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Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1 Alpha Play a Role in PM2.5 and Hypoxia-Induced Simultaneously Oxidative Damage in Human Bronchial Epithelial Cells

El Coactivador Gamma-1 Alfa del Receptor Activado por Proliferador de Peroxisomas Desempeña un Papel en PM2.5 y en el Daño Oxidativo Simultáneo Inducido por Hipoxia en Células

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SUMMARY: Peroxisome proliferator activated receptor γ co-activator 1α (PGC-1α) plays an important role in antioxidative stress. The aim of this study is to investigate the role of PGC-1α in the oxidative damage of BEAS-2B cells exposed to PM2.5 and hypoxia simultaneously and in the anti-oxidative effect of N-acetyl-L-cysteine (NAC). The PGC-1α mRNA level in human bronchial epithelial BEAS-2B cells exposed to PM2.5, hypoxia and PM2.5 combined with hypoxia were evaluated. The cells were treated for 12 h in the presence or absence of PM2.5 combined with hypoxia and in the presence or absence of NAC. The cell viability after intervention with NAC was assessed. PGC-1α was knocked down by siRNA to investigate the protective mechanism of NAC. The expression levels of PGC-1α and PPARγ were determined at both the mRNA and protein levels. The expression of NF-κBp65, malondialdehyde (MDA) and superoxide dismutase (SOD) were detected at the protein level. PM2.5 combined with hypoxia reduced the expression of PGC-1α and PPAR-γ ($P<0.01$), increased the protein levels of NF-κBp65 ($P<0.01$) and MDA($P<0.05$), and decreased the SOD activity ($P<0.05$) compared to the normal control group, and NAC ameliorated the damage($P < 0.05$). PGC-1α siRNA blocked the protective effect of NAC. PM2.5 combined with hypoxia can cause oxidative damage to BEAS-2B cells, and PGC-1α may play an important role in the anti-oxidative effect of NAC. The results of this study suggest for the first time that PGC-1α might play an important role in regulation of oxidative damage induced by PM2l.5 and hypoxia simultaneously in human bronchial epithelial cells, which provides potential ideas for the treatment of PM2.5-induced respiratory diseases.

KEY WORDS: PM2.5; Hypoxia; Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; N-acetyl-L-cysteine; Oxidative damage.

INTRODUCTION

Human bronchial epithelial cells are a type of epithelial cell that lines the airways of the lung, specifically the bronchi (Fig. 1). They play a crucial role in the respiratory system, serving as a barrier and participating in various physiological and immune functions. Oxidative damage in human bronchial epithelial cells is a significant concern, particularly in the context of respiratory diseases such as chronic obstructive pulmonary disease, asthma, and lung cancer. This damage is primarily caused by reactive oxygen species that can alter cellular components and functions. Similarly, hypoxia, a condition characterized by reduced oxygen availability, can induce oxidative damage in human bronchial epithelial cells. This paradoxical phenomenon occurs because hypoxia can enhance the production of reactive oxygen species, leading to cellular damage despite the low oxygen environment (Fig. 2).

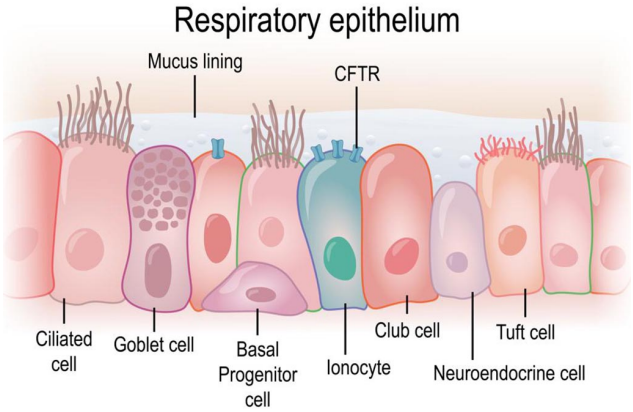

Fig. 1. Schematic view of cells of the respiratory epithelium. (CFTR: Cystic fibrosis transmembrane conductance regulator).

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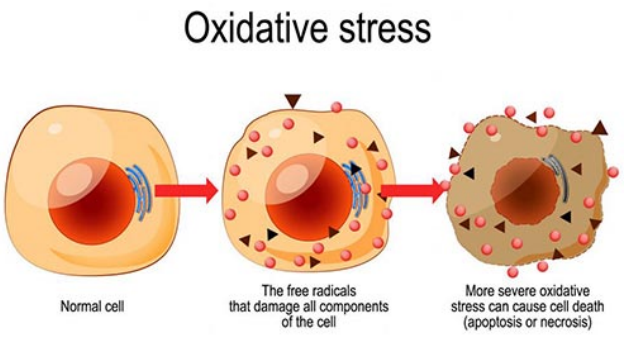

Fig. 2. Schematic view of oxidative stress formation in the respiratory cell.

Fine particulate matter with aerodynamic diameters of 2.5 μm or less (PM2.5) is a complex containing inorganic, organic, and biological components that can be easily inhaled into the airway and deposit in the alveoli, which may cause deterioration of lung structure and function (Yen *et al*., 2022). Epidemiologic studies have demonstrated that PM2.5 exposure is closely related to the morbidity and mortality of various respiratory diseases, such as chronic bronchitis, asthma, and lung cancer (Vinikoor-Imler *et al*., 2011; Tsai *et al*., 2014; Fang *et al*., 2016; Gonçalves *et al*., 2023). Inflammation- and oxidative stress-triggered immune dysfunction (Oh *et al*., 2011), DNA damage (Jin *et al*., 2023), and calcium homeostasis disequilibrium (Li *et al*., 2015) are the main mechanisms underlying PM2.5-induced pulmonary damage. Accumulative evidence indicates that increased PM2.5 exposure is associated with decreased oxygen saturation (hypoxemia), especially in elderly patients with chronic obstructive pulmonary disease (DeMeo *et al*., 2004; Gong Jr. *et al*., 2005; Luttmann-Gibson *et al*., 2014), suggesting that lung tissues are simultaneously exposed to PM2.5 and hypoxia. Thus, it is logical and indispensable to establish a model exposed to PM2.5 and hypoxia in combination to explore the underlying mechanism of PM2.5-induced pulmonary damage. Previous studies have shown that, when investigated separately, both PM2.5 and hypoxia may induce oxidative stress in alveolar epithelial cells (Faiss *et al*., 2013; Liu *et al*., 2023), suggesting that targeting oxidative damage might be a potential therapeutic strategy against PM2.5- or hypoxia-induced lung injury. However, the oxidative damage in airway epithelial cells in simultaneous response to PM2.5 and hypoxia remains unknown.

Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) plays an important role in the regulation of mitochondrial biosynthesis and oxidative metabolism by transcriptionally upregulating the expression of important antioxidative genes (St-Pierre *et al*., 2006; Kong *et al*., 2010; Lu *et al*., 2010; Huang *et al*., 2017), such as sirtuin 3, manganese superoxide dismutase (SOD2), and peroxisomal membrane protein 70 kDa, via interactions with a broad range of transcription factors including peroxisome proliferator-activated receptors (PPARs), nuclear factor-kappa B (NF-κB), estrogen related receptor a, and nuclear respiratory factor-1 (Ye *et al*., 2016a), which in turn reduces intracellular oxidative stress. In a hypoxia-induced pulmonary hypertension rat model, PGC-1α expression has been found to be suppressed in vascular endothelial cells, leading to enhancement of oxidative stress. Restoration of PGC-1α expression reduces the formation of reactive oxygen species (ROS) (Ye *et al*., 2016b), suggesting antioxidant properties of PGC-1α. However, whether PGC-1α contributes to PM2.5 and hypoxia-induced simultaneously oxidative damage is poorly understood.

In the present study, we hypothesized that PGC-1α may play a regulatory role in oxidative damage induced simultaneously by PM2.5 and hypoxia in human bronchial epithelial BEAS-2B cells. The alterations in expression of important genes in the PGC-1α signaling and the oxidative damage status were examined in BEAS-2B cells exposed to PM2.5 and hypoxia with an intervention with N-acetyl-L-cysteine (NAC), which has been found to have antioxidant effect (Lai *et al*., 2017). A loss-of-function assay using small interfering RNA of PGC-1α (siPGC-1α) was carried out to explore the role of PGC-1α in NAC-rendered protection against PM2.5 and hypoxia-induced simultaneously oxidative damage in BEAS-2B cells.

MATERIAL AND METHOD

Cell culture. Human Bronchial Epithelioid cells BEAS-2B cells (CRL-9609) authenticated by STR were purchased from the American Type Culture Collection (Manassas, VA, USA) and maintained in bronchial epithelial cell medium BEpiCM (3211, Sciencell, California, USA) with 10 % FBS (P30-3302, PAN-Biotech, Germany) in a humidified atmosphere of 5 % CO_2 at 37 °C. When cells reach 80 % confluency, they were passaged using TrypLE Express (12604013, Life Technologies, California, USA) and were seeded on fibronectin-coated flasks or plates (JET BIOFIL, Guangzhou, China). Mycoplasma testing showed negative.We have communicated with our ethics committee and have been exempted.

Preparation of PM2.5 sample. PM2.5 particles were collected as previously described (Zhao *et al*., 2012). Briefly, a medium volume air sampler (Qingdao Laoshan Institute of Applied Technology, Qingdao, China) was placed on the top of a 6-storey building located in the center of Shijiazhuang, China. A glass fiber filter (Ji LuTaiShan Technology, Shandong, China) with a diameter of 80 mm

was used for PM2.5 collection and continuous sampling was conducted 23.5 h per day for 30 days in August and September 2016, except for the rainy days. A total of 19 samples were obtained. The filter containing PM2.5 was wrapped with aluminum foil and stored at -20 °C until use. For PM2.5 extraction, the filter membrane was cut into small pieces (1 cm × 1 cm) and placed in sterile water, followed by ultrasonic vibration for 20 min × 3 times. After being filtered with 6 layers of sterile gauze, the filtrate was collected, dried, and weighed. PM2.5 stock solution (4 mg/ml) was prepared with sterile saline and stored at -20 °C until use.

PM2.5 and hypoxia exposure. The cells were seeded onto 6-well plates at 5×10^5 cells/well and cultured in a normoxic incubator for 30 h, 36 h, and 48 h as the normal control groups. After culturing in a normoxic incubator for 24 h, the cells were treated with 25, 50 and 100 mg/mL PM2.5 and cultured in a normoxic incubator (37 , 21 % O_2, 5 % CO_2) for 6 h, 12 h and 24 h, respectively, as the PM2.5 intervention groups. The cells unexposed to PM2.5 were cultured in a hypoxic incubator (37 °C, 3 % O_2, and 5 % CO_2) for 6 h, 12 h, and 24 h, as the hypoxia intervention groups. The cells exposed to 50 μg/mL PM2.5 were cultured in a hypoxic incubator for 6 h, 12 h, and 24 h as the PM2.5+hypoxia groups. In each group, there were three samples and texted in triplicate.

