



# Effects of <math><10\text{-}\mu\text{m}</math> Particulate Matter on Cultured Human Sebocytes and Outer Root Sheath Cells and Usefulness of Siegesbeckia Herba Extract

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**Received** August 25, 2021  
**Revised** November 30, 2021  
**Accepted** January 12, 2022

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**Background:** Particulate matter (PM) is one of the air pollutants that can damage human skin; the recent increase in the amount of PM may be detrimental to skin health.

**Objective:** We aimed to investigate the effects of PM on cultured human sebocytes and outer root sheath (ORS) cells and the effects of Siegesbeckia Herba extract (SHE) on PM-treated cultured cells.

**Methods:** Sebocytes and ORS cells were cultured. The cultured cells were treated with various concentrations of PM of <math><10\ \mu\text{m}</math> in size (PM10) (10  $\mu\text{g}/\text{ml}$ , 25  $\mu\text{g}/\text{ml}$ , 50  $\mu\text{g}/\text{ml}$ , and 100  $\mu\text{g}/\text{ml}$ ) for 24 h. Real-time polymerase chain reaction, measurement of reactive oxygen species (ROS), small interfering (si) RNA transfection, Oil Red O and Nile red staining, and immunofluorescence staining were performed to analyze the presence of inflammatory cytokines, matrix metalloproteinases (MMPs), aryl hydrocarbon receptor (AhR), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B), ROS, and lipid production. In addition, PM10 (100  $\mu\text{g}/\text{ml}$ )-treated cultured cells were treated with 10 mg/ml of SHE.

**Results:** PM10 upregulates the expression of inflammatory cytokines, MMPs, AhR, NF- $\kappa$ B, and ROS in cultured human sebocytes and ORS cells. The production of ROS was dramatically reduced in AhR siRNA-transfected cells. In addition, PM10 upregulates sebum production in cultured sebocytes. SHE inhibited the upregulation of inflammatory cytokines, MMPs, AhR, NF- $\kappa$ B, ROS, and sebum production in cultured human sebocytes and/or ORS cells by PM10.

**Conclusion:** Effects of PM10 on cultured human sebocytes and ORS cells can be regulated by SH.

**Keywords:** Outer root sheath cells, Particulate matter, Sebocytes, Siegesbeckia Herba extract

## INTRODUCTION

Particulate matter (PM) in the urban area consists of complex mixtures of components of various sizes originating from factories, power plants, automobiles, construction activities, and natural windblown dust<sup>1,2</sup>. PM of <math><10\ \mu\text{m}</math> in size (PM10) is one of the most harmful components of air pollutant. PM10 serves as a mediator for toxic compounds, such as transitional met-

als, endotoxins, organic chemicals, and ultrafine components. PM10 can reach the lungs and damage the respiratory and cardiovascular system. In addition, PM10 has a negative effect on the skin<sup>3</sup>.

PM10 attaches or penetrates into the skin epidermis and hair follicles, and then, it can damage the skin via the synthesis of reactive oxygen species (ROS). The generation of oxidative stress promotes skin aging, such as wrinkles,



pigmentation, and telangiectasia<sup>4-6</sup>. Furthermore, inflammatory skin conditions including atopic dermatitis, acne, and psoriasis are associated with PM10<sup>7</sup>. Antioxidants have been used to inhibit oxidative stress and improve inflammatory skin conditions. Several polyphenolic compounds have anti-oxidative and anti-inflammatory effects<sup>8-10</sup>. Also, plants are reported as a potential antioxidant and certain phytochemicals are expected to reduce cellular oxidative stress directly or indirectly. *Siegesbeckiae Herba* (SH), used as traditional medicine in Korea, Japan, China, and Vietnam, has been recently studied for their phytochemicals and biological efficacy<sup>11</sup>.

Although it is known that PM10 induces skin inflammation, insufficient research on the impact of PM10 on human sebocytes and outer root sheath (ORS) cells is available. Here, we aimed to investigate the effects of PM10 on the inflammatory cytokines, matrix metalloproteinases (MMPs), aryl hydrocarbon receptor (AhR), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B), ROS and sebum of sebocytes and ORS cells.

## MATERIALS AND METHODS

### Sebocyte and ORS cell culture

Patients provide the occipital non-balding scalp specimens through hair transplantation surgery. The study was conducted according to the Declaration of Helsinki Principles. Patients signed informed written consent of their own accord. The Medical Ethical Committee of the Kyungpook National University Hospital (Daegu, Korea) approved all of the described studies (IRB no. KNU 2018-0155).

The sebaceous glands of human occipital hair were transferred to a tissue culture dish. The isolated cells were cultivated in Sebocyte Basal Medium (Cell Application, San Diego, CA, USA) containing Sebocyte Growth Supplement in a 5% CO<sub>2</sub> incubator at 37°C. After 2 weeks of isolation, the cells were harvested with 0.25% trypsin/10 mM ethylenediaminetetraacetic acid in Hank's balanced salt solution and subcultured at EpiLife (Gibco BRL, Rockville, MD, USA).

For the cultivation of ORS cells, contamination with other cells was prevented by cutting off the hair shaft and hair bulb regions of the hair follicle. Trimmed hair follicles were immersed in Dulbecco's Modified Eagle's medium (Hyclone,

Logan, UT, USA) supplemented with 20% fetal bovine serum (Hyclone). On the third day of culture, the medium was changed to EpiLife keratinocyte growth medium-containing supplement. These experiments used sebocytes and ORS cells after the second passage.

### *Siegesbeckiae Herba* extract (SHE) and its solvent fractions

Dried *Siegesbeckiae Herba* was purchased from Sinsun Herb (Seoul, Korea) and the leaves (140 g) were ground and extracted with 0.9 L water at 90°C for 1 h. The extracted solution was evaporated under reduced pressure to obtain the crude extract (9 g). The SHE was dispersed in 150 ml water and partitioned sequentially with an equal volume of methylene chloride (MC), ethyl acetate (EA), and n-butyl alcohol (BA). Evaporation of the organic solvents yielded MC fraction (0.27 g), EA fraction (0.16 g), and BA fraction (0.46 g). The aqueous layer was filtered to remove insoluble material (1.15 g) and then evaporated to obtain a water fraction (6.64 g).

