Staphylococcus epidermidis WF2R11 Suppresses PM$_{2.5}$-Mediated Activation of the Aryl Hydrocarbon Receptor in HaCaT Keratinocytes

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Abstract
The skin supports a diverse microbiome whose imbalance is related to skin inflammation and diseases. Exposure to fine particulate matter (PM$_{2.5}$), a major air pollutant, can adversely affect the skin microbiota equilibrium. In this study, the effect and mechanism of PM$_{2.5}$ exposure in HaCaT keratinocytes were investigated. PM$_{2.5}$ stimulated the aryl hydrocarbon receptor (AhR) to produce reactive oxygen species (ROS) in HaCaT cells, leading to mitochondrial dysfunction and intrinsic mitochondrial apoptosis. We observed that the culture medium derived from a particular skin microbe, Staphylococcus epidermidis WF2R11, remarkably reduced oxidative stress in HaCaT cells caused by PM$_{2.5}$-mediated activation of the AhR pathway. Staphylococcus epidermidis WF2R11 also exhibited inhibition of ROS-induced inflammatory cytokine secretion. Herein, we demonstrated that S. epidermidis WF2R11 could act as a suppressor of AhRs, affect cell proliferation, and inhibit apoptosis. Our results highlight the importance of the clinical application of skin microbiome interventions in the treatment of inflammatory skin diseases.

Keywords Particulate matter · Keratinocyte · Aryl hydrocarbon receptor · Staphylococcus epidermidis · Skin microbiome

Introduction
Air pollution from particulate matter (PM) has detrimental effects on humans and animals [1]. Industries, cars, coal-fired power plants, and other anthropogenic activities contribute to air pollution, adversely affecting the environment and human health [2]. The US Environmental Protection Agency (EPA) classified PM according to particle size as PM$_{0.1}$ (ultrafine, ≤0.1 µm), PM$_{2.5}$ (fine, ≤2.5 µm), and PM$_{10}$ (coarse, ≤10 µm) [3]. PM$_{0.1}$ and PM$_{2.5}$ are the most prevalent among all types of PM. The adhesion of contaminants, oxidizing gases, organic compounds, or transition metals to PM$_{0.1}$ and PM$_{2.5}$ introduces toxins to the body [4, 5].

The skin, the largest organ of the human body, is a multilayered structure comprising the epidermis, dermis, and subcutaneous tissues [6, 7]. It is the primary barrier against external contaminants and serves as a repository for millions of microorganisms [8]. The composition of the skin microbial community depends upon the site and the presence of external irritants [9]. For instance, differences in fatty acid concentrations in the composition of a specific skin site cause changes in the abundance of bacterial species [10–13]. In a lipophilic environment, Propionibacterium species dominate, whereas, in moist areas, Staphylococcus and Corynebacterium species dominate. An imbalance in the composition of bacterial species—i.e., dysbiosis—destroys the diversity of the microbial community, causing deterioration and inflammation of the skin and a plethora of skin disorders [12, 14–16]. Airborne PM$_{2.5}$ can cause severe skin diseases, including eczema, upon permeating the human skin cells [17]. However, alterations in skin microbiomes as a result of PM$_{2.5}$-induced dermatitis have not been evaluated.

PAHs contained in PM$_{2.5}$ mediate many biological effects, including carcinogenicity and developmental defects [18–20]. Several PAHs are direct activators of the aryl hydrocarbon receptor (AhR) and may subsequently play a crucial role in the induction and action of cytochrome P450 (CYP) 1A1 and 1B1 [19, 21]. Most PAHs are not

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water-soluble, but substances such as anthracene, benz[a]anthracene, and benzo[a]pyrene are capable of secondary oxidation to water-soluble oxygen metabolites (oxy-PAH,) [22–24]. These PAHs can act as potent regulators of CYP1 family enzymes and trigger the activation of AhR and sub-mechanisms (Table 1) [25]. Therefore, CYP1 and Cox-2 are deemed important functional biomarkers involved in AhR-mediated signaling. In addition to the PAHs constituting PM$_{2.5}$, PM$_{2.5}$ comprises various transition metals and organic compounds, which can induce excessive ROS production in mitochondria and cell cytoplasm [26]. It induces oxidative stress directly in the epidermis and dermis, triggers inflammation, and affects cell proliferation and apoptosis [27].