Cell counting kit-8 (CCK-8) viability assay. NAC powder (H20057334) purchased from Zhejiang Jinhua Kangenbei Biological Pharmaceutical Co. LTD, Zhejiang, China was dissolved in sterile water followed by filter sterilization to prepare a 60 mg/ml stock solution. BEAS-2B cells were seeded in 24-well plates at a density of 1×10^5 cells/well and allowed to adhere under a normoxic condition for 24 h. Wells without cells were used as blank control. NAC solution was added into each well at a final concentration of 0, 1, 5, 10, 20, or 30 μg/ml. The experiment was performed in triplicate for each concentration. The medium was refreshed at 12 h post-treatment and cells were incubated with 50 μl of CCK8 solution (Beyotime Biotechnology, Shanghai, China) in each well at 37 °C for 4 h. The absorbance at a wavelength of 450 nm was measured using a microplate reader (BioTek Instruments, Vermont, USA).

siRNA transfection and intervention group. BEAS-2B cells were seeded in 6-well plates at 5×10^5 cells/well, cultured in a normoxic incubator for 24 h (cells confluence 80 %-90 % of the plate), and then transfected 24 h with Lipofectamine 3000 transfection reagent (L3000150, Invitrogen, USA) with 50 pmol of *PGC-1α siRNA* (target sequence: GACGGATTGCCCTCATTTG; Sense (5'-3'): GACGGAUUGCCCUCAUUUG dTdT; Antisense (5'-3'): CAAAUGAGGGCAAUCCGUC dTdT) or negative *control siRNA*(target sequence: TTCTCCGAACGTGTCACGT; Sense (5'-3'): UUCUCCGAACGUGUCACGU dTdT; Antisense (5'-3'): ACGUGACACGUUCGGAGAA dTdT) (RiboBio, Guangzhou, China) per well following the manufacturer's instruction. Cells transfected 24 h with 50 pmol of *CONTROL siRNA* were used as control. After culturing in a normoxic incubator for 35 h, the BEAS-2B cells or cells transfected with *PGC-1α siRNA*, *CONTROL siRNA* were treated with 5 μg/ml NAC solution to continuously culture for 1 h, then exposed to 50 μg/mL PM2.5 and cultured in a hypoxic incubator for 12 h as PM2.5+hypoxia+NAC groups, PM2.5+hypoxia+*PGC-1α siRNA*+NAC groups and PM2.5+hypoxia+*CONTROL siRNA*+NAC groups.

Quantitative real-time PCR (qRT-PCR). Total RNA was isolated from BEAS-2B cells using TRIzol reagent (Invitrogen, California, USA) and reversely transcribed for cDNA synthesis using a reverse transcription kit (Promega Corporation, Wisconsin, USA) following the manufacturer's instruction. qRT-PCR was performed using a PCR kit (Promega) and primers (Table I) on a 7500 Real Time PCR instrument (Applied Biosystems, California, USA). GAPDH was used as an housekeeping gene. The relative mRNA expression was quantified using the 2^{-DDCt} method.

Table I. Primer sequence for all genes.

Gene	Primer sequence (5'-3')
GAPDH	F: CAA TGA CCC CTT CAT TGA CC
	R: TTG ATT TTG GAG GGA TCT CG
PGC-1α	F: GTC ACC ACC CAA ATC CTT AT
	R: ATC TAC TGC CTG GAG ACC TT
PPARγ	F: GGC TTC ATG ACA AGG GAG TTT C
	R: AAC TCA AAC TTG GGC TCC ATA AAG

Western blot assay. BEAS-2B cells were lysed with RIPA buffer and the cell lysates were collected after the centrifugation. Protein samples were quantified using BCA reagent (Generay Biotech, Shanghai, China). After denaturation, the proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5 % nonfat dry milk for 2 h and then incubated with the primary antibody against PGC-1α (Abcam ab191838), PPARγ (Abcam ab209350), NF-κB p65 (Proteintech 10745-1-AP) or β-actin (Cell Signaling Technology 4970) at 4 °C overnight. The dilution ratio of primary antibody is 1:1000. After being washed with tris-buffered saline (TBS) for 5 min × 3 times, the membranes were incubated with horseradish peroxidase-conjugated secondary goat anti-rabbit IgG (Bio-Swamp PAB160011)

at room temperature for 1 h. The dilution ratio of secondary antibody is 1:10000. After additional 3 washes with TBS, The chemiluminescent signals were detected using the Odyssey infrared imaging system (LI-COR, Nebraska, USA) and quantified using Image Studio Ver 4.0 Software (LI-COR, Nebraska, USA). These indicators in the medium supernatant or cell pellets are not measured.

Enzyme-linked immunosorbent assay (ELISA). Post-lysate supernatant of cells in different conditions were collected. The SOD activity and malondialdehyde (MDA) level were determined using ELISA kits for SOD activity (SOD-1-Y, Comin Biotechnology, Suzhou, China) and MDA content (MDA-1-Y, Comin Biotechnology, Suzhou, China), respectively, following the manufacturer's instructions. SOD activity was detected at 560 nm by ELISA instrument (Bio Tek Synergy H1, USA) . MDA contents were detected at 532 nm and 600 nm by ELISA instrument (Bio Tek Synergy H1, USA) and calculated by difference value.

Statistical analysis. All experiments were independently repeated at least three times. The results were expressed as the mean ± standard deviation. Statistical analyses were carried out using SPSS 21.0 software (SPSS, Chicago, IL, USA). Comparison among multiple groups was performed using two-way analysis of variance (ANOVA) with Tukey's test. Comparison between two samples was conducted using *t* test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of PM2.5/hypoxia on PGC-1α transcription in BEAS-2B cells. In order to identify the optimal dosage and timing for PM2.5/hypoxia treatment to induce the suppression of PGC-1α expression in BEAS-2B cells, we examined the time-course and dose-response effects of PM2.5 on *PGC-1α mRNA* expression under normoxic condition. As shown in Figure 3A, 12- or 24-h treatment with PM2.5 had no significant dose-response effect on *PGC-1α mRNA* expression, as 6-h treatment (P<0.01 when 25 μg/ml/6-h PM2.5 *vs*. 100 μg/ml/6-h PM2.5 , 50 μg/ml/6-h PM2.5 *vs*. 100 μg/ml/6-h PM2.5), which suggests that *PGC-1α mRNA* expression was inhibited to the greatest degree as early as 12 h after PM2.5 treatment. In addition, treatment for 12 h with 100 μg/mL PM2.5 did not further inhibit *PGC-1α* expression regardless of the duration of PM2.5 treatment, suggesting an overdose effect of 100 μg/ml PM2.5. Taken together, treatment with 50 μg/mL PM2.5 for 12 h was an optimal condition for generating the maximum inhibitory effect on *PGC-1α mRNA* expression. The following experiments were performed under this condition.

