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ORIGINAL ARTICLE

1,25-Dihydroxy Vitamin D_3 Attenuates the Oxidative Stress-Mediated Inflammation Induced by $PM_{2.5}$ *via* the p38/NF-κB/NLRP3 Pathway

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Abstract— Vitamin D_3 is reported to be involved in the regulation of inflammatory processes. In this study, biomarkers related to oxidative stress and inflammation were investigated to clarify the protective effects and possible mechanism of 1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2D_3$) on $PM_{2.5}$-induced inflammatory response. In the *in vitro* study using human bronchial epithelial (HBE) cells, aqueous extracts of $PM_{2.5}$ could induce oxidative damage which is characterized by significant increases in production of reactive oxygen species, malonaldehyde concentration, and protein expression of HSPA1A and HO-1. Meanwhile, $PM_{2.5}$ caused secretion of inflammatory factors (IL-6, IL-8) in the culture medium as well as phosphorylation of p38, nuclear factor-kappa B (NF-κB) inhibitor alpha (IκBα), and NF-κB p65 proteins. Increases in NLRP3 expression was also observed in HBE cells after $PM_{2.5}$ exposure. However, all these biomarkers were remarkably attenuated by a 24-h pretreatment of 1 nM 1,25-$(OH)_2D_3$. Furthermore, 1,25-$(OH)_2D_3$ also reduced transcriptional activation of NF-κB induced by $PM_{2.5}$ as indicated by a significant decrease in luciferase activity in HBE cells stably transfected with the NF-κB response element (RE)-driven luciferase reporter. Taken together, our findings provided novel experimental evidences supporting that vitamin D_3 could reduce the predominantly oxidative stress-mediated inflammation induced by $PM_{2.5}$ *via* the p38/NF-κB/NLRP3 signaling pathway.

KEY WORDS: vitamin D_3; $PM_{2.5}$; oxidative stress; inflammation; NF-κB; bronchial epithelial cells.

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INTRODUCTION

Ambient air pollution, one of the most difficult challenges with which human beings are confronted, is now considered as a leading cause of death and lost disability-adjusted life years. According to the recent Global Burden of Disease report, outdoor air pollution can cause an estimated > 3 million premature deaths per year globally [1, 2]. The ubiquitous brown-haze, a characteristic feature of air pollution, has now reached alarming levels and gained prominence as a global public health concern [3]. Specifically, increase in environmental particulate matter (PM), especially the fine particulate

matter ($PM_{2.5}$), is becoming a leading risk factor for global disease [4, 5]. Mounting epidemiological evidences suggest that $PM_{2.5}$ is associated with decreased lung function and increased hospitalization, morbidity, and/or mortality of certain chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD), and also increases the risk of lung cancer [6–8].

Airborne $PM_{2.5}$, generally produced from both natural and anthropogenic emission sources, is increasing with economic development and usage of fossil fuels [9]. One of the most critical acute effects of $PM_{2.5}$ is to trigger airway and systemic inflammation, which accounts for several forms of respiratory diseases and cardiovascular disorders [10, 11]. The nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome, an intracellular danger-sensing protein complex, can respond to a variety of cellular stresses caused by various sterile danger signals, including environmental particles and nanoparticles [12]. Recent studies showed that epithelial cells of the respiratory tract are capable of initiating inflammatory events after exposure to pathogenic particles *via* NLRP3 inflammasome [13]. Nuclear factor-kappa B (NF-κB), an important molecule for immunity and inflammatory response, is critical as the priming signal in activation of NLRP3 inflammasome, to upregulate NLRP3 and pro-interleukin 1β (IL-1β) protein expression [14]. As one of the major subgroups of mitogen-activated protein kinases (MAPKs), p38 is also an important protein playing a role in mediating signals triggered by environmental stress and inflammatory cytokines [15]. Thus, $PM_{2.5}$-induced inflammatory response might be mediated through the p38/NF-κB/NLRP3 signaling pathway.

Vitamin D_3, a multifunctional steroid hormone, is involved in regulation of calcium homeostasis, oxidative stress, inflammatory processes, and cancer prevention [16, 17]. As reported in previous studies, vitamin D_3 not only protects human endothelial cells from oxidative stress [18], but also dampens inflammation by modulating the immune system in a number of cell types including monocytes, macrophages, and T cells [19–21]. The synthesis of biologically active vitamin D_3 always begins in the skin, and is converted to 1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2D_3$), its active metabolite, by 1α-hydroxylase in the kidneys. Recently, increasing evidence has shown that the respiratory epithelial cells also contain 1α-hydroxylase and can convert vitamin D_3 to its active form [22, 23]. Interestingly, epidemiological studies suggest that vitamin D_3 deficiency (levels of plasma 25-hydroxyvitamin D_3 (25-$(OH)_2D_3$) < 20 ng/mL) occurs in 25–50% of the human population [24, 25], and is associated with the onset and progression of COPD [26, 27]. Levels of plasma 25-$(OH)_2D_3$ have also been reported to be inversely associated with the occurrence of upper respiratory tract infections and reduced hospitalization of asthma-related complications [28, 29]. Therefore, vitamin D_3 may play an important role in the maintenance of the respiratory health.

The present study was undertaken to investigate the protective effects of vitamin D_3 on ambient $PM_{2.5}$-induced inflammation in human bronchial epithelial (HBE) cells. Air sampling was collected at a major industrial park in Suzhou, an important city located in the Yangtze River Delta Region in China. Chemical components of $PM_{2.5}$ were characterized by ion chromatograph (IC), inductively coupled plasma mass spectrometry (ICP-MS), and gas chromatography-mass spectrometry (GS-MS). Toxicity of $PM_{2.5}$ particles and effects of 1,25-$(OH)_2D_3$ on $PM_{2.5}$-induced oxidative damage and inflammatory response were evaluated by reactive oxygen species (ROS) production, lipid peroxidation, and secretion of inflammatory cytokines. To explore the possible mechanisms driving the effects of 1,25-$(OH)_2D_3$ on $PM_{2.5}$-induced toxicity, critical molecules involving in oxidative stress and the inflammation process were identified as potential intervention target.

MATERIALS AND METHODS

Chemicals

Dulbecco's modified Eagle's minimal (DMEM) essential medium, trypsin, and fetal bovine serum (FBS) were obtained from GIBCO (Grand Island, NY, USA). 1,25-$(OH)_2D_3$ was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell Counting Kit-8 (CCK-8) was purchased from Beyotime Institute of Biotechnology (Haimen, China). Human interleukin-6 (IL-6) and interleukin-8 (IL-8) enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D systems (Minneapolis, MN, USA). Cell malonaldehyde (MDA) assay kit was purchased from Jiancheng Bio Company (Nanjing, China). pGL4.32 [*luc*2P/NF-κB-RE/Hygro] vector and luciferase assay system were obtained from Promega (Madison, WI, USA). Rabbit anti-phospho-p38, phospho-IκBα, phospho-NF-κB p65, NLRP3, and HO-1 antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Mouse anti-HSPA1A antibody was obtained from StressGen Biotechnologies Corporation Company (Victoria, British Columbia, Canada).

$PM_{2.5}$ Collection and Physicochemical Characterization

Particulate matter < 2.5 μm ($PM_{2.5}$) was collected by a medium-volume air sampler (TH-150C, Tianhong Instrument Co.LTD, Wuhan, China) on a Teflon filter (90 × 90 mm) at China-Signapore Suzhou industrial park. Sampling was conducted over four to five separated days and for 8.5 h per day (100 L/min), using three parallel sampling lines, in the winter season of 2016. The sampling day with the highest $PM_{2.5}$ concentration (μg/m^3) was chosen for further physicochemical analysis and toxicity tests to ensure sufficient availability of particles.