Simultaneously, increased free radical production in skin tissue stimulates the release of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukins (ILs). These cytokines induce activated neutrophil infiltration and phagocytic production of radicals [28, 29]. Excessive accumulation of ROS triggers apoptosis through Cox-2 and TNF-α. Nuclear factor-kappa B (NF-κB) translocation to the nucleus induces apoptosis by downregulating Bcl-2, upregulating Bax, releasing cytochrome-c from mitochondria, and activating caspases [30–32]. We investigated whether *Staphylococcus epidermidis* WF2R11, a member of the human skin microbiome, alleviates the oxidative stress generated due to PM$_{2.5}$-mediated ROS accumulation, and whether *S. epidermidis* WF2R11 inhibits PM$_{2.5}$-induced apoptosis in human skin keratinocytes (HaCaT) in vitro.

**Methods**

**AhR Complex Gene Set Score Analysis of Gene Expression Data from Public Resources**

All processed gene expression data used in this study were procured from the Gene Expression Omnibus repository with accession number GSE107871 [30]. This RNA-seq dataset contained 24 samples, which consisted of the lesion site of a psoriasis patient, non-lesion area of the psoriasis patient, and skin from normal control groups [30]. The processes of patient recruitment, sampling, and RNA sequencing have been previously described by Mohan [30]. To

| PAHs                  | Mass fraction (mg/kg) | Water-soluble (at 25 °C) | AhR regulation | CYP regulation |
|-----------------------|-----------------------|--------------------------|----------------|----------------|
| Phenanthrene          | 4.86 ± 0.17           | Soluble (1.15 mg/L)      | Activation     | Activation     |
| 2-Methylphenanthrene  | 0.96 ± 0.12           | Insoluble                |                |                |
| 3-Methylphenanthrene  | 0.614 ± 0.067         | Insoluble                |                |                |
| Fluoranthene          | 8.07 ± 0.14           | Soluble (256 μg/L)       | Activation     | Inhibition     |
| Pyrene                | 5.88 ± 0.07           | Insoluble                |                |                |
| Benzo[g,h,i]fluoranthene | 1.17 ± 0.05       | Insoluble                |                |                |
| Benz[a]anthracene     | 2.71 ± 0.15           | Insoluble                |                |                |
| Chrysene              | 6.12 ± 0.06           | Insoluble                |                |                |
| Triphenylene          | 2.04 ± 0.13           | Soluble (6.6 μg/L)       |                |                |
| Benzo[k]fluoranthene  | 3.03 ± 0.24           | Insoluble                |                |                |
| Benzo[e]pyrene        | 4.85 ± 0.07           | Soluble (0.2 to 6.2 μg/L)|                |                |
| Benzo[a]pyrene        | 2.57 ± 0.10           | Soluble (0.2 to 6.2 μg/L)| Activation     | Activation     |
| Perylene              | 0.742 ± 0.048         | Nearly insoluble         |                |                |
| Benzo[g,h,i]perylene  | 5.00 ± 0.18           | Insoluble                |                |                |
| Indeno[1,2,3,-cd]pyrene | 4.17 ± 0.17       | Insoluble                |                |                |
| Dibenz[a,j]anthracene | 0.407 ± 0.039        | Soluble (0.00166 mg/L)   | Activation     | Activation     |
| Benzo[b]chrysene      | 0.405 ± 0.041         | Insoluble                |                |                |
| Picene                | 0.586 ± 0.058         | Insoluble                |                |                |
| Coronene              | 2.28 ± 0.10           | Soluble (0.14 μg/L)      | Activation     | Activation     |
| Dibenzo[b,k]fluoranthene | 0.947 ± 0.054     | Insoluble                |                |                |
| Dibenzo[a,e]pyrene    | 0.622 ± 0.045         | Insoluble                |                |                |
calculate the AhR score, gene set variation analysis (GSVA) was performed using the AhR complex gene set of harmony database using the GSVA package in R Project 4.2.0 program [33]. In the GSVA package, the “ssgsea” method was used, and the analysis was carried out between a minimum size of 10 and a maximum size of 500 [34].