Human bronchial epithelial BEAS-2B cells were seeded in 6-well plates at a density of 5×10^5 cells/well and were cultured under a normoxic condition (21 % O_2, 5 % CO_2) at 37 °C for 24 h. For the control group, cells were cultured under a normoxic condition for additional 6, 12, or 24 h. For the PM2.5 intervention group, cells were treated with various concentrations of PM2.5 (25, 50, or 100 μg/mL) for 6, 12, or 24 h (A). For the hypoxia intervention group, cells were cultured under hypoxic condition (3 % O_2, 5 % CO_2) in the absence of PM2.5 for 6, 12, or 24 h (B). For the PM2.5+hypoxia group, cells were treated with 50 μg/mL PM2.5 and cultured under hypoxic condition for 6, 12, or 24 h (B). mRNA expression of *PGC-1α* was determined by quantitative real-time RT-PCR (qRT-PCR). *GAPDH* was used as an internal control. Data are expressed as the mean ± standard deviation (SD). *P < 0.05 *vs*. the control for 6 h, **P < 0.01 *vs*. the control for 12 h,▼P < 0.01 *vs*. the control for 24 h; △P < 0.01 *vs*. 25 μg/ml/6-h PM2.5;☆P <0.05 *vs*. 50 μg/ml/6-h PM2.5; ▽P < 0.01 *vs*. 100 μg/ml/6-h PM2.5; •P < 0.05 *vs*. 6-h PM2.5+hypoxia; ◆P < 0.01 *vs* 6-h PM2.5+hypoxia; ▲P < 0.01 *vs*. 12-h PM2.5+hypoxia; ■P < 0.01 *vs*. 24-h PM2.5+hypoxia. n = 3. PGC-1α, peroxisome proliferator activated receptor γ co-activator 1α; PM, PM2.5.

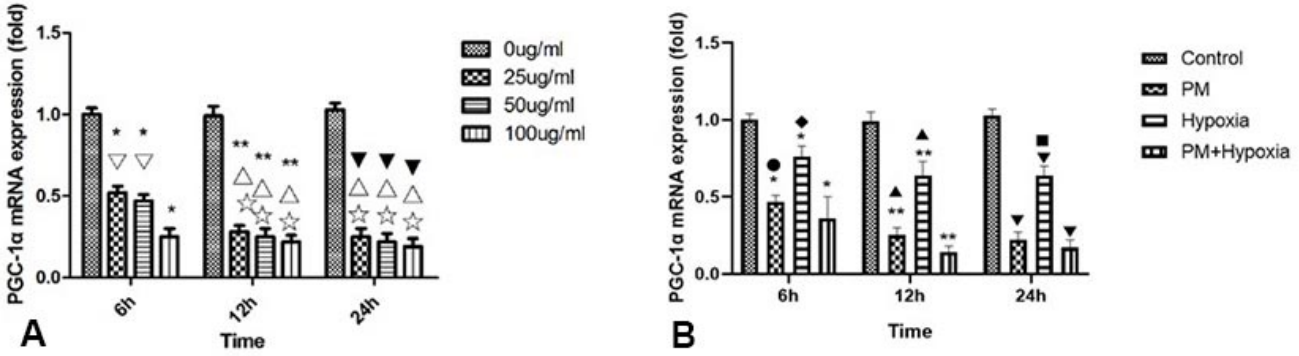

Fig. 3. Effects of PM2.5/hypoxia on mRNA expression of peroxisome proliferator activated receptor γ co-activator 1α (PGC-1α) in BEAS-2B cells.

Similarly, hypoxia also significantly decreased *PGC-1α mRNA* expression in a generally time-dependent manner compared with normoxia (Fig. 3B, P<0.05). However, no significant change was observed between 12 h and 24 h exposure durations (Fig. 3B, P>0.05), suggesting that 12 h of hypoxia was sufficient to induce maximal suppression of *PGC-1α mRNA* expression in BEAS-2B cells. In addition, more potent inhibition of *PGC-1α mRNA* expression was observed in response to PM2.5 (50 μg/ml) plus hypoxia (Fig. 3B, P<0.05 *vs* control), suggesting a synergy between hypoxia and PM2.5 in inhibiting PGC-1α expression. This synergy was further confirmed by the findings that a combination of PM2.5 (50 μg/ml) and hypoxia had a greater suppressive effect on *PGC-1α mRNA* expression than PM2.5 (50 μg/ml) or hypoxia alone (Fig. 3B, P<0.05). Accordingly, 50 μg/ml PM2.5 plus 12-h hypoxia were used for the following experiments.

Cytotoxic effect of NAC on BEAS-2B cells. NAC has been found to play a protective role against PM2.5-induced oxidative stress in rats (Yang *et al*., 2018), and thus may counteract the adverse effects of PM2.5 on BEAS-2B cells. However, it remains unknown whether NAC itself is cytotoxic to BEAS-2B cells. By performing CCK-8 assay, we found that NAC significantly reduced BEAS-2B cell viability at a dose of up to 10 μg/ml, compared with 0 μg/ml group (Fig. 4, P<0.05). Therefore, 5 μg/ml NAC was used to treat BEAS-2B cells as the dose under study.