$PM_{2.5}$ samples were extracted with ultrapure water by sonication to collect the water-soluble components. Then, a Malvern Zetasizer Nano S90 instrument (Southborough, MA, USA) was used to assess the size distribution of PM in water extracts. After 1 h-centrifugation at 12,000*g*, 4 °C, the supernatant was used to measure the concentrations of ionic species by IC. To obtain $PM_{2.5}$ suspensions, the above water extracts were vacuum freeze dried in a refrigerated Centrivap® concentrator and weighed.

To analyze the metal elements and organic compounds absorbed onto the particles, another $PM_{2.5}$ sample was taken and divided equally. One half was digested in nitric acid, and the amount of elemental metals was determined by ICP-MS. The other half was extracted with dichloromethane, and quantitative chemical analysis of 25 kinds of polycyclic aromatic hydrocarbons (PAHs) was performed by GS-MS.

$1,25\text{-}(OH)_2D_3$ Preparation

A $1,25\text{-}(OH)_2D_3$ solution (2.4×10^{-4} M) was prepared by dissolving 0.1 mg $1,25\text{-}(OH)_2D_3$ (molecular weight 416.64) in 1 mL 95% ethanol. Then, 100 μL of the $1,25\text{-}(OH)_2D_3$ solution was added to 23.9 mL 95% ethanol to get a stock solution of $1,25\text{-}(OH)_2D_3$ (10^{-6} M). All stock solutions were stored at − 20 °C in the dark prior to usage. To prepare the 1 nM $1,25\text{-}(OH)_2D_3$ solution, 1 μL of the 10^{-6} M $1,25\text{-}(OH)_2D_3$ solution was diluted with 999 μL DMEM medium supplemented with 10% FBS.

$PM_{2.5}$ Preparation

The concentrated $PM_{2.5}$ particles extracted with ultrapure water were UV-irradiated overnight to sterilize them and inactivate any contaminating endotoxin, as indicated in the paper of Peeters et al. [30]. Then, the $PM_{2.5}$ particles were suspended with sterile water to a concentration of 5 mg/mL and stored at 4 °C. Prior to each treatment, the stock $PM_{2.5}$ suspensions were diluted to a final concentration of 200 μg/mL with the cell culture medium, oscillated and mixed completely.

Cell Culture

HBE cells were kindly provided by Dr. Wen Chen (Sun Yat-sen University, China). HBE cells and the developed luciferase cells were maintained in DMEM essential medium supplemented with 10% FBS, 2 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2-humidified cell culture incubator. The culture medium was changed every 2–3 days.

Generation of Stable NF-κB-RE-Driven Luciferase Reporter Cells

The pGL4.32 [*luc*2P/NF-κB-RE/Hygro] vector was stably transfected into HBE cells by using Lipofectamine® 2000 (Thermo Fisher Scientific) according to the manufacturer's instructions. Cells were trypsinized and resuspended in culture medium to a final concentration of 1.5×10^5 cells/mL. To each well of a white six-well culture plate, 2-mL cell suspension was added. After incubating at 37 °C for 24 h, a mixture containing 1 μg plasmid and 2.5 μL lipofectamine was added to each well for transfection reaction. The transfected cells were then incubated with culture medium containing 50 μg/mL hygromycin for 2 weeks to select and amplify the stable reporter cell line. This cell line was now called HBE-luciferase cells.

Cell Treatment

HBE and HBE-luciferase cells were seeded in triplicate at a density of 0.5×10^4/well in 96-well plates (for CCK-8 assay), 2.5×10^4/well in 48-well plates (for luciferase reporter assay), and 2.5×10^5/well in six-well plates (for ROS production, Cell MDA, ELISA, and Western blot assays). Confluent mono-layers were pretreated with 1 nM $1,25\text{-}(OH)_2D_3$ or ethanol (0.1% *v*/*v*) for 24 h. Then, fresh DMEM culture medium was reapplied, and cells were simulated with 200 μg/mL aqueous extracts of $PM_{2.5}$ for another 48 h.

Cell Viability Assay

The number of viable HBE cells in culture was determined using the CCK-8 assay performed as previously described [31]. After $PM_{2.5}$ treatment, cells were incubated with 10 μL CCK-8 solution at 37 °C for another 4 h. The absorbance was measured at 450 nm by a SYNERGY 2

microplate reader (Bio-Tek, USA). The ethanol-treated cells were considered 100% viable as solvent control.

ROS Production Assay

The generation of ROS was measured using a dichlorofluorescein diacetate (DCFH-DA) detection kit according to the manufacturer's recommendations. After $PM_{2.5}$ treatment, cells were harvested and incubated with 10 μM DCFH-DA for 30 min at 37 °C. Then, the DCF-derived fluorescence was detected by a flow cytometer (FC500, Beckman Coulter, Quanta, SC, USA).

MDA Assay

MDA, an end product of peroxidation of fatty acid, is considered to be an important indicator of lipid peroxidation. MDA concentration in whole-cell lysates was measured by using the Cell MDA assay kit. Results are reported as MDA concentration and calculated as percent of solvent control.

Cytokine Analysis

Concentrations of IL-6 and IL-8 released into the cell culture supernatant after $PM_{2.5}$ treatment were evaluated using commercially available ELISA kits according to the manufacturer's instructions.

Western Blot Assay

After $PM_{2.5}$ treatment, HBE cells were lysed with RIPA (Beyotime, China) buffer containing protease and phosphatase inhibitors to obtain the whole-cell extracts. Proteins were quantified using the Bicinchoninic Acid assay (Thermo Scientific), separated electrophoretically on a 10% SDS-polyacrylamide gel, and transferred to nitrocellulose membranes. Membranes was then blocked with 5% fat-free milk at room temperature for 1 h, before being incubated with 1:1000 dilution of primary rabbit or mouse monoclonal antibodies overnight at 4 °C. After extensive washing, anti-rabbit (CST, 7074P2) and anti-mouse (CST, 7076P2) HRP-conjugated secondary antibody (1:3000 dilution) was added and incubated at 37 °C for 1 h. After washing, the specific antibody-antigen complexes were detected using the ECL luminescence reagent (Absin, China).

Luciferase Reporter Gene Assay

Luciferase activities in HBE-luciferase cells after $PM_{2.5}$ treatment were detected by the luciferase assay system as previously described [31]. Briefly, cells were rinsed with 0.5 M phosphate-buffered saline (pH 7.4) twice, and then, 100 μL of cell lysis buffer was added to each well and lysed for 15 min. After mixing with the luciferase assay reagent, luciferase activity of each sample was measured using a SYNERGY 2 microplate reader. Results were expressed as relative luciferase activity *versus* control (test sample/solvent control). In each treatment, the relative luciferase activities from three replicates were averaged.

