in C. elegans [39, 48], we next want to find out the interaction between the lifespan and oxidative stress or UPR, using antioxidant NAC and UPR inhibitor 4-PBA to remove oxidants or reduce ER stress at the same time worms exposed to PM2.5. The lifespan analysis demonstrated that NAC has a slight effect of extending the lifespan of worms, and 4-PBA has no significant effects on the lifespan (Figure 7); however, both NAC and 4-PBA could rescue the shortened lifespan when cotreated with PM1.5, indicating that the removal of oxidants and ER stress reduction could extend the lifespan of C. elegans and the shortening...
of life caused by PM$_{2.5}$ was closely associated with oxidative stress and ER stress.

Taken together, our experiments demonstrated that fine Particle Matter (PM$_{2.5}$) in the air pollution declines the lifespan of *C. elegans* through triggering UPR and elevated oxidative stress. NAC and UPR inhibitor 4-PBA could recover the lifespan shortened by PM$_{2.5}$ through alleviating UPR and reducing the oxidative stress.

**Data Availability**

All data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (21707002), the Excellent Young Talents Fund Program of Higher Education Institutions of Anhui Province (gxyqZD2016162), the Natural Science Foundation for Colleges and Universities of Anhui Province (KJ2017A227 and KJ2018A1021), and the Natural Science Foundation of Anhui Province (1908085MH257).

**Supplementary Materials**

Supplementary Table s1: metals detected in PM$_{2.5}$ samples. The data are displayed as the mean ± SEM. Supplementary Table s2: organic compositions detected in PM$_{2.5}$ samples. The data are displayed as the mean ± SEM. Supplementary Table s3: the primers used in this study. These primers were used for the verification of the expression of hsp-4 and hsp-6. Figure s1: analysis of mRNA expression for hsp-4 and hsp-6. (Supplementary Materials)

**References**

[1] H. Sies, “Oxidative stress: a concept in redox biology and medicine,” *Redox Biology*, vol. 4, pp. 180–183, 2015.

[2] A. Kupsco and D. Schlenk, “Oxidative stress, unfolded protein response, and apoptosis in developmental toxicity,” *International Review of Cell and Molecular Biology*, vol. 317, pp. 1–66, 2015.

[3] G. Pizzino, N. Irrera, M. Cucinotta et al., “Oxidative stress: harms and benefits for human health,” *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 8416763, 13 pages, 2017.

[4] J. Yin, R. Liu, Z. Jian et al., “Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in *Caenorhabditis elegans*,” *Ecotoxicology and Environmental Safety*, vol. 163, article S0147651318306626, pp. 298–306, 2018.

[5] S. Soberanes, D. Urich, C. M. Baker et al., “Mitochondrial complex III-generated oxidants activate ASK1 and JNK to induce alveolar epithelial cell death following exposure to particulate matter air pollution,” *Journal of Biological Chemistry*, vol. 284, no. 4, pp. 2176–2186, 2009.

[6] D. Dubey, A. K. Srivastav, J. Singh et al., “Photoexcited triclosan induced DNA damage and oxidative stress via p38 MAP kinase signaling involving type I radicals under sunlight/UVB exposure,” *Ecotoxicology and Environmental Safety*, vol. 174, pp. 270–282, 2019.

[7] R. Ushioda and K. Nagata, “Redox-mediated regulatory mechanisms of endoplasmic reticulum homeostasis,” *Cold Spring Harbor Perspectives in Biology*, vol. 11, no. 5, article a033910, 2019.

[8] M. Xu, X. Bi, X. He, X. Yu, M. Zhao, and W. Zang, “Inhibition of the mitochondrial unfolded protein response by acetylcholine alleviated hypoxia/reoxygenation-induced apoptosis of endothelial cells,” *Cell Cycle*, vol. 15, no. 10, pp. 1331–1343, 2016.

[9] F. R. M. Laurindo, T. L. S. Araujo, and T. B. Abrahão, “Nox NADPH oxidases and the endoplasmic reticulum,” *Antioxidants & Redox Signaling*, vol. 20, no. 17, pp. 2755–2775, 2014.

[10] P. Walter and D. Ron, “The unfolded protein response: from stress pathway to homeostatic regulation,” *Science*, vol. 334, no. 6059, pp. 1081–1086, 2011.

[11] X. Shen, R. E. Ellis, K. Lee et al., “Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development,” *Cell*, vol. 107, no. 7, pp. 893–903, 2001.

[12] O. V. Tsyusko, J. M. Unrine, D. Spurgeon et al., “Toxicogenomic responses of the model organism *Caenorhabditis elegans* to gold nanoparticles,” *Environmental Science & Technology*, vol. 46, no. 7, pp. 4115–4124, 2012.