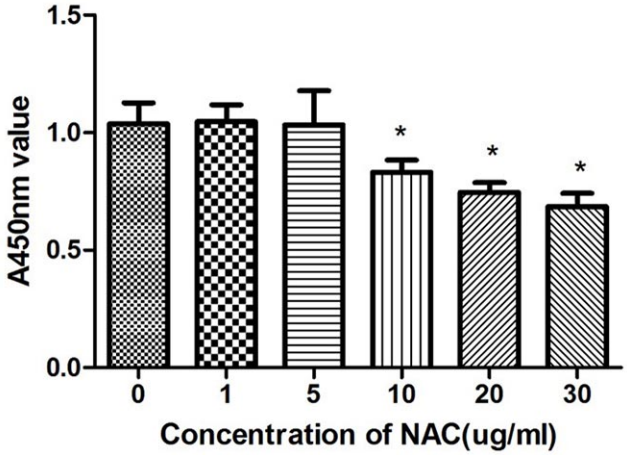

Fig. 4. Cytotoxic effect of N-acetyl-L-cysteine (NAC) on BEAS-2B cell viability.

BEAS-2B cells were cultured under a normoxic condition for 24 h and then treated with various concentrations of NAC (0, 1, 5, 10, 20, or 30 μg/ml) for 12 h. 50 μl of CCK8 solution was added into each well followed by incubation at 37°C for 4 h. The absorbance at a wavelength of 450 nm was measured using a microplate reader. Data are expressed as the mean ± SD. *P < 0.05 *vs.* untreated cells. n = 3. NAC, N-acetyl-L-cysteine.

The PGC-1α signaling may contribute to NAC-mediated inhibition of cellular oxidative damage in response to PM2.5 plus hypoxia. We next sought to determine whether NAC has an effect on PM2.5 plus hypoxia-induced suppression of PGC-1α expression. As shown in Figure 5A, NAC dramatically restored PM2.5 plus hypoxia-suppressed *PGC-1α mRNA* expression(P<0.05), suggesting that NAC may antagonize the effect of PM2.5 plus hypoxia on the PGC-1α signaling. Indeed, NAC reversed the negative effect of PM2.5 plus hypoxia on mRNA expression of PPARγ, a direct downstream target of PGC-1α (Fig. 5B, P<0.05). The similar trends were also observed in protein expression of PGC-1α and PPARγ (Fig. 5C–5E, P<0.05). Consistently, PGC-1α, as a negative upstream regulator of NF-κB, significantly inhibited PM2.5 plus hypoxia-induced NF-κB p65 protein expression (Fig. 5C and 5F, P<0.05). In addition, NAC/ PM2.5 plus hypoxia markedly regulated cellular oxidative damage as evidenced by NAC-induced reversal of the MDA level and SOD activity in PM2.5 plus hypoxia-treated BEAS-2B cells (Fig. 5G and 5H, P<0.05). These data further suggest a possible involvement of the PGC-1α signaling in regulation of cellular oxidative damage by NAC/ hypoxia plus PM2.5.

BEAS-2B cells were divided into 3 groups: control group (48-h normoxia), PM+hypoxia group (36-h normoxia, and then 12-h PM2.5 (50 μg/ml)+hypoxia), and PM+hypoxia+NAC group (35-h normoxia, 1-h NAC (5 μg/ml), followed by 12-h PM2.5 (50 μg/ml)+hypoxia). mRNA (A and B) and protein (C) expression of PGC-1α, peroxisome proliferator-activated receptor gamma (PPAR-γ), and nuclear factor kappa B (NF-κB) p65 were determined using qRT-PCR and Western blot assay, respectively. (D–F) Quantification of (C). The malondialdehyde (MDA) content (G) and superoxide dismutase (SOD) activity (H) in BEAS-2B cells were measured using enzyme-linked immunosorbent assay (ELISA). Data are expressed as the mean ± SD. *P < 0.05 *vs*. the control group; △P < 0.05 *vs* PM+hypoxia group. n = 3. PGC-1α, peroxisome proliferator activated receptor γ co-activator 1α; PM, PM2.5; PPAR-γ, peroxisome proliferator-activated receptor gamma; NF-κB, nuclear factor kappa B; MDA, malondialdehyde; SOD, superoxide dismutase; NAC, N-acetyl-L-cysteine.

PGC-1α is essential for NAC-mediated inhibition of oxidative damage induced by PM2.5 plus hypoxia. To further investigate the role of the PGC-1α signaling in the regulation of cellular oxidative damage by NAC/PM2.5 plus hypoxia, a loss-of-function assay was performed. As shown in Figure 6A, 6C and 6D (P<0.05), *siPGC-1α* transfection significantly decreased both mRNA and protein expression of PGC-1α, compared with *siCONTROL*, confirming the high efficiency of PGC-1α