Statistical Analysis

Results were expressed as mean values or mean ± SD of three cultures from a representative experiment. Statistical analysis was performed by one-way analysis of variance test. In all tests, a value of P less than 0.05 was considered to be statistically significant. All the data analysis was carried out using the statistical analysis software SPSS 17.0 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Basic Characteristics and Chemical Composition of the $PM_{2.5}$ Sample

Basic characteristics of the ambient $PM_{2.5}$ during the sampling day including GPS coordinates, volume of sampled air, and concentrations of PM_{10} and $PM_{2.5}$ are summarized in Table 1. As the air sampling was conducted in the winter season, high concentrations of PM were recorded, 212.0 μg/m^3 for PM_{10} and 135.9 μg/m^3 for $PM_{2.5}$. As shown in Table 2, 99% of the total PM number was ≤ 2.5 μm in the $PM_{2.5}$ sample. The highest frequency of particle number was detected with diameters from 0.1 to 0.2 μm.

Table 1. Basic Characteristics of PM Sampling in China

Site (GPS coordinates)	Sampling date	Air volumes (m^3)	PM_{10} (μg/m^3)	$PM_{2.5}$ (μg/m^3)	Temp (°C)
Suzhou (31° 16′ 30.79″ N, 120° 43′ 38.53″ E)	2016.12.30	47.1	212.0	135.9	13.4

Table 2. Size Distribution of $PM_{2.5}$ Sampling in China, Depicted as Relative Frequency (%)

$PM_{2.5}$ size range (μm)					
Relative frequency (%)	0.1–0.2	0.2–0.3	0.3–0.5	0.5–2.5	2.5–10
	85.454	12.904	1.623	0.018	0.002

To evaluate the chemical characterization of ambient $PM_{2.5}$, several classes of inorganic and organic contaminants were analyzed (Table 3). Among the inorganic elements detected by ICP-MS, Mg, Al, Cu, Zn, and Pb were the most abundantly present. The ionic species were also founded in the $PM_{2.5}$ sample, among them, $SO_4^{2-} > Cl^-$, on the one hand, and $K^+ > Na^+$, on the other hand. However, F^- was not detected in this sample. Quantification of PAHs in the organic extracts showed relatively high concentrations of phenanthrene, fluoranthene, benzo[b + k]fluoranthene, and benzo[ghi]perylene in the ambient $PM_{2.5}$. Pyrene, a marker of pyrogenic sources of PAHs, was also detected at a high concentration of 9.2 ng/m^3.

Effect of 1,25-$(OH)_2D_3$ on Reduction of Cell Viability Induced by $PM_{2.5}$

After 1,25-$(OH)_2D_3$ and $PM_{2.5}$ treatments, the viable cell number, a direct measurement of cell proliferation, was determined by the CCK-8 assay. As shown in Fig. 1, 24-h treatment of 1 nM 1,25-$(OH)_2D_3$ had no significant effect on cell viability as compared with the ethanol-treated solvent control. However, in the presence of 1,25-$(OH)_2D_3$, cell viability of HBE cells treated with 200 μg/mL $PM_{2.5}$ for 48 h was significantly increased from 85.92 ± 0.92% to 89.87 ± 2.33%, relative to the solvent control, cell viability of which was 100% (Fig. 1, $P < 0.01$). These results suggested that cytotoxicity could be induced by $PM_{2.5}$ and well inhibited by 1,25-$(OH)_2D_3$ pretreatment.

Effects of 1,25-$(OH)_2D_3$ on Oxidative Stress Induced by $PM_{2.5}$

To determine if 1,25-$(OH)_2D_3$ could attenuate $PM_{2.5}$-induced oxidative damage, ROS accumulation, lipid peroxidation (*i.e.*, MDA concentration), and expression levels of two heat shock proteins (HSPA1A, HO-1) were studied in HBE cells. Our results showed that, compared with the solvent control, $PM_{2.5}$ could significantly increase levels of ROS, MDA concentration, and HSPA1A and HO-1 protein expressions (Figs. 2 and 3, $P < 0.05$). Although 1,25-$(OH)_2D_3$ pretreatment could not fully attenuate the oxidative damage induced by $PM_{2.5}$, significant decreases in ROS production, MDA concentration, and levels of HSPA1A and HO-1 were observed in the presence of 1,25-$(OH)_2D_3$ (Figs. 2 and 3, $P < 0.05$). Thus, vitamin

Table 3. Concentrations of Metal Elements, Water-Soluble Components, and PAHs in the $PM_{2.5}$ Sampling in China

Metal element (ng/m^3)	Water-soluble components (μg/m^3)	PAHs (ng/m^3)	PAHs (ng/m^3)
Mg 127.57	F^- 0	Naphthalene 0.124	Chrysene 2.335
Al 348.78	Cl^- 4.5	Acenaphthylene 0.150	Benzo[b + k]fluoranthene 5.907
Ti 17.46	SO_4^{2-} 23.3	Acenaphthene 0.796	Benzo[a]fluoranthene 0.210
V 14.64	Na^+ 0.4	Fluorene 0.501	Benzo[e]pyrene 2.520
Cr 42.20	K^+ 0.9	Phenanthrene 7.385	Benzo[a]pyrene 0.862
Mn 40.97		Anthracene 0.614	Perylene 0.144
Co 28.98		Fluoranthene 11.033	1,3,5-triphenylbenzene 0.653
Ni 12.17		Acephenanthrene 0.547	Anthanthracene 0.914
Cu 222.58		Pyrene 9.213	Benzo[123-cd]pyrene 1.567
Zn 126.94		Retene 0.821	Benzo[ghi]perylene 4.015
Ga 11.80		Benzo[ghi]fluoranthene 2.333	Dibenz[ah]anthracene 0.228
As 16.14		Cyclopenta[cd] pyrene 0.423	Coronene 1.496
Cd 1.97		Benz[a]anthracene 1.032	
Pb 90.88			

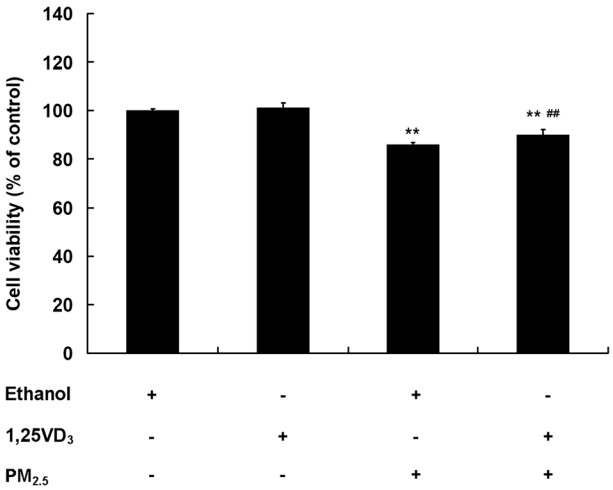

Fig. 1. Effect of 1,25-$(OH)_2D_3$ on $PM_{2.5}$-induced cytotoxicity detected by the CCK-8 assay. The HBE cells were pretreated with ethanol (0.1% *v*/*v*) or 1 nM 1,25-$(OH)_2D_3$ for 24 h. Then, fresh media was reapplied and cells were simulated with 200 μg/mL of $PM_{2.5}$ for another 48 h. Data represent mean ± SD of triplicate determinations. $^{**}P < 0.01$, compared with the solvent control. $^{\#\#}P < 0.01$, compared with the group treated with both ethanol and $PM_{2.5}$.

D_3 was found to potentially decrease $PM_{2.5}$-induced oxidative damage.