Human Skin Sample Collection and Preparation

The study for the isolation of permanent bacteria on the skin was approved by the Institutional Review Board (IRB; P01-201,605–31-003) of Korea National Institute for Bioethics Policy (KONIBP). All study protocols adhered to relevant ethical guidelines. In addition, all participants provided written informed consent before enrolment. In addition, this study protocols adhered to relevant ethical guidelines and regulations. Skin samples were collected from donors who had no history of skin disease. Before collecting the skin samples, the participants were asked not to wash their faces for longer than 12 h. Samples were obtained by rubbing the donor’s face for 1 min or 20 times vigorously with a sterilized cotton swab soaked in distilled water and were then placed in a 10-mL trophic soy broth (TSB) solution.

Microbial Sample Isolation and 16S rRNA PCR Amplification and Sequencing

The skin samples collected from ten donors were diluted to 10⁻¹–10⁻³ fold using phosphate-buffered saline (PBS). Then, 100 µL of each diluted solution was spread onto R2A, TSB, Luria–Bertani, MRS (De Man, Rogosa, and Sharpe), and blood (Columbia agar with 5% sheep blood) agar plates (Bio-Rad, Hercules, CA, USA). The inoculated plates were incubated at 37 °C for up to 72 h; whereafter, the single colonies on the plates were picked up, and their 16S rRNA genes were amplified using colony PCR. The applied parameters for the PCR were as follows: initial denaturation at 95 °C for 15 min, then 32 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min and 45 s, and then a final extension step at 72 °C for 5 min. The primers for PCR were 27F (5'-AGAGTTTGATCCTGCTGCTAG-3') and 1492R (5'-GGTTACCTTGATCAGTTCTAG-3'). Purification of the amplified DNA was carried out using the EZ-pure PCR Purification Kit (Ver 2, Enzymomics, Daejeon, South Korea), and the nucleotide sequences of the genes were determined using the ABI 3730xl system (Macrogen, Seoul, South Korea). For phylogenetic analysis, the 16S rRNA gene sequences were analyzed using the nucleotide BLAST program available at the NCBi website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

Hemolysis Tests for Isolated Bacteria

For hemolysis testing of the isolated bacteria, 73 single colonies were obtained by streaking bacterial samples onto TSB plates using an inoculation loop. The colonies were re-streaked onto blood agar plates (trypticase soy agar with 5% sheep blood) and incubated at 37 °C. The color of the colonies, indicating the hemolysis pattern, was noted at 48 h. From the isolation and subsequent analysis of 16S rRNA gene sequences, 50 species and 135 strains, including a variety of skin bacteria, were identified (data not shown). Hemolysis analysis of the isolated bacteria showed that most of the microbes had a β (35 strains) or γ pattern (37 strains), and the α pattern (1 strain) was not frequently observed (data not shown). For further analyses, six strains that showed γ (gamma) hemolysis patterns were selected.

Preparation of PM2.5

Urban PM NIST 1648a (PM2.5) was purchased from Sigma-Aldrich, St. Louis, MO, USA. The PM2.5 dose used in this study was established based on USEPA air quality standards for particulate pollution and AQI (Air Quality Index) revisions. The composition and AQI category of Urban PM NIST 1648a are indicated in Table 1. PM2.5 stock solutions (50, 100, and 200 µg/mL) were prepared in DMEM and sonicated for 10 min to avoid agglomeration of the suspended PM2.5 particles. All experiments were performed within 1 h of stock preparation to avoid variability in PM2.5 compositions in the solution.