### MTT assay

We used the MTT assay to check cell viability. We seeded the sebocytes and ORS cells in 96-well collagen-coated plates at a density of 5,000 cells/well (Becton Dickinson, Franklin, NJ, USA) for 24 h. We added various concentrations (5  $\mu$ g/ml, 10  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml, and 100  $\mu$ g/ml) of PM10 (Sigma-Aldrich, St. Louis, MO, USA) and various concentrations (0.5 mg/ml, 1 mg/ml, 10 mg/ml, 30 mg/ml, and 50 mg/ml) of SHE to the well plates of the sebocytes and ORS cells for 1 day or 3 days each and the MTT solution (3-[4,5]dimethylthiazol-2,5-diphenyltetrazolium bromide) at 70  $\mu$ g/well for 3 h. We solubilized the formazan produced with dimethyl sulfoxide (DMSO) and measured the optical density at 570 nm.

### Real-time polymerase chain reaction

Cells were treated with a variety of concentrations of PM10 (10  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml, and 100  $\mu$ g/ml) for 24 h. PM10 (PM10-like, European reference material ERM-CZ120) was obtained from Sigma-Aldrich. PM10 (100  $\mu$ g/ml)-treated cells were also treated with 10 mg/ml of SHE for 24 h. Total RNA was isolated using an RNeasy Mini Kit (Qiagen, Hilden, Germany), and cDNA was synthesized from 3  $\mu$ g of total RNA with a cDNA synthesis kit containing ImProm-II<sup>TM</sup> reverse transcriptase and oligo (dT) primers based on the protocol suggested by the manufacturer (Promega, Mad-

ison, WI, USA).

Real-time polymerase chain reaction (PCR) was conducted with Step One Plus Real-Time PCR Assay (Applied Biosystems, Foster City, CA, USA). All reactions were conducted with Power SYBR Green premix (Applied Biosystems) using 50 ng cDNA and 10 pM primers. The cycling conditions for amplification were as follows: 95°C for 10 min, 40 cycles at 95°C for 15 s, and 60°C for 60 s. The products of the PCR were evaluated with the StepOnePlus Real-time PCR analysis software (Applied Biosystems). The primers used in the study are shown in Supplementary Table 1.

### Measurement of ROS

The cellular production of ROS was assessed by measuring 2',7'-dichlorodihydrofluorescein diacetate. The cells were plated onto 6-well collagen coated plates (Becton Dickinson) at  $5 \times 10^5$  cells/well for 24 h. Cells were pre labeled with 10  $\mu$ M DCF-DA (Invitrogen, Carlsbad, CA, USA) for 30 min. Plates were washed with phosphate-buffered saline (PBS) and then treated with various concentrations of PM10 (25  $\mu$ g/ml, 50  $\mu$ g/ml, and 100  $\mu$ g/ml) or with PM10 (100  $\mu$ g/ml) and SHE (10 mg/ml) and incubated at 37°C for 1 h protected from light. Cells were extracted with 20 mM Tris-Cl buffer containing 1% sodium dodecyl sulfate and 2.5 mM ethylenediamine-N,N,N',N'-tetraacetic acid. The extracts were centrifuged at 13,000 rpm for 15 min, and the supernatants were detected using a fluorescence microplate reader (Molecular Devices, Sunnyvale, CA, USA) with excitation at 485 nm and emission at 538 nm.

### Small interfering RNA transfection

The small interfering (si) RNAs used in this study were purchased from Bioneer (Daejeon, Korea). Cultured sebocytes and ORS cells were transfected with either control siRNA or a mixture of AhR siRNAs at a final concentration of 10 nmol/L in the presence of Lipofectamine<sup>TM</sup> RNAiMAX reagent (Invitrogen) for 48 h. After 2 days, the cells were pre labeled with 10  $\mu$ M DCF-DA (Invitrogen) for 30 min and treated with 100  $\mu$ g/ml PM10 for 1 h. The level of ROS production was visualized by fluorescent microscopy.

### Quantification of lipid production: Oil Red O and Nile red

The Oil Red O staining kit (Abcam, Cambridge, UK) was used according to the manufacturer's instructions. The slides

were incubated in propylene glycol for 2 min and in Oil Red O solution for 30 min. The slides were immersed in 85% propylene glycol for 1 min and washed twice in water and stained hematoxylin for 2 min. Then, the slides were washed with water and mounted.

AdipoRed assay reagent (Lonza, Walkersville, MD, USA) was used for Nile red. The slides were incubated in Adipo red solution (1:100 dilution) for 10 min at dark, washed with water, and mounted.

### Immunofluorescence staining

The hair follicle specimens were maintained in 100  $\mu$ g/ml Williams E media without phenol red (Sigma, St. Louis, MI, USA) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> for 2 days.

Sebocytes and ORS cells were seeded into eight chambers at a density of 30,000 cells per well (Nuck, Roskilde, Denmark) for 24 h. The cells were treated with or without 100  $\mu$ g/ml PM10 for 24 h. The fixation and blocking processes were the same as above. The cells were incubated with a rabbit anti-interleukin (IL)-1 $\alpha$  antibody (1:100 dilution; Abcam), anti-IL-1 $\beta$  antibody (1:100 dilution; Abcam), anti-IL-6 antibody (1:100 dilution; Abcam), anti-IL-8 antibody (1:100 dilution; MybioSource), anti-MMP-1 antibody (1:100 dilution; Abcam), anti-MMP-3 (1:100 dilution; Abcam), anti-MMP-12 antibody (1:100 dilution; Abcam), anti-p-NF- $\kappa$ B (1:100 dilution; Cell signaling, Beverly, MA, USA), goat anti-tumor necrosis factor (TNF)- $\alpha$  antibody (1:100 dilution; R and D) and mouse anti-AhR (1:100 dilution; Santa Cruz, Delaware, CA, USA) at 4°C overnight. Then, these were washed three times with PBS and incubated with Alexa Flour 488-labeled donkey anti-rabbit secondary antibody (Molecular Probes), 555-labeled donkey anti-goat secondary antibody (Molecular Probes) or 555-labeled donkey anti-mouse secondary antibody (Molecular Probes) for 1 h. The slides were counterstained with 4',6-diamidino-2-phenylindole.

### Statistical analysis

Data are expressed as follows: means $\pm$ standard deviation. Experiment was done three times independently. Statistical analysis for data was done with analysis of variance (SPSS Statistics ver. 18.0; SPSS Inc., Chicago, IL, USA). *p*-values of <0.05 was considered statistically significant.

## RESULTS

### MTT assay to determine PM10 concentration and SHE concentration

Effects of PM10 and SHE on the cell viability of the human sebocytes and ORS cells were shown in MTT assay according to varying concentrations (5  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ , 25  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , and 100  $\mu\text{g/ml}$ ) of PM10 (Fig. 1A) and according to varying concentrations (0.5 mg/ml, 1 mg/ml, 10 mg/ml, 30 mg/ml, and 50 mg/ml) of SHE (Fig. 1B).