Effects of 1,25-$(OH)_2D_3$ on Inflammatory Cytokine Production Induced by $PM_{2.5}$

Figure 4 showed that inflammatory mediators were secreted into the culture medium of HBE cells. The aqueous extracts of $PM_{2.5}$ significantly induced IL-6 and IL-8 protein expression relative to the solvent control (Fig. 4, $P < 0.01$). Compared with the solvent control, levels of the two cytokines were significantly decreased in the 1,25-$(OH)_2D_3$-pretreated group (Fig. 4, $P < 0.05$). Meanwhile, $PM_{2.5}$-induced elevation of inflammatory cytokine induction was significantly attenuated in the presence of 1,25-$(OH)_2D_3$ (Fig. 4, $P < 0.01$). The above results showed that vitamin D_3 could potentially inhibit $PM_{2.5}$-induced inflammatory cytokine production.

Effects of 1,25-$(OH)_2D_3$ on Activation of the p38/NF-κB/NLRP3 Signaling Pathway Induced by $PM_{2.5}$

To explore the mechanisms by which $PM_{2.5}$ triggered HBE cells for inflammatory response, we tested whether $PM_{2.5}$ could activate inflammation-linked stress proteins (p38, IκBα, NF-κB p65, and NLRP3) or not. The effects of 1,25-$(OH)_2D_3$ on

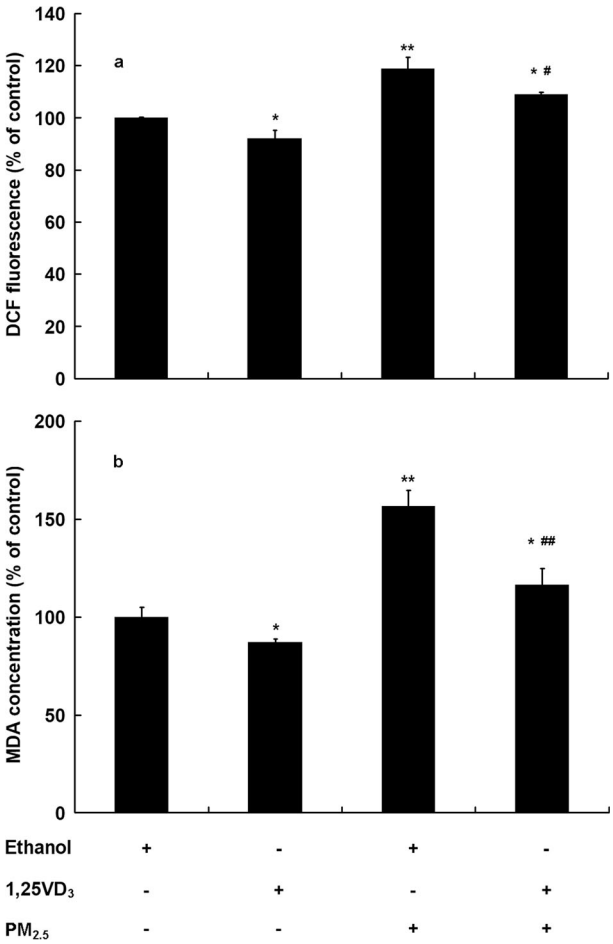

Fig. 2. Effect of 1,25-$(OH)_2D_3$ on $PM_{2.5}$-induced oxidative damage in HBE cells. ROS production (**a**) and MDA concentration (**b**) in HBE cells treated with ethanol, 1 nM 1,25-$(OH)_2D_3$, and a combination of $PM_{2.5}$ with ethanol or 1,25-$(OH)_2D_3$ were determined by DCFH-DA detection kit and cell MDA assay, respectively. Data represent mean ± SD of triplicate determinations. $^{*}P < 0.05$, $^{**}P < 0.01$, respectively, compared with the solvent control. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, respectively, compared with the group treated with both ethanol and $PM_{2.5}$.

$PM_{2.5}$-induced protein expression were then analyzed. As expected, the phosphorylation level of p38, IκBα, and NF-κB p65 and the expression level of the NLRP3 protein were significantly elevated after 48 h $PM_{2.5}$ exposure (Fig. 5, $P < 0.01$). However, 1,25-$(OH)_2D_3$ pretreatment could significantly attenuate phosphorylation of the two stress kinases (p38 and IκBα) and NF-κB p65 (Fig. 5, $P < 0.05$). Similar 1,25-$(OH)_2D_3$ effects were also observed in $PM_{2.5}$-induced NLRP3 expression. These results showed that vitamin D_3 could suppress the p38/NF-κB/NLRP3 signaling pathway activated by $PM_{2.5}$.

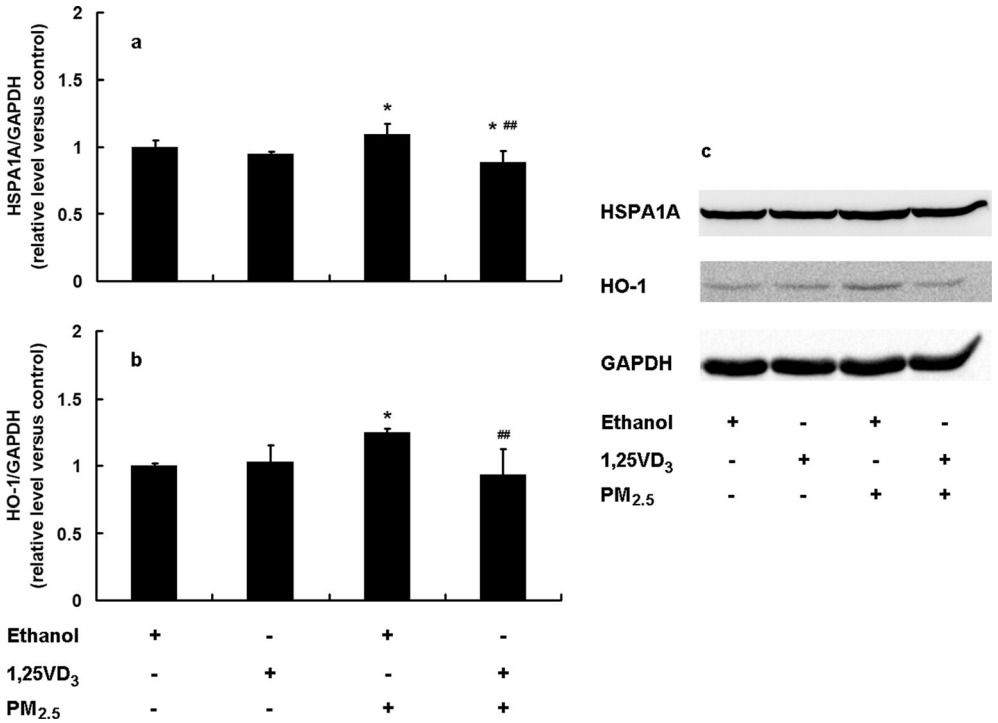

Fig. 3. Effect of 1,25-$(OH)_2D_3$ on HSPA1A and HO-1 expression. HBE cells were treated with ethanol, 1 nM 1,25-$(OH)_2D_3$, and a combination of $PM_{2.5}$ with ethanol or 1,25-$(OH)_2D_3$. Levels of HSPA1A (**a**, **c**) and HO-1 (**b**, **c**) proteins were detected by Western blot using a chemiluminescence system and densitometric analysis. GAPDH was used as the loading control. Data represent mean ± SD of triplicate determinations. $^{*}P < 0.05$, compared with the solvent control. $^{\#\#}P < 0.01$, compared with the group treated with both ethanol and $PM_{2.5}$.