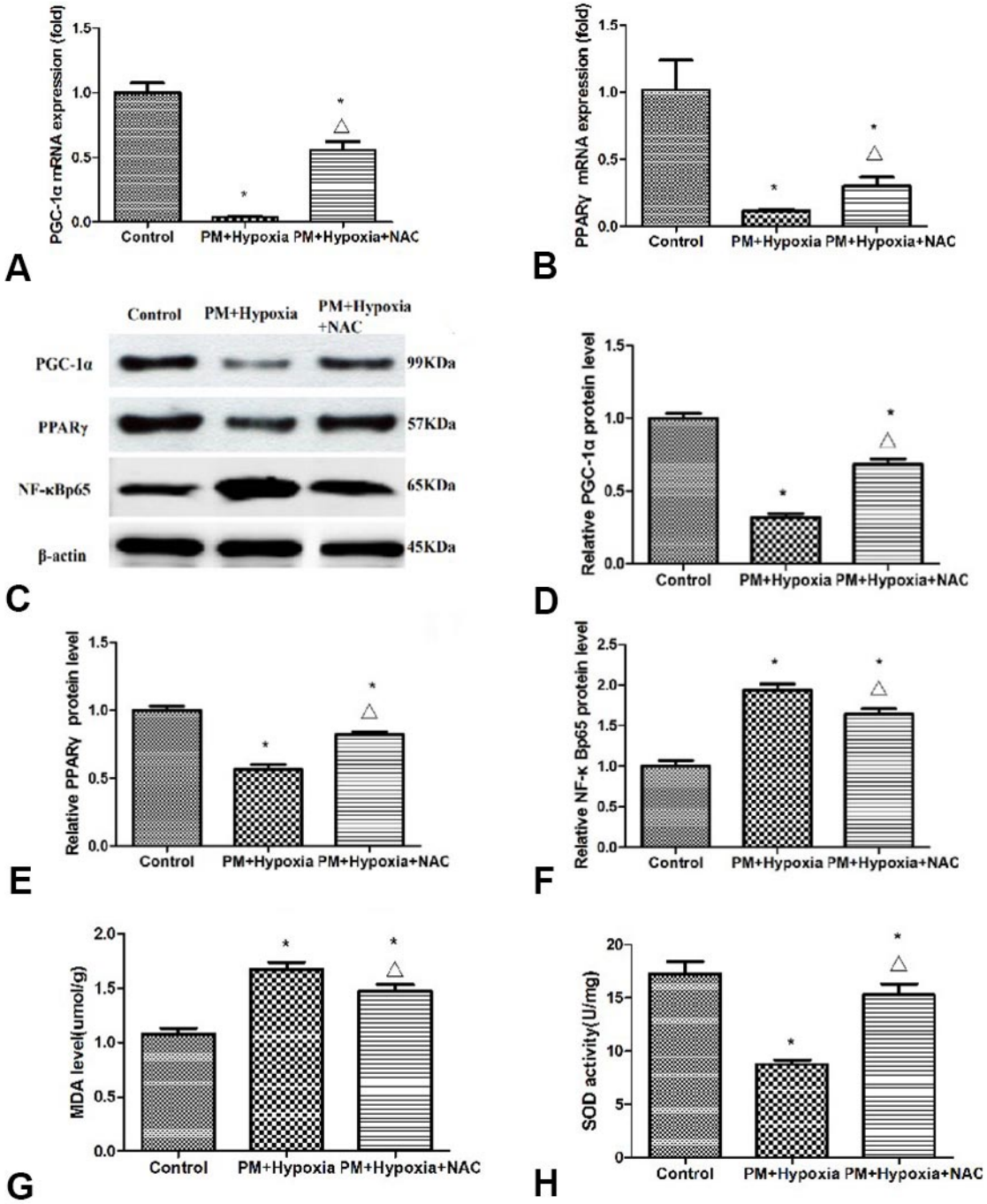

Fig. 5. The PGC-1α signaling in NAC-mediated inhibition of cellular oxidative stress in response to PM2.5 plus hypoxia.

knockdown by siPGC-1α. In addition, PGC-1α knockdown led to downregulation of PPAR-γ and upregulation of NF-κB p65, respectively (Fig. 6B, 6C, 6E and 6F, $P<0.05$). Importantly, the SOD activity and MDA level were positively and negatively regulated in PGC-1α-deficient cells, respectively (Fig. 6G and 6H, $P<0.05$), which suggests that PGC-1α contributes to suppression of cellular oxidative damage. Collectively, our data demonstrate that NAC PGC-1α-dependently protects BEAS-2B cells against PM2.5 plus hypoxia-induced oxidative damage, suggesting an important role of PGC-1α in oxidative damage protection.