### PM10 upregulates the expression of inflammatory cytokine in cultured human sebocytes and ORS cells and SHE inhibits the effects of PM10

The cells were treated with various concentrations of PM10 for 24 h to investigate the effects of PM10 on inflammatory responses in the human primary sebocytes and ORS cells. The expressions of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  were increased in PM10-treated sebocytes and ORS cells for 24 h in real-time PCR (Fig. 2A, B). A concentration-dependent increase was observed in the expression of inflammatory cytokines. SHE (10 mg/ml) decreased the upregulation of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  in PM10 (100  $\mu\text{g/ml}$ )-treated sebocytes and ORS cells for 24 h in real-time PCR (Fig. 2C, D).

Next, the protein levels of inflammatory cytokines in the human primary sebocytes and ORS cells were analyzed using immunostaining. The expression of inflammatory cytokines

showed an increase in sebocytes and ORS cells after treatment with 100  $\mu\text{g/ml}$  PM10 for 24 h (Fig. 2E, F).

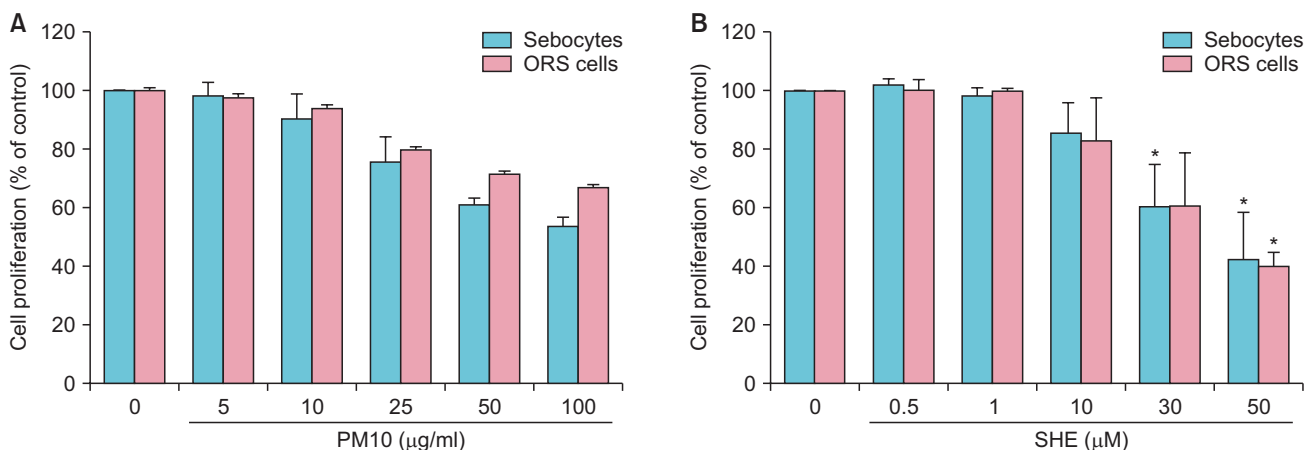
### PM10 upregulates the expression of MMPs in cultured human sebocytes and ORS cells and SHE inhibits the effects of PM10

The MMP family is known to play a critical role in skin aging. The cells were treated with various concentrations of PM10 for 24 h to investigate the effects of PM10 on the expression of MMP-1, MMP-3, and MMP-12. The expressions of MMP-1, MMP-3, and MMP-12 were increased in PM10-treated sebocytes and ORS cells for 24 h in real-time PCR (Fig. 3A, B). A concentration-dependent increase was observed in the expression of MMP genes. SHE (10 mg/ml) decreased the upregulation of MMP-1, MMP-3, and MMP-12 in PM10 (100  $\mu\text{g/ml}$ )-treated sebocytes and ORS cells for 24 h in real-time PCR (Fig. 3C, D).

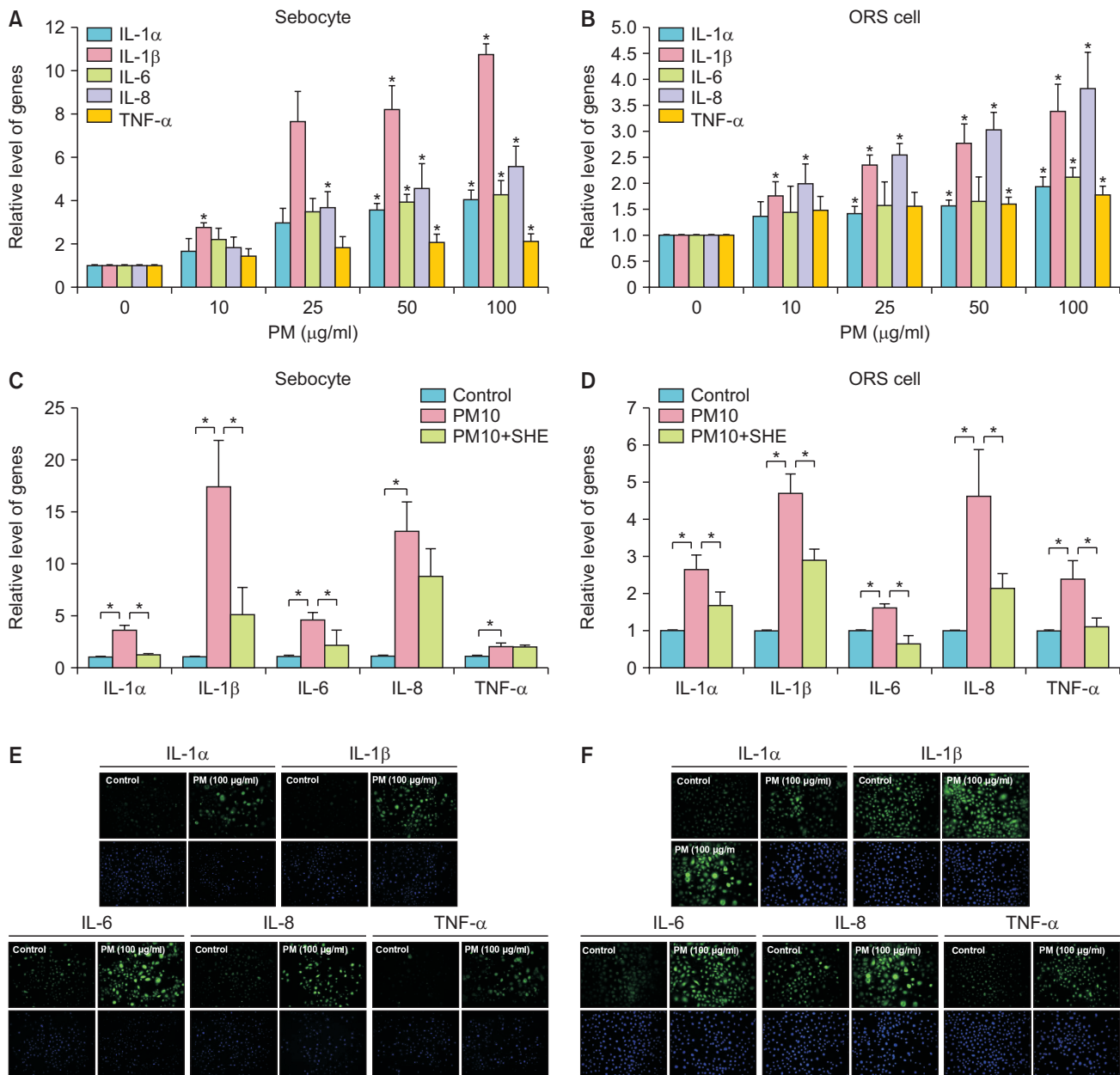
Immunostaining showed that the expression of MMP-1, MMP-3, and MMP-12 were increased in sebocytes and ORS cells after treatment with 100  $\mu\text{g/ml}$  PM10 for 24 h (Fig. 3E, F).