Effects of 1,25-$(OH)_2D_3$ on Luciferase Activity of NF-κB RE-Driven Luciferase Reporter HBE Cells

To further illustrate the importance of NF-κB underlying the protective role of 1,25-$(OH)_2D_3$ in $PM_{2.5}$-induced oxidative damage and inflammatory response, the luciferase activities in NF-κB RE-driven luciferase reporter HBE cells were determined. Our results showed that, in accordance with the phosphorylation of NF-κB p65, $PM_{2.5}$ induced a low but detectable and significant increase in relative luciferase activity (1.27 × of control) (Fig. 6, $P < 0.01$). Whereas, a 24-h pretreatment of 1,25-$(OH)_2D_3$ clearly attenuated NF-κB activation as indicated by the significant decrease in relative luciferase activity (Fig. 6, $P < 0.05$).

DISCUSSION

$PM_{2.5}$ has been reported to have potential adverse effects on functions of airway epithelial cells. Increasing evidences show that oxidative stress coupled with inflammation are the primary responses to $PM_{2.5}$ by induction of lipid peroxidation, ROS overproduction, and a broad range of inflammatory factors (*i.e.*, IL-8) [5, 32–34]. Although adverse effects of $PM_{2.5}$ and multifunction of vitamin D_3 are well known, the relationship between these two issues has been largely unreported. Therefore, this study was to investigate whether 1,25-$(OH)_2D_3$ supplementation can directly influence the oxidative stress and inflammatory response induced by $PM_{2.5}$. The major findings suggested that addition of exogenous 1,25-$(OH)_2D_3$ can remarkably attenuate the adverse effects of $PM_{2.5}$ on HBE cells by decreasing levels of ROS production and lipid peroxidation, secretion of IL-6 and IL-8, and expression or phosphorylation levels of oxidative stress and inflammation-related crucial proteins. Meanwhile, we identified the p38/NF-κB/NLRP3 signaling pathway as a potential intervention target of 1,25-$(OH)_2D_3$ in the inhibition of oxidative stress-mediated inflammation induced by $PM_{2.5}$.

$PM_{2.5}$ particles can be easily inhaled and deposited in our respiratory tract and alveolar area, and thus, epithelial cells in the respiratory airways are vulnerable targets of

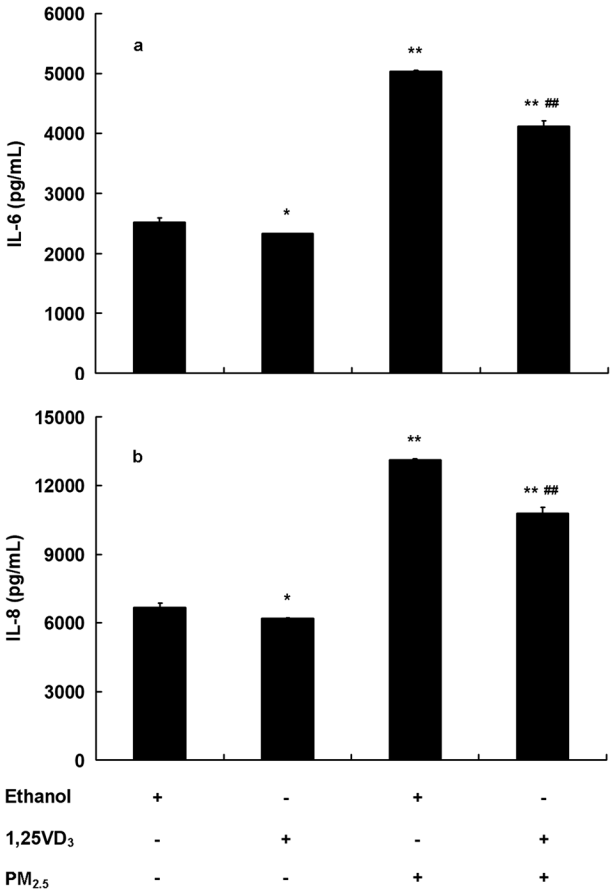

Fig. 4. Effect of 1,25-$(OH)_2D_3$ on $PM_{2.5}$-induced inflammatory responses in HBE cells. Quantity of cytokine secretion (IL-6 (**a**) and IL-8 (**b**)) in cell culture supernatant of HBE cells treated with ethanol, 1,25-$(OH)_2D_3$, and a combination of $PM_{2.5}$ with ethanol or 1,25-$(OH)_2D_3$ was estimated using the commercially available ELISA kits. Data represent mean ± SD of triplicate determinations. $^{*}P < 0.05$, $^{**}P < 0.01$, respectively, compared with the solvent control. $^{\#\#}P < 0.01$, compared with the group treated with both ethanol and $PM_{2.5}$.

PM. It is well known that oxidative stress plays a pivotal role in many adverse health effects induced by particles [35]. In the current study, oxidative damage induced by the ambient $PM_{2.5}$ particles (200 μg/mL) clearly increased ROS accumulation and MDA concentration. Two oxidative stress markers, HSPA1A and HO-1 proteins, were also increased in $PM_{2.5}$-treated HBE cells. According to several previous studies, transition metals including V, Cr, Co, Ni, and Ti which were present in the $PM_{2.5}$ sample combined with Cu and Zn could catalyze Fenton-type reactions, which can result in oxidative stress [36]. Additionally, PAHs, often linked to ROS production through activation of cytochrome P450 1A1 [37], were also detected in our $PM_{2.5}$ particles. Taken together, these results indicated that oxidative damage induced by the ambient $PM_{2.5}$ mainly arose from the inorganic or organic contaminants absorbed onto the particles.

Previous studies have assumed that activation of MAPK activities is dependent on the generation of oxidative stress [38]. According to some reports, metals and PAH-rich $PM_{2.5}$ can activate p38 MAPK and NF-κB accompanying with induction of oxidative damage and inflammatory factors in rat lungs and human and mouse macrophage cell lines [15, 39, 40]. Pyrene, a typical PAH, could increase IL-8 promoter activity and gene and protein expressions, which were regulated by NF-κB binding sites [41]. Thus, the excessive oxidative stress caused by PAHs and metals coated onto $PM_{2.5}$ can activate the MAPK and NF-κB signaling pathways [42], which thereby triggers a cascade of events closely associated with inflammation [43]. In the present study, in accordance with the physicochemical characteristics of $PM_{2.5}$ and the particles induced oxidative damage, aqueous extracts of $PM_{2.5}$ finally initiated an inflammatory response in HBE cells with enhanced production of inflammatory mediators (IL-6, IL-8). Activation of the p38/NF-κB signaling pathway was also observed as indicated by phosphorylation of p38, IκBα, and NF-κB p65. Meanwhile, expression of the NLRP3 protein, which is directly regulated by NF-κB, was also remarkably induced by $PM_{2.5}$ in HBE cells. Thus, these results suggested that activation of p38, IκBα, NF-κB p65, and NLRP3 by ROS induced by metals and PAHs absorbed onto $PM_{2.5}$ finally caused secretion of inflammatory cytokines.