[13] R. Chen, L. Huo, X. Shi et al., “Endoplasmic reticulum stress induced by zinc oxide nanoparticles is an earlier biomarker for nanotoxicological evaluation,” *ACS Nano*, vol. 8, no. 3, pp. 2562–2574, 2014.

[14] M. Shenkman, S. Tolchinsky, and G. Z. Lederkremer, “ER stress induces alternative nonproteasomal degradation of ER proteins but not of cytosolic ones,” *Cell Stress & Chaperones*, vol. 12, no. 4, pp. 373–383, 2007.

[15] Y. Zhao, Q. Wu, Y. Li, and D. Wang, “Translocation, transfer, and *in vivo* safety evaluation of engineered nanomaterials in the non-mammalian alternative toxicity assay model of nematode *Caenorhabditis elegans,*” *RSC Advances*, vol. 3, no. 17, pp. 5741–5757, 2013.

[16] Y. Zhao, Z. Lin, R. Jia, G. Li, Z. Xi, and D. Wang, “Transgenerational effects of traffic-related fine particulate matter PM$_{2.5}$ on nematode *Caenorhabditis elegans,*” *Journal of Hazardous Materials*, vol. 274, pp. 106–114, 2014.

[17] B. Ient, R. Edwards, R. Mould, M. Hannah, L. Holden-Dye, and V. O’Connor, “HSP-4 endoplasmic reticulum (ER) stress pathway is not activated in a *C. elegans* model of ethanol intoxication and withdrawal,” *Invertebrate Neuroscience*, vol. 12, no. 2, pp. 93–102, 2012.

[18] C. F. Bennett, H. Vander Wende, M. Simko et al., “Activation of the mitochondrial unfolded protein response does not predict longevity in *Caenorhabditis elegans,*” *Nature Communications*, vol. 5, no. 1, 2014.

[19] N. L. Mills, K. Donaldson, P. W. Hadoke et al., “Adverse cardiovascular effects of air pollution,” *Nature Clinical Practice Cardiovascular Medicine*, vol. 6, no. 1, pp. 36–44, 2009.

[20] C. Wang, Y. Tu, Z. Yu, and R. Lu, “PM2.5 and cardiovascular diseases in the elderly: an overview,” *International Journal of...*
E. Eruslanov and S. Kusmartsev, “Identification of ROS using oxidized DCFDA and flow-cytometry,” Methods in Molecular Biology, vol. 594, pp. 57–72, 2010.

R. Mendez, Z. Zheng, Z. Fan, S. Rajagopalan, Q. Sun, and K. Zhang, “Exposure to fine airborne particulate matter induces macrophage infiltration, unfolded protein response, and lipid deposition in white adipose tissue,” American Journal of Translational Research, vol. 5, no. 2, pp. 224–234, 2013.

M. Kampa and E. Castanas, “Human health effects of air pollution,” Environmental Pollution, vol. 151, no. 2, pp. 362–367, 2008.

L. Dai, P. Koutrakis, B. A. Coull, D. Sparrow, P. S. Vokonas, and J. D. Schwartz, “Use of the adaptive LASSO method to identify PM2.5 components associated with blood pressure in elderly men: the Veterans Affairs Normative Aging Study,” Environmental Health Perspectives, vol. 124, no. 1, pp. 120–125, 2016.

Y. Li, T. Shimizu, Y. Hirata et al., “PM2.5-induced oxidative stress triggers autophagy in human lung epithelial A549 cells,” Toxicology in Vitro, vol. 27, no. 6, pp. 1762–1770, 2013.

S. Hussain, S. Boland, A. Baeza-Squiban et al., “Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: role of particle surface area and internalized amount,” Toxicology, vol. 260, no. 1-3, pp. 142–149, 2009.

S. Laing, G. Wang, T. Briazova et al., “Airborne particulate matter selectively activates endoplasmic reticulum stress response in the lung and liver tissues,” American Journal of Physiology-Cell Physiology, vol. 299, no. 4, pp. C736–C749, 2010.

X. Ding, M. Wang, H. Chu et al., “Global gene expression profiling of human bronchial epithelial cells exposed to airborne fine particulate matter collected from Wuhan, China,” Toxicology Letters, vol. 228, no. 1, pp. 31–38, 2014.

M. Rodriguez, L. B. Snoek, J. A. Riksen, R. P. Bevers, and J. E. Kammenga, “Genetic variation for stress-response hormesis in Caenorhabditis elegans,” Experimental Gerontology, vol. 47, no. 8, pp. 581–587, 2012.

J. N. Sampayo, N. L. Jenkins, and G. J. Lithgow, “Use of the adaptive LASSO method to identify stress and longevity gene regulation by adapted stress and longevity gene regulation by Caenorhabditis elegans,” Integrative Biology, vol. 6, no. 1, pp. 35–43, 2014.