### PM10 upregulates the expression of AhR and NF- $\kappa\text{B}$ in cultured sebocytes and ORS cells and SHE inhibits the effects of PM10

PM contains a significant amount of polycyclic aromatic hydrocarbons, which can induce ROS production via AhR. Oxidative stress can also activate NF- $\kappa\text{B}$ . We next assessed



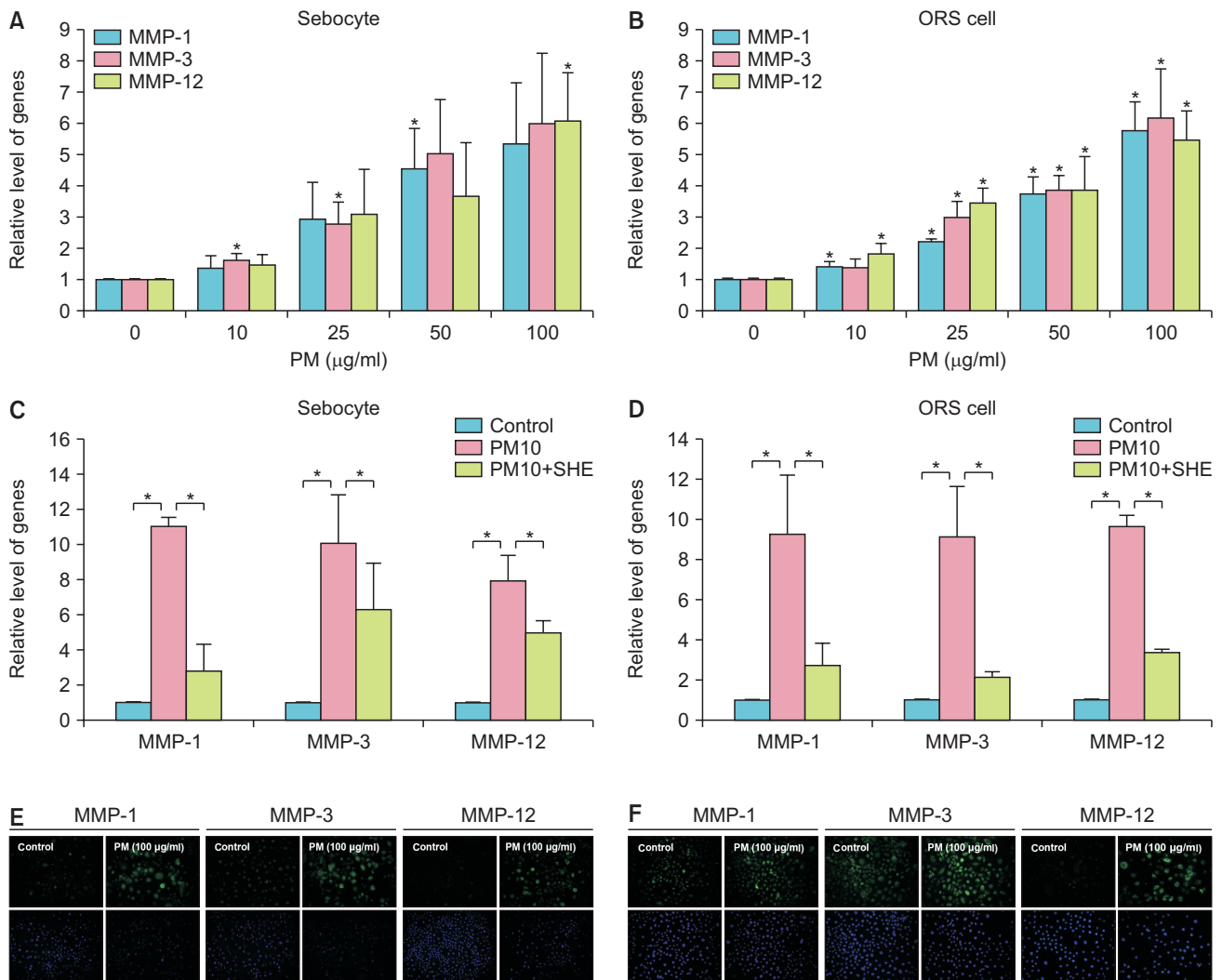
**Fig. 1.** (A) Effects of particulate matter (PM) 10 in human-cultured sebocytes and outer root sheath (ORS) cells. We treated the cells with PM10 (5  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ , 25  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , and 100  $\mu\text{g/ml}$ ) for 1 day, using the 3-[4,5]dimethylthiazol-2,5-diphenyltetrazolium bromide assay to measure cell viability. (B) We treated sebocytes and outer root sheath cells with varying concentrations (0.5 mg/ml, 1 mg/ml, 10 mg/ml, 30 mg/ml, and 50 mg/ml) of Siegesbeckia Herba extract (SHE). Data in the bar graphs represent the means  $\pm$  standard deviation from the three independent experiments (\* $p < 0.05$ ).