Vitamin D_3, a lipophilic micronutrient, has been largely reported to inhibit oxidative stress [18] as well as modulate inflammatory cell processes [44]. It also plays an important role in airway remodeling which is the hallmark of severe asthmatics and patients with COPD. Therefore, we then tried to investigate the potential effects of vitamin D_3 on $PM_{2.5}$-induced inflammatory response in HBE cells. In the current study, our data showed that a 24-h pretreatment of 1,25-$(OH)_2D_3$ could remarkably attenuate $PM_{2.5}$-induced oxidative stress and inflammation in HBE cells as indicated by increase in cell viability, decreases in ROS accumulation, MDA concentration, and expression of IL-6 and IL-8. Meanwhile, protein expression of two oxidative stress markers, HSPA1A and HO-1, was also significantly decreased by 1,25-$(OH)_2D_3$. Thus, these results may suggest that vitamin D_3 is an effective regulator of HBE cells through increasing cell survival rates, decreasing oxidative damage, and secretion of inflammatory cytokines. Such

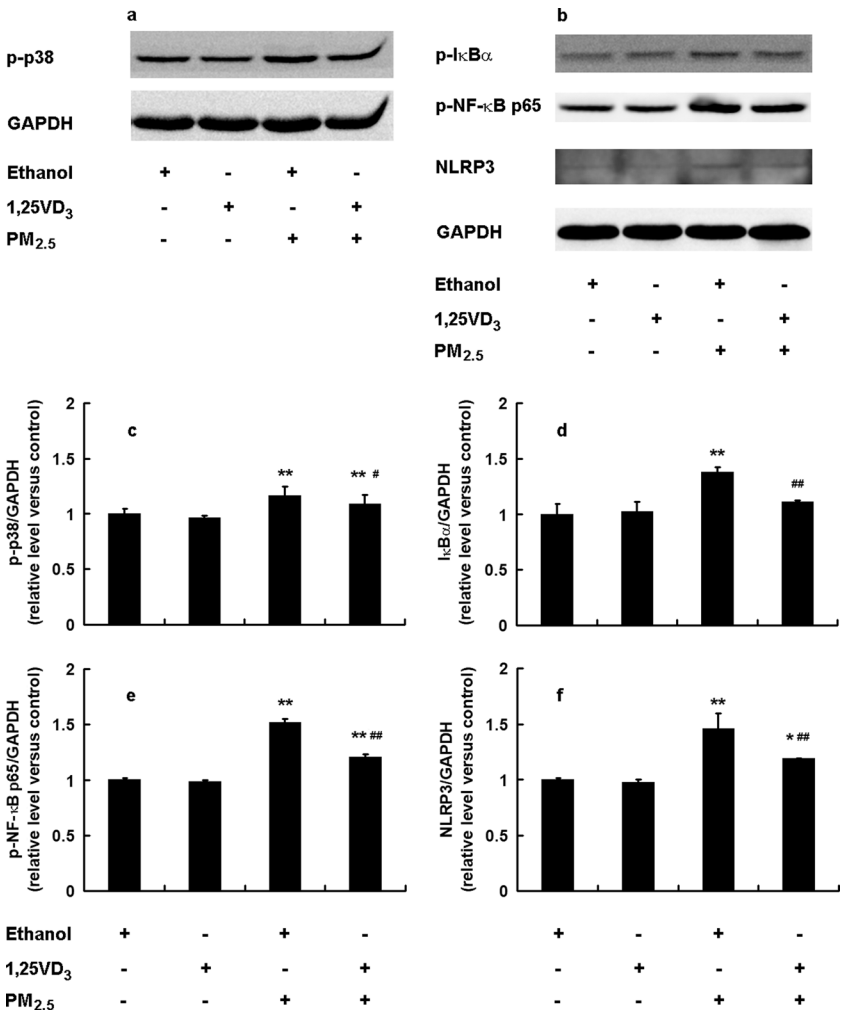

Fig. 5. Effect of 1,25-$(OH)_2D_3$ on p38, IκBα, and NF-κB p65 phosphorylation, and NLRP3 expression. Phosphorylation of p38 (**a**, **c**), IκBα (**b**, **d**), and NF-κB p65 (**b**, **e**) and NLRP3 expression (**b**, **f**) were detected by Western blot using a chemiluminescence system and densitometric analysis. GAPDH was used as the loading control. Data represent mean ± SD of triplicate determinations. $^{*}P < 0.05$, $^{**}P < 0.01$, respectively, compared with the solvent control. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, respectively, compared with the group treated with both ethanol and $PM_{2.5}$.

effects are consistent with oxidative stress suppression and immune modulation by vitamin D_3 in a variety of cell types, including endothelial cells, airway epithelial cells, and T cells [19, 45, 46].

NF-κB, a vital transcription factor in inflammation, immunity, and cell proliferation, has been shown to be a major signaling pathway in the development of various inflammation-related diseases [47]. Interestingly, 1,25-$(OH)_2D_3$, the active form of vitamin D_3, has been reported to decrease the secretion of inflammatory cytokines and chemokines in macrophages or airway epithelial cells, potentially by interfering with the NF-κB pathway [19, 21]. For the *in vitro* experiments, our results showed that pretreatment of 1,25-$(OH)_2D_3$ could substantially attenuate the phosphorylation of p38, IκBα, and NF-κB p65 as well as the expression of the NLRP3 protein in HBE cells. Meanwhile, the transcriptional activity of NF-κB induced by $PM_{2.5}$ as indicated by relative luciferase activity in the NF-κB RE-driven luciferase reporter cells was also remarkably decreased by 1,25-$(OH)_2D_3$. These results suggested the p38/NF-κB/NLRP3 signaling pathway as a potential intervention target of 1,25-$(OH)_2D_3$ in the suppression of $PM_{2.5}$-induced inflammatory response. However, HBE cells might be not sensitive enough to oxidative

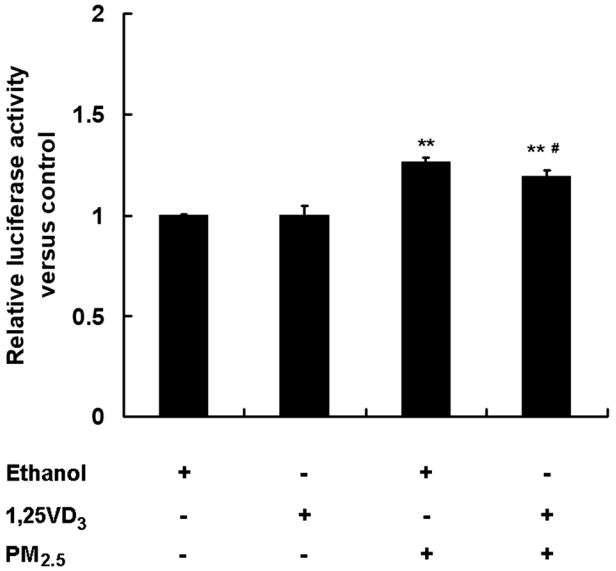

Fig. 6. Effect of 1,25-$(OH)_2D_3$ on $PM_{2.5}$-induced luciferase activity in HBE-luciferase cells stably transfected with the NF-κB RE-driven luciferase reporter plasmid. Luciferase activities in HBE-luciferase cells treated with ethanol, 1 nM 1,25-$(OH)_2D_3$, and a combination of $PM_{2.5}$ with ethanol or 1,25-$(OH)_2D_3$ were evaluated by the luciferase assay system. Data represent mean ± SD of triplicate determinations. $^{**}P < 0.01$, compared with the solvent control. $^{\#}P < 0.05$, compared with the group treated with both ethanol and $PM_{2.5}$.

stress and inflammatory response induced by $PM_{2.5}$. Some inflammatory factors including IL-1β were not detectable in HBE cells either. Therefore, other cell lines or animal models should be used to further address the specific mechanisms underlying these effects in our future studies.