Cell Culture and Treatment of PM2.5 with Se Solution

HaCaT cells were purchased from PromoCell (Heidelberg, Germany). A total of 3 x 10⁵ HaCaT cells were maintained at 37 °C in an incubator with a humidified atmosphere of 5% CO₂ and are tested four times per year for mycoplasma using PCR. All studies were conducted within 6 months of the latest test date (11–2020). HaCaT cells were cultured in DMEM with 10% heat-inactivated FBS and an antibiotic–antimycotic (100 units/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) for no longer than 4 weeks before use. For PM2.5 treatment, HaCaT cells were inoculated in 6-well plates, incubated in an atmosphere of 5% CO₂ at 37 °C, and grown till they achieved 80% confluence. After 12 h, the cells were washed once with PBS, and 2 mL of each concentration of PM2.5 stock solution was added to the cells, depending on the purpose of the in vitro assays. Then, 10% of conditioned S. epidermidis WF2R11 medium (i.e., Se solution) was added to the cells together with a supplement-free medium simultaneously as PM2.5 treatment.
AhR, CYP1A1 Gene Knockdown

siRNA against human AhR and CYP1A1 mRNA was commercially synthesized by Bioneer, Daejeon, South Korea: siAhR (forward, 5′-CACUCAGACUCACACAU-3′, reverse, 5′-UGUGUGUAGUCGAGUG-3′); siCYP1A1 (forward, 5′-GUAGGGUAGAGGAGGC-3′, reverse, 5′-AGACUCCAGAACCCAGGC-3′). To transfet the siRNA oligo, we used Lipofectamine™ RNAiMAX (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Briefly, 24 h before transfection, 3 × 10⁵ HaCaT cells were seeded onto 6-well plates with 2.5 mL of growth medium without antibiotics to reach 50–60% confluence at the time of transfection. AhR and CYP1A1 siRNA were incubated with HaCaT cells for 24 h. The efficiency of gene silencing by siRNA was evaluated using real-time PCR (qPCR).

Cell Viability Assay

HaCaT cells (3 × 10⁵) were seeded into 48-well plates and incubated for 24 h in 2 mL of the complete medium. Then, 1, 5, 10, 20, or 30% of S. epidermidis WF2R11 culture supernatant was added to the cells, respectively, and incubated for another 48 h. After washing the cells once with PBS, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well, and the plates were incubated for another 48 h. After washing the cells once with PBS, the supernatant was added to the cells, respectively, and incubated for 4 h. Then, the medium was discarded, and dimethyl sulfoxide was added to dissolve the formazan crystals. Optical density was measured at 570 nm using a microplate reader and was normalized relative to the untreated control.

RNA Isolation and qPCR

Total RNA was isolated from cells using TRIzol reagent (TaKaRa, Shiga, Japan) according to the manufacturer’s instructions. cDNA was synthesized from 1 μg total RNA using the Reverse Transcription Premix (Elpis-Biotech, Daejeon, South Korea) under the following reaction conditions: 45 °C for 45 min and 95 °C for 5 min. Gene expression was quantified using qPCR, and data were analyzed using the StepOne Plus™ software (Applied Biosystems, Foster City, CA, USA). qPCR amplification reactions were performed using SYBR Green PCR Master Mix with premixed ROX (Applied Biosystems, Foster City, CA, USA). The following primer pairs (Bioneer, Daejeon, South Korea) were used in the ABI 7300 Cycler internal reaction according to the manufacturer’s protocol (Online Resource 7). The reaction conditions were as follows: 40 cycles for 2 min at 50 °C, 10 min at 95 °C, 10 s at 95 °C, and 1 min at 60 °C. 18S rRNA was used as an internal control.

Measurement of Proinflammatory Cytokine Concentrations

The proinflammatory cytokines (IL-1β, IL-6, IL-8, and IFN-γ) produced were measured using an ELISA assay. HaCaT cells (6 × 10⁵), seeded in 6-well culture plates, were pretreated with PM2.5 (50, 100, and 200 μg/mL) for 12 h and then treated with the supernatant of the S. epidermidis WF2R1112 h. After the treatment period, aliquots of samples (100 μL/well) were collected from the experimental medium, and the production of cytokines (IL-1β, IL-6, IL-8, and IFN-γ) was measured using a Human ILs Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions.