**Fig. 2.** (A) Particulate matter of  $<10\ \mu\text{m}$  in size (PM10) upregulates the expression of inflammatory cytokines in cultured sebocytes in real-time polymerase chain reaction (PCR). (B) PM10 upregulates the expression of inflammatory cytokines in outer root sheath (ORS) cells in real-time PCR. (C) Siegesbeckia Herba extract (SHE) (10 mg/ml) decreased the upregulation of inflammatory cytokines in PM10 (100  $\mu\text{g}/\text{ml}$ )-treated sebocytes. (D) SHE (10 mg/ml) decreased the upregulation of inflammatory cytokines in PM10 (100  $\mu\text{g}/\text{ml}$ )-treated ORS cells. (E, F) Immunostaining showed the expression of inflammatory cytokines were increased in sebocytes and ORS cells after treatment with 100  $\mu\text{g}/\text{ml}$  PM10 for 24 h. The data in the bar graphs represent mean  $\pm$  standard deviation from three independent experiments ( $*p < 0.05$ ). IL: interleukin, TNF: tumor necrosis factor.

whether PM10 treatment increased AhR and NF- $\kappa$ B expression levels in cultured human sebocytes and ORS cells. The expressions of AhR and NF- $\kappa$ B were increased in PM10-treated sebocytes and ORS cells for 24 h in real-time PCR (Fig. 4A). A concentration-dependent increase was observed in the

expression of AhR and NF- $\kappa$ B. In addition, nuclear AhR and NF- $\kappa$ B were increased by treatment with 100  $\mu\text{g}/\text{ml}$  PM10 for 24 h by immunostaining (Fig. 4B). These results demonstrated that PM10 treatment causes nuclear activation of AhR and NF- $\kappa$ B pathway. SHE (10 mg/ml) decreased the upregulation



**Fig. 3.** (A) Particulate matter of  $<10\ \mu\text{m}$  in size (PM10) upregulates the expression of matrix metalloproteinases (MMPs) in cultured sebocytes. (B) PM10 upregulates the expression of MMPs in outer root sheath (ORS) cells. (C) Siegesbeckia Herba extract (SHE) (10 mg/ml) decreased the upregulation of MMPs in PM10 (100  $\mu\text{g}/\text{ml}$ )-treated sebocytes. (D) SHE (10 mg/ml) decreased the upregulation of MMPs in PM10 (100  $\mu\text{g}/\text{ml}$ )-treated ORS cells. (E, F) Immunostaining showed the expression of MMP-1, MMP-3, and MMP-12 were increased in sebocytes and ORS cells after treatment with 100  $\mu\text{g}/\text{ml}$  PM10 for 24 h. The data in the bar graphs represent mean  $\pm$  standard deviation from three independent experiments ( $*p < 0.05$ ).

of AhR and NF- $\kappa$ B in PM10 (100  $\mu\text{g}/\text{ml}$ )-treated sebocytes and ORS cells for 24 h in real-time PCR (Fig. 4C, D).

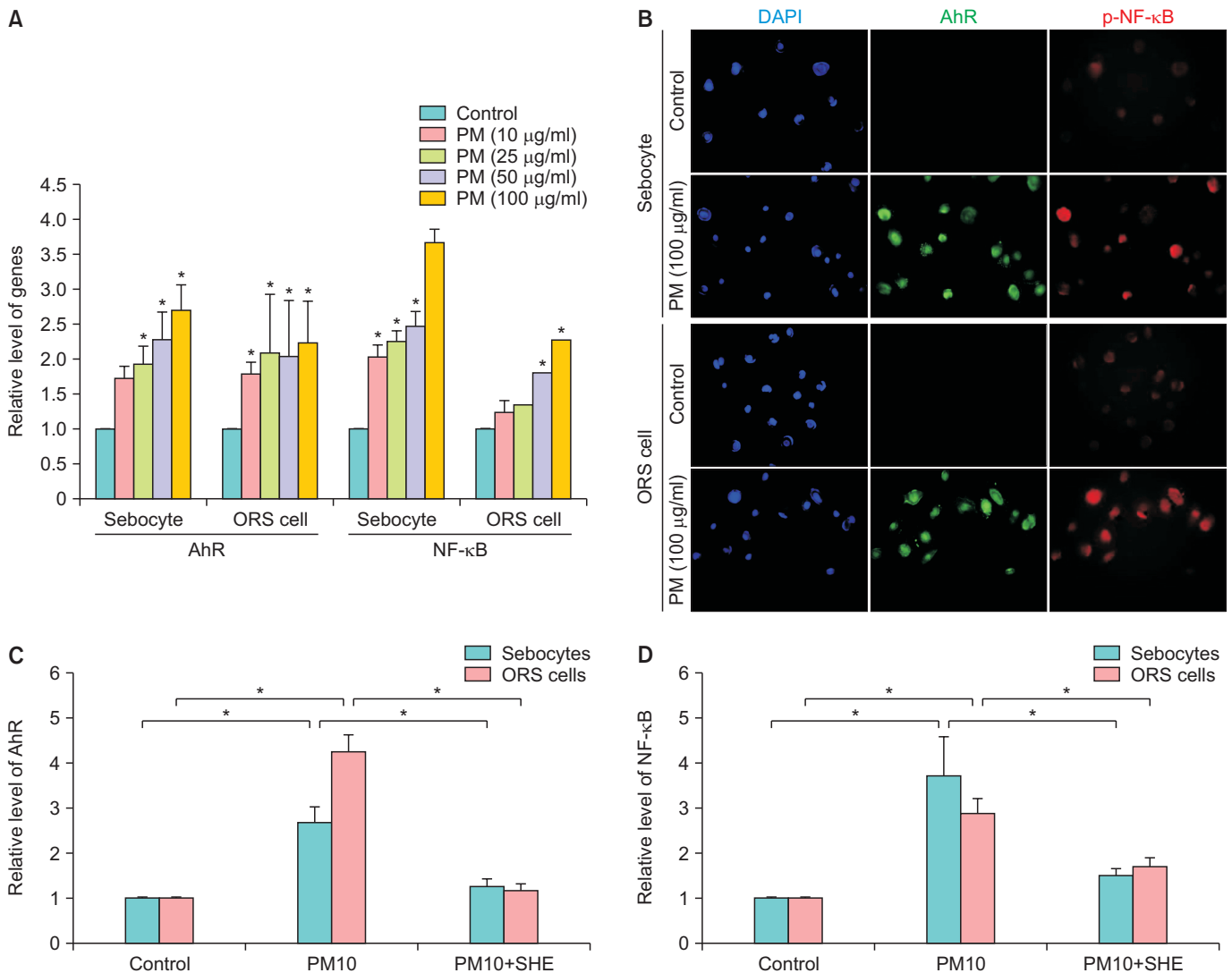
### PM10 upregulates the production of ROS in cultured sebocytes and ORS cells and SHE inhibits the effects of PM10

The production of ROS was increased in a concentration-dependent manner (Fig. 5A, B). Next, we investigated whether ROS induction is mediated by AhR. The production of ROS was dramatically reduced in AhR siRNA-transfected cells (Fig. 5C, D). These results demonstrated that ROS production is

mediated by AhR in cultured sebocytes and ORS cells. SHE (10 mg/ml) decreased the upregulation of ROS in PM10 (100  $\mu\text{g}/\text{ml}$ )-treated sebocytes and ORS cells (Fig. 5E).