CONCLUSION

Taken together, *in vitro* short-term exposure to airborne $PM_{2.5}$ collected in Suzhou City, China, could induce oxidative damage and inflammatory response in HBE cells. However, vitamin D_3 could remarkably attenuate the predominantly oxidative stress-mediated inflammation *via* the p38/NF-κB/NLRP3 signaling pathway.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

REFERENCES

1. Forouzanfar, M.H., L. Alexander, H.R. Anderson, et al. 2015. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 386: 2287–2323.
2. Thurston, G.D., H. Kipen, and I. Annesi-Maesano, et al. 2017. A joint ERS/ATS policy statement: what constitutes an adverse health effect of air pollution? An analytical framework. *European Respiratory Journal* 49.
3. Leung, P.Y., H.T. Wan, M.B. Billah, J.J. Cao, K.F. Ho, and C.K.C. Wong. 2014. Chemical and biological characterization of air particulate matter 2.5, collected from five cities in China. *Environmental Pollution* 194: 188–195.
4. Brauer, M., G. Freedman, J. Frostad, A. van Donkelaar, R.V. Martin, F. Dentener, R. Dingenen, K. Estep, H. Amini, J.S. Apte, K. Balakrishnan, L. Barregard, D. Broday, V. Feigin, S. Ghosh, P.K. Hopke, L.D. Knibbs, Y. Kokubo, Y. Liu, S. Ma, L. Morawska, J.L.T. Sangrador, G. Shaddick, H.R. Anderson, T. Vos, M.H. Forouzanfar, R.T. Burnett, and A. Cohen. 2016. Ambient air pollution exposure estimation for the Global Burden of Disease 2013. *Environmental Science & Technology* 50: 79–88.
5. Chen, Z.H., Y.F. Wu, P.L. Wang, Y.P. Wu, Z.Y. Li, Y. Zhao, J.S. Zhou, C. Zhu, C. Cao, Y.Y. Mao, F. Xu, B.B. Wang, S.A. Cormier, S.M. Ying, W. Li, and H.H. Shen. 2016. Autophagy is essential for ultrafine particle-induced inflammation and mucus hyperproduction in airway epithelium. *Autophagy* 12: 297–311.
6. Gehring, U., O. Gruzieva, R.M. Agius, R. Beelen, A. Custovic, J. Cyrys, M. Eeftens, C. Flexeder, E. Fuertes, J. Heinrich, B. Hoffmann, J.C. de Jongste, M. Kerkhof, C. Klümper, M. Korek, A. Mölter, E.S. Schultz, A. Simpson, D. Sugiri, M. Svartengren, A. von Berg, A.H. Wijga, G. Pershagen, and B. Brunekreef. 2013. Air pollution exposure and lung function in children: The ESCAPE project. *Environmental Health Perspectives* 121: 1357–1364.
7. Karakatsani, A., A. Analitis, D. Perifanou, J.G. Ayres, R.M. Harrison, A. Kotronarou, I.G. Kavouras, J. Pekkanen, K. Hämeri, G.P.A. Kos, J.J. de Hartog, G. Hoek, and K. Katsouyanni. 2012. Particulate matter air pollution and respiratory symptoms in individuals having either asthma or chronic obstructive pulmonary disease: A European multicentre panel study. *Environmental Health* 11: 75.
8. Raaschou-Nielsen, O., Z.J. Andersen, R. Beelen, E. Samoli, M. Stafoggia, G. Weinmayr, B. Hoffmann, P. Fischer, M.J.

Nieuwenhuijsen, B. Brunekreef, W.W. Xun, K. Katsouyanni, K. Dimakopoulou, J. Sommar, B. Forsberg, L. Modig, A. Oudin, B. Oftedal, P.E. Schwarze, P. Nafstad, U. de Faire, N.L. Pedersen, C.G. Östenson, L. Fratiglioni, J. Penell, M. Korek, G. Pershagen, K.T. Eriksen, M. Sørensen, A. Tjønneland, T. Ellermann, M. Eeftens, P.H. Peeters, K. Meliefste, M. Wang, B. Bueno-de-Mesquita, T.J. Key, K. de Hoogh, H. Concin, G. Nagel, A. Vilier, S. Grioni, V. Krogh, M.Y. Tsai, F. Ricceri, C. Sacerdote, C. Galassi, E. Migliore, A. Ranzi, G. Cesaroni, C. Badaloni, F. Forastiere, I. Tamayo, P. Amiano, M. Dorronsoro, A. Trichopoulou, C. Bamia, P. Vineis, and G. Hoek. 2013. Air pollution and lung cancer incidence in 17 European cohorts: Prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE). *The Lancet Oncology* 14: 813–822.