H. K. DeBardeleben, L. E. Lopes, M. P. Nessel, and D. M. Raizen, “Stress-induced sleep after exposure to ultraviolet light is promoted by p53 in Caenorhabditis elegans,” Genetics, vol. 207, no. 2, p. genetics.300070.2017, 2017.

Y. Zhao, Q. Wu, and D. Wang, “An epigenetic signal encoded protection mechanism is activated by graphene oxide to inhibit its induced reproductive toxicity in Caenorhabditis elegans,” Biomaterials, vol. 79, pp. 15–24, 2016.

E. Eruslanov and S. Kusmartsev, “Identification of ROS using oxidized DCFDA and flow-cytometry,” Methods in Molecular Biology, vol. 594, pp. 57–72, 2010.

M. S. Zaqout, T. Sumizawa, H. Igisu, D. Wilson, T. Myojo, and S. Ueno, “Binding of titanium dioxide nanoparticles to lactate dehydrogenase,” Environmental Health and Preventive Medicine, vol. 17, no. 4, pp. 341–345, 2012.

L. DiPeso, D. X. Ji, R. E. Vance, and J. V. Price, “Cell death and cell lysis are separable events during pyroptosis,” Cell Death Discovery, vol. 3, no. 1, 2017.

F. Li, Z. Liang, X. Zheng, W. Zhao, M. Wu, and Z. Wang, “Toxicity of nano-TiO2 on algae and the site of reactive oxygen species production,” Aquatic Toxicology, vol. 158, pp. 1–13, 2015.

X. Li, O. Matilainen, C. Jin, K. M. Glover-Cutter, C. I. Holmberg, and T. K. Blackwell, “Specific SKN-1/Nrf stress responses to perturbations in translation elongation and proteasome activity,” PLoS Genetics, vol. 7, no. 6, article e1002119, 2011.

M. Abdollahi, M. Y. Moridani, O. I. Aruoma, and S. Mostafalou, “Oxidative stress in aging,” Oxidative Medicine and Cellular Longevity, vol. 2014, Article ID 876834, 2 pages, 2014.

R. P. Oliveira, J. Porter Abate, K. Dilks et al., “Condition-adapted stress and longevity gene regulation by Caenorhabditis elegans SKN-1/Nrf,” Aging Cell, vol. 8, no. 5, pp. 524–541, 2009.

K. N. Prasad and S. C. Bondy, “Evaluation of role of oxidative stress on aging in Caenorhabditis elegans: a brief review,” Current Aging Science, vol. 6, no. 3, pp. 215–219, 2013.

M. Rodriguez, L. B. Snoek, J. A. Riksen, R. P. Bevers, and J. E. Kammenga, “Genetic variation for stress-response hormesis in C. elegans lifespan,” Experimental Gerontology, vol. 47, no. 8, pp. 581–587, 2012.

D. Chen, P. Li, B. A. Goldstein et al., “Germline signaling mediates the synergistically prolonged longevity produced by double mutations indaf-2 and rks-1 in C. elegans,” Cell Reports, vol. 5, no. 6, pp. 1600–1610, 2013.

J. N. Sampayo, N. L. Jenkins, and G. J. Lithgow, “Using stress resistance to isolate novel longevity mutations in Caenorhabditis elegans,” Annals of the New York Academy of Sciences, vol. 908, pp. 324–326, 2000.

A. D. Kapos, P. Bruckmann, T. Eikmann et al., “Health effects of particles in ambient air,” International Journal of Hygiene and Environmental Health, vol. 207, no. 4, pp. 399–407, 2004.

D. J. Cattie, C. E. Richardson, K. C. Reddy et al., “Mutations in nonessential elf3k and elf3l genes confer lifespan extension and enhanced resistance to ER stress in Caenorhabditis elegans,” PLoS Genetics, vol. 12, no. 9, article e1006326, 2016.

K. Zhang, “Mutations in N-acetylcysteine and vitamin E rescue animal longevity and cellular oxidative stress in preclinical models of mitochondrial complex I disease,” Molecular Genetics and Metabolism, vol. 123, no. 4, pp. 449–462, 2018.

J. N. Sampayo, N. L. Jenkins, and G. J. Lithgow, “Using stress resistance to isolate novel longevity mutations in Caenorhabditis elegans,” Annals of the New York Academy of Sciences, vol. 908, pp. 324–326, 2000.

A. D. Kapos, P. Bruckmann, T. Eikmann et al., “Health effects of particles in ambient air,” International Journal of Hygiene and Environmental Health, vol. 207, no. 4, pp. 399–407, 2004.

D. J. Cattie, C. E. Richardson, K. C. Reddy et al., “Mutations in nonessential elf3k and elf3l genes confer lifespan extension and enhanced resistance to ER stress in Caenorhabditis elegans,” PLoS Genetics, vol. 12, no. 9, article e1006326, 2016.