### PM10 upregulates sebum production in cultured sebocytes and SHE inhibits the effects of PM10

The increased expressions of peroxisome proliferator-activated receptor (PPAR)- $\gamma$ , sterol regulatory element-binding protein 1a (SREBP1a), SREBP1c, and stearyl-CoA desaturase (SCD) were observed in PM10-treated sebocytes in real-time PCR for 24 h (Fig. 6A). A concentration-dependent increase was

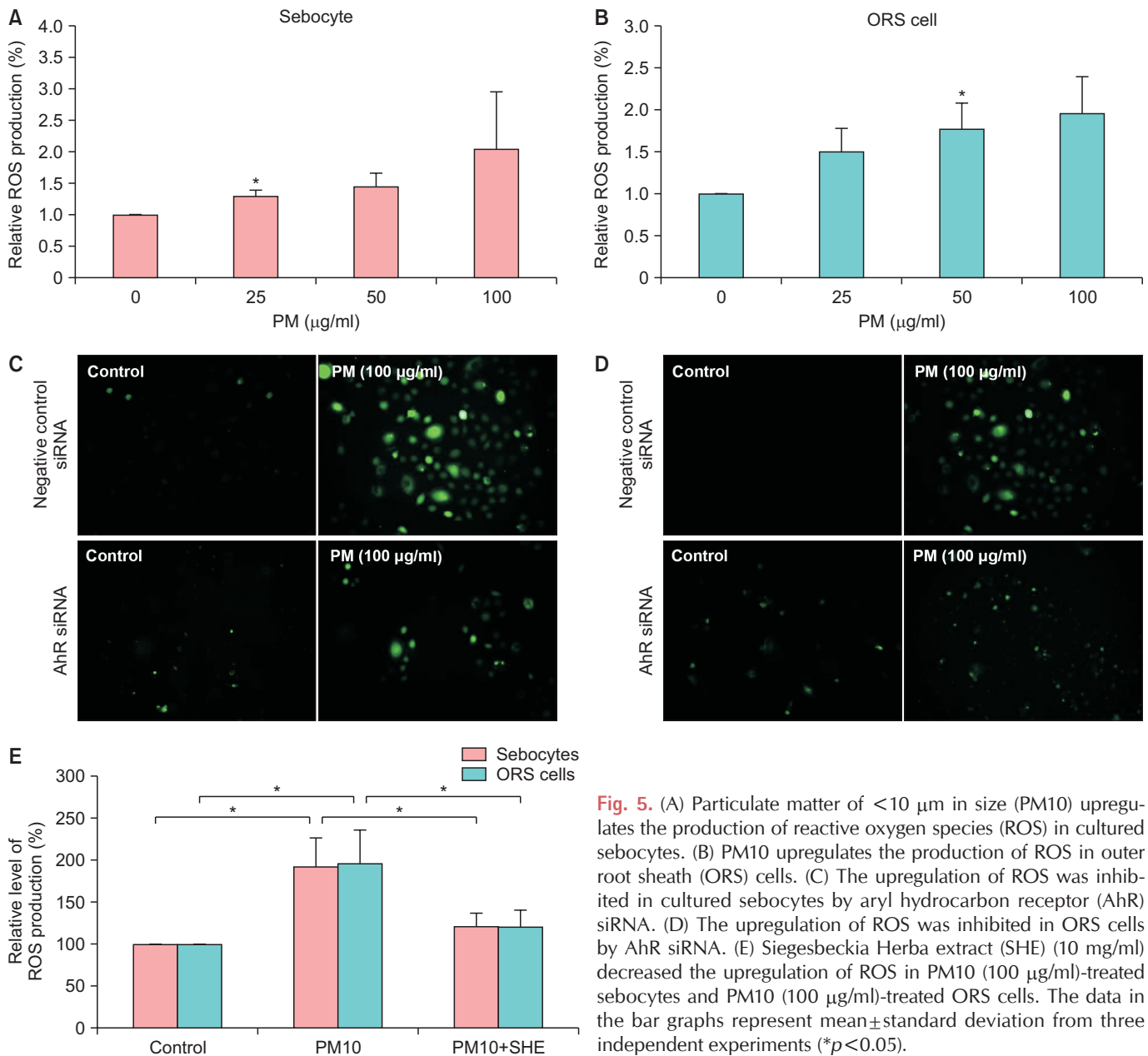


**Fig. 4.** (A) Particulate matter of <math><10\ \mu\text{m}</math> in size (PM10) upregulates the gene expression of aryl hydrocarbon receptor (AhR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in cultured sebocytes and outer root sheath (ORS) cells. (B) PM10 upregulates protein expression of AhR and NF-κB in cultured sebocytes and ORS cells. (C) *Siegesbeckia Herba* extract (SHE) (10 mg/ml) decreased the upregulation of AhR in PM10 (100 μg/ml)-treated sebocytes. (D) SHE (10 mg/ml) decreased the upregulation of NF-κB in PM10 (100 μg/ml)-treated ORS cells. The data in the bar graphs represent mean ± standard deviation from three independent experiments (\* $p < 0.05$ ).

observed in the expression of sebum production-related genes. We examined the expression of lipids through Oil Red O staining and Nile red staining after PM10 treatment of human scalp skin for 48 h to verify the production of sebum-related genes in sebocytes. Increased sebum production was observed in the sebaceous glands of the human scalp skin organ culture after treatment with 100 μg/ml of PM10 in Oil Red O and Nile red staining (Fig. 6B). SHE (10 mg/ml) downregulated the expressions of PPAR-γ, SREBP1a, SREBP1c, and SCD in PM10 (100 μg/ml)-treated sebocytes ( $p < 0.05$ ) (Fig. 6C).