9. Wang, P., J.J. Cao, Z.X. Shen, Y.M. Han, S.C. Lee, Y. Huang, C.S. Zhu, Q.Y. Wang, H.M. Xu, and R.J. Huang. 2015. Spatial and seasonal variations of $PM_{2.5}$ mass and species during 2010 in Xi'an, China. *Sci Total Environ* 508: 477–487.
10. Pope, C.A., III, R.T. Burnett, D. Krewski, M. Jerrett, Y. Shi, E.E. Calle, and M.J. Thun. 2009. Cardiovascular mortality and exposure to airborne fine particulate matter and cigarette smoke: Shape of the exposure-response relationship. *Circulation* 120: 941–948.
11. Rusconi, F., D. Catelan, G. Accetta, M. Peluso, R. Pistelli, F. Barbone, E. di Felice, A. Munnia, P. Murgia, L. Paladini, A. Serci, and A. Biggeri. 2011. Asthma symptoms, lung function, and markers of oxidative stress and inflammation in children exposed to oil refinery pollution. *The Journal of Asthma* 48: 84–90.
12. Baroja-Mazo, A., F. Martin-Sanchez, A.I. Gomez, et al. 2014. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nature Immunology* 15: 738–748.
13. Sayan, M., and B.T. Mossman. 2016. The NLRP3 inflammasome in pathogenic particle and fibre-associated lung inflammation and diseases. *Particle and Fibre Toxicology* 13: 51.
14. Jo, E.K., J.K. Kim, D.M. Shin, and C. Sasakawa. 2016. Molecular mechanisms regulating NLRP3 inflammasome activation. *Cellular & Molecular Immunology* 13: 148–159.
15. He, M., T. Ichinose, S. Yoshida, T. Ito, C. He, Y. Yoshida, K. Arashidani, H. Takano, G. Sun, and T. Shibamoto. 2017. PM2.5-induced lung inflammation in mice: Differences of inflammatory response in macrophages and type II alveolar cells. *Journal of Applied Toxicology* 37: 1203–1218.
16. Scaranti, M.C., Gde Júnior, and A.O. Hoff. 2016. Vitamin D and cancer: Does it really matter? *Current Opinion in Oncology* 28: 205–209.
17. Hewison, M. 2012. Vitamin D and immune function: An overview. *The Proceedings of the Nutrition Society* 71: 50–61.
18. Uberti, F., D. Lattuada, V. Morsanuto, U. Nava, G. Bolis, G. Vacca, D.F. Squarzanti, C. Cisari, and C. Molinari. 2014. Vitamin D protects human endothelial cells from oxidative stress through the autophagic and survival pathways. *The Journal of Clinical Endocrinology and Metabolism* 99: 1367–1374.
19. Hansdottir, S., M.M. Monick, N. Lovan, L. Powers, A. Gerke, and G.W. Hunninghake. 2010. Vitamin D decreases respiratory syncytial virus induction of NF-κB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *Journal of Immunology* 184: 965–974.
20. Zhang, Y., D.Y. Leung, B.N. Richers, et al. 2012. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *Journal of Immunology* 188: 2127–2135.
21. Korf, H., M. Wenes, B. Stijlemans, T. Takiishi, S. Robert, M. Miani, D.L. Eizirik, C. Gysemans, and C. Mathieu. 2012. 1,25-Dihydroxyvitamin D_3 curtails the inflammatory and T cell stimulatory capacity of macrophages through an IL-10-dependent mechanism. *Immunobiology* 217: 1292–1300.
22. McNally, P., C. Coughlan, G. Bergsson, M. Doyle, C. Taggart, L. Adorini, M.R. Uskokovic, B. el-Nazir, P. Murphy, P. Greally, C.M. Greene, and N.G. McElvaney. 2011. Vitamin D receptor agonists inhibit pro-inflammatory cytokine production from the respiratory epithelium in cystic fibrosis. *Journal of Cystic Fibrosis* 10: 428–434.
23. Hansdottir, S., M.M. Monick, S.L. Hinde, N. Lovan, D.C. Look, and G.W. Hunninghake. 2008. Respiratory epithelial cells convert inactive vitamin D to its active form: Potential effects on host defense. *Journal of Immunology* 181: 7090–7099.
24. Kennel, K.A., M.T. Drake, and D.L. Hurley. 2010. Vitamin D deficiency in adults: When to test and how to treat. *Mayo Clinic Proceedings* 85: 752–757 quiz 757-758.
25. Breysse, C., P. Guillot, and G. Berrut. 2015. Study of vitamin D supplementation in people over 65 years in primary care. *Geriatrie et psychologie neuropsychiatrie du vieillissement* 13: 123–132.
26. Janssens, W., R. Bouillon, B. Claes, C. Carremans, A. Lehouck, I. Buysschaert, J. Coolen, C. Mathieu, M. Decramer, and D. Lambrechts. 2010. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. *Thorax* 65: 215–220.
27. Afzal, S., P. Lange, S.E. Bojesen, J.J. Freiberg, and B.G. Nordestgaard. 2014. Plasma 25-hydroxyvitamin D, lung function and risk of chronic obstructive pulmonary disease. *Thorax* 69: 24–31.
28. Ginde, A.A., J.M. Mansbach, and Jr. Camargo CA. 2009. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Archives of Internal Medicine* 169: 384–390.
29. Brehm, J.M., J.C. Celedón, M.E. Soto-Quiros, L. Avila, G.M. Hunninghake, E. Forno, D. Laskey, J.S. Sylvia, B.W. Hollis, S.T. Weiss, and A.A. Litonjua. 2009. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *American Journal of Respiratory and Critical Care Medicine* 179: 765–771.
30. Peeters, P.M., I.M. Eurlings, T.N. Perkins, et al. 2014. Silica-induced NLRP3 inflammasome activation in vitro and in rat lungs. *Particle and Fibre Toxicology* 11: 58.
31. Xin, L., J. Wang, L.W. Zhang, B. Che, G. Dong, G. Fan, and K. Cheng. 2016. Development of *HSPA1A* promoter-driven luciferase reporter gene assays in human cells for assessing the oxidative damage induced by silver nanoparticles. *Toxicology and Applied Pharmacology* 304: 9–17.
32. Nakayama Wong, L.S., H.H. Aung, M.W. Lamé, T.C. Wegesser, and D.W. Wilson. 2011. Fine particulate matter from urban ambient and wildfire sources from California's San Joaquin Valley initiate differential inflammatory, oxidative stress, and xenobiotic responses in human bronchial epithelial cells. *Toxicology In Vitro* 25: 1895–1905.
33. Deweirdt, J., J.F. Quignard, B. Crobeddu, A. Baeza-Squiban, J. Sciare, A. Courtois, S. Lacomme, E. Gontier, B. Muller, J.P. Savineau, R. Marthan, C. Guibert, and I. Baudrimont. 2017. Involvement of oxidative stress and calcium signaling in airborne particulate matter - induced damages in human pulmonary artery endothelial cells. *Toxicology In Vitro* 45: 340–350.
34. Crobeddu, B., L. Aragao-Santiago, L.C. Bui, S. Boland, and A. Baeza Squiban. 2017. Oxidative potential of particulate matter 2.5 as predictive indicator of cellular stress. *Environmental Pollution* 230: 125–133.
35. Nel, A.E., D. Diaz-Sanchez, and N. Li. 2001. The role of particulate pollutants in pulmonary inflammation and asthma: Evidence for the involvement of organic chemicals and oxidative stress. *Current Opinion in Pulmonary Medicine* 7: 20–26.
36. Shi, T., R.P. Schins, A.M. Knaapen, et al. 2003. Hydroxyl radical generation by electron paramagnetic resonance as a new method to

monitor ambient particulate matter composition. *Journal of Environmental Monitoring* 5: 550–556.

37. Perret, A., and D. Pompon. 1998. Electron shuttle between membrane-bound cytochrome P450 3A4 and b_5 rules uncoupling mechanisms. *Biochemistry* 37: 11412–11424.
38. Ng, D., N. Kokot, T. Hiura, et al. 1998. Macrophage activation by polycyclic aromatic hydrocarbons: Evidence for the involvement of stress-activated protein kinases, activator protein-1, and antioxidant response elements. *Journal of Immunology* 161: 942–951.
39. Li, R., L. Zhao, J. Tong, Y. Yan, and C. Xu. 2017. Fine particulate matter and sulfur dioxide coexposures induce rat lung pathological injury and inflammatory responses via TLR4/p38/NF-κB pathway. *International Journal of Toxicology* 36: 165–173.
40. Zhang, Y., S. Wang, J. Zhu, C. Li, T. Zhang, H. Liu, Q. Xu, X. Ye, L. Zhou, and L. Ye. 2018. Effect of atmospheric PM2.5 on expression levels of NF-κB genes and inflammatory cytokines regulated by NF-κB in human macrophage. *Inflammation* 41: 784–794.
41. Bömmel, H., M. Haake, P. Luft, J. Horejs-Hoeck, H. Hein, J. Bartels, C. Schauer, U. Pöschl, M. Kracht, and A. Duschl. 2003. The diesel exhaust component pyrene induces expression of IL-8 but not of eotaxin. *International Immunopharmacology* 3: 1371–1379.
42. Li, N., M. Hao, R.F. Phalen, et al. 2003. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clinical Immunology* 109: 250–265.
43. Totlandsdal, A.I., F.R. Cassee, P. Schwarze, M. Refsnes, and M. Låg. 2010. Diesel exhaust particles induce CYP1A1 and pro-inflammatory responses via differential pathways in human bronchial epithelial cells. *Particle and Fibre Toxicology* 7: 41.
44. Adams, J.S., and M. Hewison. 2010. Update in vitamin D. *The Journal of Clinical Endocrinology and Metabolism* 95: 471–478.
45. Cantorna, M.T., L. Snyder, Y.D. Lin, and L.L. Yang. 2015. Vitamin D and $1,25(OH)_2D$ regulation of T cells. *Nutrients* 7: 3011–3021.
46. Haas, M.J., M. Jafri, K.R. Wehmeier, L.M. Onstead-Haas, and A.D. Mooradian. 2016. Inhibition of endoplasmic reticulum stress and oxidative stress by vitamin D in endothelial cells. *Free Radical Biology & Medicine* 99: 1–10.
47. Tak, P.P., and G.S. Firestein. 2001. NF-κB: A key role in inflammatory diseases. *The Journal of Clinical Investigation* 107: 7–11.