Western Blotting for Measuring Protein Expression

Total HaCaT cells protein was extracted using lysis buffer (Thermo Fisher Scientific, Walthan, MA, USA) supplemented with protease/phosphatase inhibitor cocktail on ice for 30 min. BCA kit was used to measure the concentration of the proteins and for protein quantification. Proteins (20 μg) were mixed with loading buffer, separated using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). The PVDF membranes were blocked with 5% skim milk in Tris-buffered saline with Tween (TBST) for 2 h at 37 °C and incubated with primary antibodies against β-actin (1:2000), Bax (1:1000 dilution), Bcl-2 (1:500 dilution), p38 (1:1000 dilution), p-p38 (1:1000 dilution), JNK (1:1000 dilution), p-JNK (1:1000 dilution), ERK (1:1000 dilution), p-ERK (1:1000 dilution), and p-ERK (1:1000 dilution) at 4 °C overnight. After washing 3 times with TBST, PVDF membranes were incubated with appropriate secondary antibodies for 1 h at room temperature. The immunoblots were visualized with a chemiluminescence detection system. The optical density (OD) of western blot bands was measured using ImageJ software (National Institute of Health, Bethesda, MD, USA). The data was normalized to the level of reference proteins and then averaged and presented as a relative fold change of control, from at least three independent experiments.

Annexin V-Pi Assay for Mitochondrial Apoptosis

For Annexin V-Pi assays, HaCaT cells were stained with Annexin V-FITC and PI and assessed for mitochondrial apoptosis using flow cytometry according to the
Mitochondrial Superoxide Detection

For the measurement of mitochondrial peroxide, HaCaT cells treated with different concentrations of PM2.5 were detected with fluorescence microscopy using MitoSOX™ Red Mitochondrial Superoxide indicator (Thermo Fisher, St. Louis, MO, USA) as a specific fluorescence probe. HaCaT cells treated with PM2.5 were incubated with 5 μM of the probe for 30 min at 37 °C in the dark. Then, cells were thoroughly washed with warm Hank’s balanced salt solution (HBSS) buffer and mounted for imaging. MitoSOX™ Red-stained cells were visualized at an excitation wavelength of 510 nm and an emission wavelength of 580 nm.

Mitochondrial Membrane Potential (Δψm) Measurement

The mitochondrial membrane potential and early stage of apoptosis were analyzed via fluorescence microscopy after staining with 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide (JC-1, Invitrogen, Carlsbad, CA, USA), a lipophilic cationic fluorescence dye. JC-1 dye is characterized by green fluorescence emission at ~529 nm in the monomeric form of the probe, which changes to red (~590 nm) with the concentration-dependent formation of red J-aggregates.

Diff-Quik Staining for Observing Cytological Features

Diff-quik (catalog no. 111661; Merck, Darmstadt, Germany) staining preparations of HaCaT cells were obtained by spreading 3 × 10^5 normal cells onto LAB-TEK® chamber slides (Thermo Fisher, St. Louis, MO, USA), treating the cells with 100 μg/mL PM2.5, and incubating the slides for 24 h at 37 °C. A thin smear of each HaCaT was stained using the diff-quik staining by manufacturer’s instructions. Briefly, the air-dried smears were sequentially dipped five times in a methanol fixative solution I, five more times in stain solution II (color reagent red), then dipped five times in stain solution III (color reagent blue), and gently rinsed with distilled water. The overall staining process took ~40 s. The slides were then cleared in xylol and mounted in a non-aqueous mounting medium. A diff-quik-stained smear of each HaCaT slide was analyzed by a board-certified pathologist (MGN).