## DISCUSSION

Nitrogen dioxide (NO<sub>2</sub>) stimulates AhR and activates human keratinocytes resulting in the release of increased amounts of proinflammatory cytokines through the creation of free radicals. In this study, the synthesis of ROS was increased in the sebocytes and ORS cells by PM10. AhR was upregulated in the sebocytes and ORS cells in this study. We found that the production of ROS was decreased after the inhibition of AhR with siRNA. AhR is a receptor for PM10 resulting in the produc-

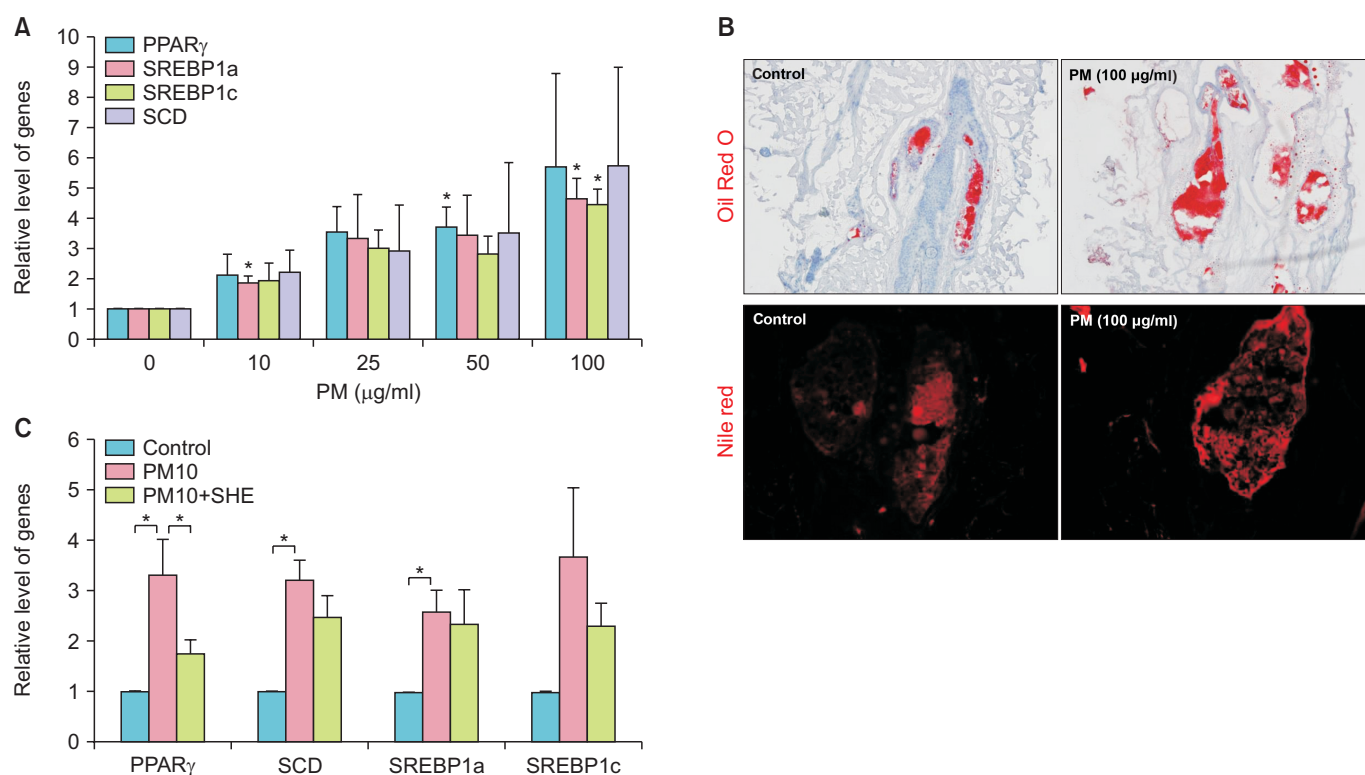


**Fig. 5.** (A) Particulate matter of  $<10\ \mu\text{m}$  in size (PM10) upregulates the production of reactive oxygen species (ROS) in cultured sebocytes. (B) PM10 upregulates the production of ROS in outer root sheath (ORS) cells. (C) The upregulation of ROS was inhibited in cultured sebocytes by aryl hydrocarbon receptor (AhR) siRNA. (D) The upregulation of ROS was inhibited in ORS cells by AhR siRNA. (E) Siegesbeckia Herba extract (SHE) (10 mg/ml) decreased the upregulation of ROS in PM10 (100  $\mu\text{g/ml}$ )-treated sebocytes and PM10 (100  $\mu\text{g/ml}$ )-treated ORS cells. The data in the bar graphs represent mean  $\pm$  standard deviation from three independent experiments ( $*p < 0.05$ ).

tion of ROS. SHE also reduced the upregulation of AhR and ROS in the cultured sebocytes and ORS cells. ROS then subsequently stimulates the release of proinflammatory mediators<sup>12</sup>. Oxidative stress induces complex biological processes that lead to the activation of transcription factors, such as activator protein 1 and NF- $\kappa$ B, cell growth and differentiation, and the degradation of the connective tissue of the dermis. The expression of NF- $\kappa$ B was increased in the sebocytes and ORS cells by PM10 in this study. SHE also reduced the upregulation of NF- $\kappa$ B in the cultured sebocytes and ORS cells. PM10

increases the expression of proinflammatory cytokines and MMP-1 in human epidermal keratinocytes<sup>13</sup>. In this study, the expression of proinflammatory mediators, such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$ , was upregulated in the sebocytes and ORS cells. Furthermore, the expression of MMPs in the cultured sebocytes and ORS cells was increased. Some studies have indicated that PM2.5 activated NF- $\kappa$ B and the induction of inflammatory genes, such as IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8<sup>14,15</sup>. SHE also reduced the upregulation of IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  and MMPs in the cultured sebocytes and





**Fig. 6.** Particulate matter of  $<10\ \mu\text{m}$  in size (PM10) upregulates sebum production in cultured sebocytes. (A) PM10 upregulates the expression of sebum production-related genes in cultured sebocytes. (B) Sebum production was increased in sebaceous glands of the human scalp skin organ culture after treatment with  $100\ \mu\text{g/ml}$  of PM10 in Oil Red O staining and Nile red staining ( $\times 20$ ). (C) Siegesbeckia Herba extract downregulated these expressions in the cultured sebocytes ( $*p < 0.05$ ). PPAR: peroxisome proliferator-activated receptor, SREBP: sterol regulatory element-binding protein, SCD: stearyl-CoA desaturase.

ORS cells.