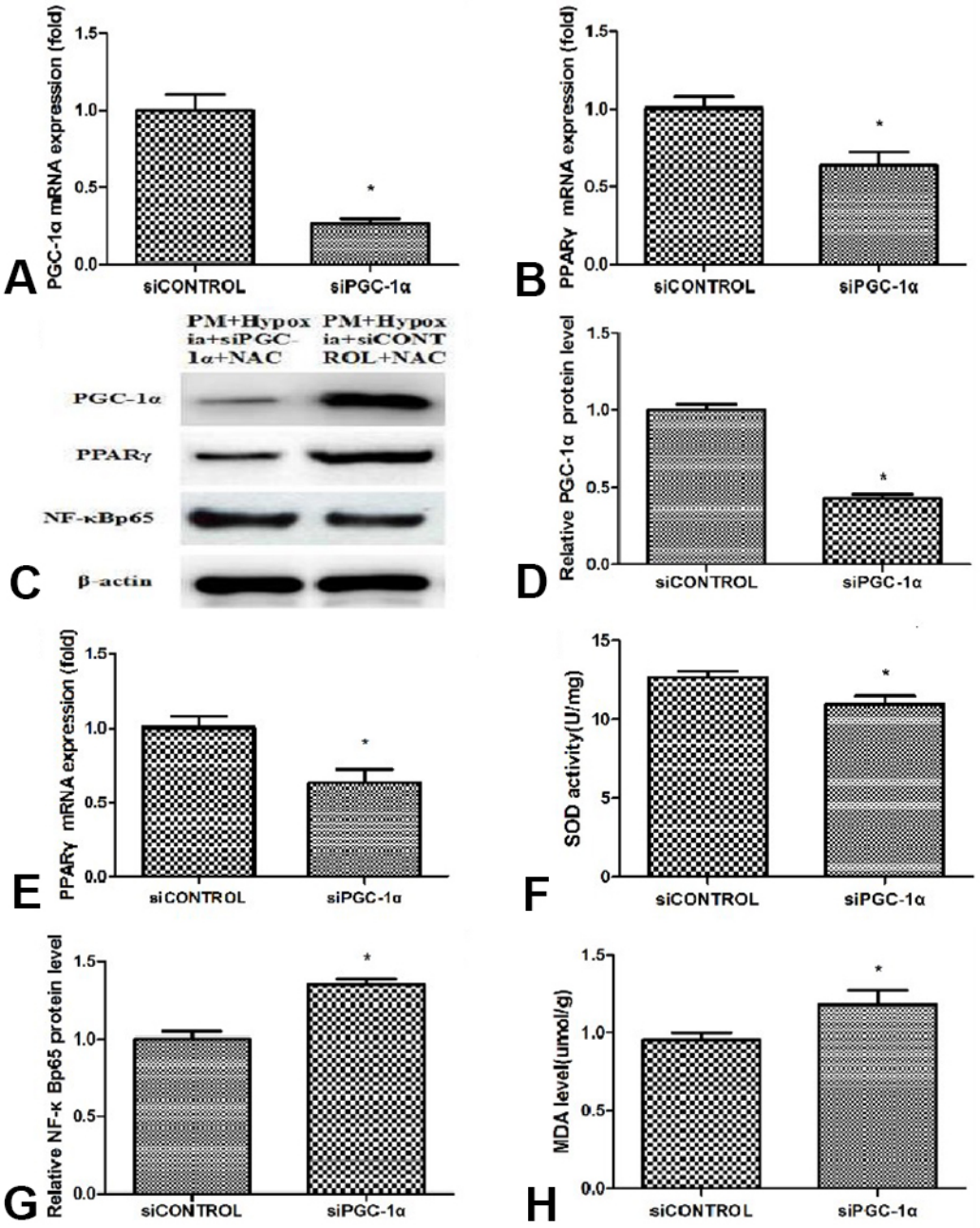

Fig. 6. PGC-1α is required for NAC inhibition of oxidative stress.

BEAS-2B cells were seeded in 6-well plates at a density of 5×10^5 cells/well and cultured in a normoxic incubator for 24 h, followed by transfection with 50 pmol of small interfering RNA of PGC-1α (*siPGC-1α*) or *siCONTROL* per well using Lipofectamine 3000. After 4 h of incubation, cells were refreshed with 5 μg/ml NAC-containing media and incubated in normoxia for additional 1 h, followed by exposure to 50 μg/mL PM2.5+hypoxia for 12 h. mRNA (A and B) and protein (C) expression of PGC-1α, PPAR-γ, and NF-κB p65 were determined using qRT-PCR and Western blot assay, respectively. (D–F) Quantification of (C). The SOD activity (G) and MAD content (H) in BEAS-2B cells were measured using ELISA. Data are expressed as the mean ± SD. *P < 0.05 *vs. siCONTROL*; n = 3. PGC-1α, peroxisome proliferator activated receptor γ co-activator 1α; PM, PM2.5; PPAR-γ, peroxisome proliferator-activated receptor gamma; NF-κB, nuclear factor kappa B; MDA, malondialdehyde; SOD, superoxide dismutase; NAC, N-acetyl-L-cysteine.

DISCUSSION

The results of the present study demonstrated that?in human BEAS-2B bronchial epithelial cells, oxidative damage resulted from PM2.5 plus hypoxia exposure was associated with the suppression of the PGC-1α signaling. NAC-induced reversal could be suppressed by knockdown of PGC-1α, suggesting that PGC-1α plays an essential role in the regulation of PM2.5 plus hypoxia-induced oxidative damage in BEAS-2B cells.

Fuentes-Mattei *et al*. (2010) exposed BEAS-2B cells to different concentrations of PM2.5 (1, 5, 10, 25, 50, and 100 μg/ml) to assess cytotoxicity, levels of cytokines and relative gene expression. In addition, they studied BEAS-2B cells exposed to hypoxia (0.5–3 % O_2) compared with normoxia (21 % O_2) (Linder *et al*., 2003). In a study, they exposed guinea pigs to CS smoke (four cigarettes/day; 3 months) and to chronic hypoxia (12 % O_2, 15 days) alone or in combination and evaluated airways remodeling and resistance (Olea *et al*., 2011). Based on the above research, we designed to expose BEAS-2B cells to PM2.5(25, 50 and 100 μg/mL), hypoxia(3 % O_2) and PM2.5 plus hypoxia respectively and conducted experimental research.

Oxidative stress is used to describe various detrimental processes caused by the imbalance between the formation and elimination of ROS and/or reactive nitrogen species (RNS) (Poljsak & Fink, 2014). Under stress, the activity of antioxidant enzymes, such as SOD, is inhibited, and excessive ROS tends to attack the cell membrane to form MDA, which is a typical product of lipid peroxidation (LPO) (Khoubnasabjafari *et al*., 2015). A previous study showed that the SOD activity in the lung tissue of Wistar rats administered with PM2.5 via intratracheal instillation was decreased whereas the MDA level was increased (Luo *et al*., 2014). Similar results were also observed in mice exposed to hypoxia: the MDA level of the lung tissue was increased, while the SOD activity and total antioxidant capacity (T-AOC) was decreased (Wang *et al*., 2015). These findings indicate that individual administration of PM2.5 or hypoxia may induce oxidative stress in lung tissues. Considering the association between PM2.5 exposure and declined oxygen saturation (Luttmann-Gibson *et al*., 2014), we established an *in vitro* model by exposing human bronchial epithelial cells to PM2.5 and hypoxia in combination. Although we did not assess the direct effect of PM2.5 or hypoxia on intracellular MDA level and SOD activity, we did find that PM2.5 plus hypoxia had a greater suppressive effect on PGC-1α mRNA expression than PM2.5 or hypoxia alone. PGC-1α plays an antioxidant role by recruiting nuclear receptors or transcription factors to upregulate the transcription of the downstream genes involved in ROS scavenging (Girnun, 2012; Kadlec *et al*., 2016). Our findings suggest a synergy between hypoxia and PM2.5 in inducing cellular antioxidant enzymes through downregulating the expression of PGC-1α and its downstream target genes. It has reported that PGC-1α can modulate ROS scavenging enzymes, including SOD1 and SOD2, *etc.* (Marmolino *et al*., 2010). We will continue to explore whether PM2.5 and hypoxia exposure cause changes in SOD1 or SOD2 through PGC-1α in subsequent studies.