Interestingly, the present study showed that PM10 increased the production of sebum and the expression of sebum-related transcription factors, such as PPAR- $\gamma$ , SREBP1a, SREBP1c, and SCD. Furthermore, SHE reduced the expression of the sebum production-related biomarkers in the cultured sebocytes. Increased exposure to PM2.5 was associated with an increased sebum secretion in human skin<sup>16</sup>. Some studies revealed that the prevalence of oily skin was considerably higher in areas with a high level of air pollution in China and Mexico<sup>17</sup>. In addition, some air pollutants decreased sebum secretion<sup>18</sup>. PM10 showed fluctuations in the sebum levels. Some studies highlighted that PM2.5 reduced sebum synthesis in human SZ95 sebocytes<sup>19</sup>.

Based on this study, PM10 can be considered in clinical area including hair loss or hair disease. Jun et al.<sup>20</sup> reported particulate matters induce apoptosis through increased production of ROS and inflammatory cytokines in human hair follicular keratinocytes, leading to impairment of hair growth. Antioxidant and anti-inflammatory agents such as SHE may

be useful for PM-related clinical areas. Lee and Lee<sup>21</sup> described that susceptibility to environmental factors may provide a clinical evidence to support the pathogenesis of alopecia areata. New research, which was presented at the 28th European Academy of Dermatology and Venereology (EADV) Congress in Madrid, revealed air pollution can be linked to hair loss.

In conclusion, PM10 can affect the biologic process of sebocytes and ORS cells, including inflammation and sebum production and SHE can inhibit the effects of PM10 on sebocytes and ORS cells.

## SUPPLEMENTARY MATERIALS

Supplementary data can be found via <http://anndermatol.org/src/sm/ad-34-163-s001.pdf>.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

## FUNDING SOURCE

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute and funded by the Ministry of Health and Welfare, Republic of Korea (grant number: HP20C0004).

## DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## REFERENCES

1. Pöschl U. Atmospheric aerosols: composition, transformation, climate and health effects. *Angew Chem Int Ed Engl* 2005;44:7520-7540.
2. Dagouassat M, Lanone S, Boczkowski J. Interaction of matrix metalloproteinases with pulmonary pollutants. *Eur Respir J* 2012;39:1021-1032.
3. Morakinyo OM, Mokgobu MI, Mukhola MS, Hunter RP. Health outcomes of exposure to biological and chemical components of inhalable and respirable particulate matter. *Int J Environ Res Public Health* 2016;13:592.
4. Lademann J, Schaefer H, Otberg N, Teichmann A, Blume-Peytavi U, Sterry W. [Penetration of microparticles into human skin]. *Hautarzt* 2004;55:1117-1119. German.
5. Vierkötter A, Schikowski T, Ranft U, Sugiri D, Matsui M, Krämer U, et al. Airborne particle exposure and extrinsic skin aging. *J Invest Dermatol* 2010;130:2719-2726.
6. Mills NL, Miller MR, Lucking AJ, Beveridge J, Flint L, Boere AJ, et al. Combustion-derived nanoparticulate induces the adverse vascular effects of diesel exhaust inhalation. *Eur Heart J* 2011;32:2660-2671.
7. Kim J, Kim EH, Oh I, Jung K, Han Y, Cheong HK, et al. Symptoms of atopic dermatitis are influenced by outdoor air pollution. *J Allergy Clin Immunol* 2013;132:495-498.e1.
8. Park S, Seok JK, Kwak JY, Suh HJ, Kim YM, Boo YC. Anti-inflammatory effects of pomegranate peel extract in THP-1 cells exposed to particulate matter PM10. *Evid Based Complement Alternat Med* 2016;2016:6836080.
9. Uysal U, Seremet S, Lamping JW, Adams JM, Liu DY, Swerdlow RH, et al. Consumption of polyphenol plants may slow aging and associated diseases. *Curr Pharm Des* 2013;19:6094-6111.
10. Lee SI, Kim HJ, Boo YC. Effect of green tea and (-)-epigallocatechin gallate on ethanol-induced toxicity in HepG2 cells. *Phytother Res* 2008;22:669-674.
11. Ha JW, Boo YC. Siegesbeckiae herba extract and chlorogenic acid ameliorate the death of HaCaT keratinocytes exposed to airborne particulate matter by mitigating oxidative stress. *Antioxidants (Basel)* 2021;10:1762.
12. Choi H, Shin DW, Kim W, Doh SJ, Lee SH, Noh M. Asian dust storm particles induce a broad toxicological transcriptional program in human epidermal keratinocytes. *Toxicol Lett* 2011;200:92-99.
13. Seok JK, Lee JW, Kim YM, Boo YC. Punicalagin and (-)-epigallocatechin-3-gallate rescue cell viability and attenuate inflammatory responses of human epidermal keratinocytes exposed to airborne particulate matter PM10. *Skin Pharmacol Physiol* 2018;31:134-143.
14. Romani A, Cervellati C, Muresan XM, Belmonte G, Pecorelli A, Cervellati F, et al. Keratinocytes oxidative damage mechanisms related to airborne particle matter exposure. *Mech Ageing Dev* 2018;172:86-95.
15. Zhang Y, Zheng L, Tuo J, Liu Q, Zhang X, Xu Z, et al. Analysis of PM<sub>2.5</sub>-induced cytotoxicity in human HaCaT cells based on a microfluidic system. *Toxicol In Vitro* 2017;43:1-8.
16. Rembiesa J, Ruzgas T, Engblom J, Holefors A. The impact of pollution on skin and proper efficacy testing for anti-pollution claims. *Cosmetics* 2018;5:4.
17. Krutmann J, Moyal D, Liu W, Kandahari S, Lee GS, Nopadon N, et al. Pollution and acne: is there a link? *Clin Cosmet Investig Dermatol* 2017;10:199-204.
18. Oh SJ, Yoon D, Park JH, Lee JH. Effects of particulate matter on healthy skin: a comparative study between high- and low-particulate matter periods. *Ann Dermatol* 2021;33:263-270.
19. Liu Q, Wu J, Song J, Liang P, Zheng K, Xiao G, et al. Particulate matter 2.5 regulates lipid synthesis and inflammatory cytokine production in human SZ95 sebocytes. *Int J Mol Med* 2017;40:1029-1036.
20. Jun MS, Kwack MH, Kim MK, Kim JC, Sung YK. Particulate matters induce apoptosis in human hair follicular keratinocytes. *Ann Dermatol* 2020;32:388-394.
21. Lee YB, Lee WS. Alopecia areata and particulate matter: a 5-year retrospective study in Korea. *J Eur Acad Dermatol Venereol* 2020;34:e751-e754.