PPARγ is one of the downstream transcription factors that are coactivated by PGC-1α and has been found to inhibit the NF-κB pathway to reduce inflammation and stimulate the nuclear factor E2-related factor 2/antioxidant responsive element axis to neutralize oxidative stress (Cai *et al*., 2018). Indeed, our data showed that the expression of both PGC-1α and PPARγ were downregulated whereas the expression of NF-κB p65, a subunit of NF-κB, was upregulated in BEAS-2B cells exposed to PM2.5 plus hypoxia, which indicate that oxidative stress and inflammation may both be enhanced. This is consistent with a previous study showing that mRNA expression of a decrease of PPARγ and an increase of interleukin 6 in human adipocytes in response to hypoxia (Olli *et al*., 2013). Similarly, exposure to PM2.5 could inhibit the expression of PPARγ and PPARα in the liver of mice, which initiates the inflammatory response through c-Jun N-terminal kinase, NF-κB, and toll-like receptor 4 (Zheng *et al*., 2013). Furthermore, our results demonstrated that knockdown of PGC-1α led to decreased PPARγ and increased NF-κB p65 expression respectively, which indicates that PPARγ and NF-κB p65 expression are dependent on PGC-1α activation in BEAS-2B cells. This agrees with a previous study showing that PGC-1α activation inhibits the transcriptional activity of NF-κB, which in turn reduces inflammation and oxidative stress (Eisele *et al*., 2013), although this experiment was done in skeletal muscle cells. Interestingly, it has been reported that the activation of NF-κB signaling in cardiac myocytes enhances the physical interaction between p65 and PGC-1α, thereby downregulating the expression of PGC-1α (Alvarez-Guardia *et al*., 2010). Although the cells used in these studies are different from BEAS-2B, it appears that there is a negative feedback loop between PGC-1α and NF-κB, which mediates the interplay between oxidative damage and inflammation.

Because a recent study demonstrated that a potent antioxidant NAC can reduce PM2.5-induced oxidative damage in the lung tissues of rats (Yang *et al*., 2018), to further investigate the regulatory effect of PGC-1α in PM2.5 plus hypoxia-induced damage, we used NAC to counteract the effect of oxidative stress (Su *et al*., 2017). We found that NAC may protect the cells from oxidative damage induced by PM2.5 plus hypoxia as evidenced by increased PGC-1α

and PPARγ expression and SOD activity as well as reduced protein levels of NF-κB p65 and MDA. Moreover, these protective effects of NAC were suppressed in PGC-1α-deficient cells, which suggest that NAC can activate PGC-1α and exert a protective role in BEAS-2B cells exposed PM2.5 and hypoxia simultaneously. Although NAC can act as antioxidant, it also can promote GSH synthesis. NAC supplementation might then increase the cellular GSH pool. Therefore, does NAC work by increasing GSH levels and whether PM2.5 and hypoxia have the same damaging effect on the alveoli *in vivo* will be further explored in our follow-up research.

CONCLUSIONS

Our results demonstrate that PGC-1α plays an essential role in the regulation of PM2.5 plus hypoxia-induced oxidative stress in human bronchial epithelial cells, which provides new information for the intervention of PM2.5-induced lung diseases. Further *in vivo* studies are required to elucidate the function of the PGC-1α signaling more precisely.

TIAN, Q.; GONG, X.; DUAN, Z. & YUAN, Y. El coactivador gamma-1 alfa del receptor activado por proliferador de peroxisomas desempeña un papel en PM2.5 y en el daño oxidativo simultáneo inducido por hipoxia en células epiteliales bronquiales humanas. *Int. J. Morphol., 42(6)*:1653-1662, 2024.

RESUMEN: El coactivador 1α del receptor γ activado por el proliferador de peroxisomas (PGC-1α) juega un papel importante en el estrés antioxidante. El objetivo de este estudio fue investigar el papel de PGC-1α en el daño oxidativo de las células BEAS-2B expuestas a PM2.5 e hipoxia simultáneamente y en el efecto antioxidante de la N-acetil-L-cisteína (NAC). Se evaluó el nivel de ARNm de PGC-1α en células BEAS-2B epiteliales bronquiales humanas expuestas a PM2.5, hipoxia y PM2.5 combinada con hipoxia. Las células se trataron durante 12 h en presencia o ausencia de PM2,5 combinada con hipoxia y en presencia o ausencia de NAC. Se evaluó la viabilidad celular después de la intervención con NAC. PGC-1α fue bloqueado por ARNip para investigar el mecanismo protector de NAC. Los niveles de expresión de PGC-1α y PPARγ se determinaron tanto a nivel de ARNm como de proteína. Se detectó que la expresión de NF-κBp65, malondialdehído (MDA) y superóxido dismutasa (SOD) a nivel de proteína. PM2.5 combinado con hipoxia redujo la expresión de PGC-1α y PPAR-γ (P<0.01), aumentó los niveles de proteína de NF-κBp65 (P<0.01) y MDA (P<0.05) y disminuyó la actividad de SOD (P <0,05) en comparación con el grupo control normal, y NAC mejoró el daño (P <0,05). El ARNip de PGC-1α bloqueó el efecto protector de la NAC. PM2.5 combinado con hipoxia puede causar daño oxidativo a las células BEAS-2B, y PGC-1α puede desempeñar un papel importante en el efecto antioxidante de la NAC. Los resultados de este estudio sugieren por primera vez que PGC-1α podría desempeñar un papel importante en la regulación del daño oxidativo inducido por PM21.5 y la hipoxia simultáneamente en células epiteliales bronquiales humanas, lo que proporciona ideas potenciales para el tratamiento de enfermedades respiratorias inducidas por PM2.5

PALABRAS CLAVE: PM2.5; Hipoxia; Coactivador-1 alfa del receptor gamma activado por proliferador de peroxisomas; N-acetil-L-cisteína; Daño oxidativo.

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