Ki-67 Immunohistochemistry

HaCaT cells were prepared by spreading 3 × 10^5 normal cells onto LAB-TEK® chamber slides (Thermo Fisher, St. Louis, MO, USA), followed by treatment with 100 μg/mL PM2.5, and incubation for 24 h at 37 °C. After removing the chamber wall, all the slides were fixed in neutral-buffered formalin. Endogenous peroxidase activity was blocked by incubating with 3% hydrogen peroxidase in methanol for 5 min. Epitope retrieval of Ki-67 was carried out by boiling in a pressure cooker in Tris–EDTA solution buffer at pH 9.0 for 15 min at 99 °C. After washing with PBS, the sections were incubated with the anti-human Ki-67 polyclonal antibody (ab15580, rabbit anti-human; Abcam, Cambridge, MA, USA) at a dilution of 1:600 using an antibody diluent (GBI Labs, Bothell, WA, USA) for 60 min at 23–25.5 °C. The primary antibody against Ki-67 was detected with Polink-2 Plus HRP Rabbit with DAB Kit (GBI Labs, Bothell, WA, USA) for 30 min at approximately 23–25.5 °C. Diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) was used as the chromogen and incubated for 3 min at 25 °C. The slides were counterstained with hematoxylin. For the positive control, a normal tonsil tissue was used; for the negative control, the primary antibody was omitted, and PBS was used in each experiment. The Ki-67 index was quantified by estimating the number of positive HaCaT cells expressing nuclear Ki-67 (brown colored) among the total number of HaCaT cells. Both weakly and strongly labeled nuclei were included in the estimates of proliferating cells. All immunostained slides were evaluated twice by a blinded board-certified pathologist (MGN). A semi-quantitative assessment of the average percentage of Ki-67 positive cells in several fields comprising more than 1000 cells was performed.

Statistical Analysis

All data were tested for normality, and the dataset was analyzed using one-way ANOVA. The post hoc analysis was then carried out using the Bonferroni test for comparison between pairs. All results are presented as the mean ± SEM. Correlations were determined using Pearson’s correlation analysis. All statistical analyses were performed using GraphPad Prism 5.02 (GraphPad Software, San Diego, CA, USA) and R-4.2.0 for Windows. The statistical significance was set at p < 0.05.
PM2.5 activates the AhR signaling pathway, leading to ROS production. (a) Correlation analysis of AhR with ARNT expression reveals their positive correlation at the mRNA level. (b) Measurement of the mRNA level of AhR relative to 18S rRNA. (c) Measurement of the mRNA level of ARNT relative to 18S rRNA. (d) Correlation analysis of AhR with CYP1AI expression reveals their positive correlation at the mRNA level. (e) Measurement of the mRNA level of CYP1AI relative to 18S rRNA. (f) Correlation analysis of AhR with Cox-2 expression reveals their positive correlation at the mRNA level. (g) Measurement of the mRNA level of Cox-2 relative to 18S rRNA. (h) Correlation analysis of AhR with the hallmark of oxidative stress reveals their positive correlation at the mRNA level. PM2.5 treatment in HaCaT cells, mRNA expression reveals their positive correlation at the mRNA level.

PM2.5 activates the AhR signaling pathway, leading to ROS production. (a) Correlation analysis of AhR with ARNT expression reveals their positive correlation at the mRNA level. (b) Measurement of the mRNA level of AhR relative to 18S rRNA. (c) Measurement of the mRNA level of ARNT relative to 18S rRNA. (d) Correlation analysis of AhR with CYP1AI expression reveals their positive correlation at the mRNA level. (e) Measurement of the mRNA level of CYP1AI relative to 18S rRNA. (f) Correlation analysis of AhR with Cox-2 expression reveals their positive correlation at the mRNA level. (g) Measurement of the mRNA level of Cox-2 relative to 18S rRNA. (h) Correlation analysis of AhR with the hallmark of oxidative stress reveals their positive correlation at the mRNA level. PM2.5, AhR, ARNT, CYP1AI, and Cox-2 relative to 18S rRNA was measured after 12 h of PM2.5 treatment for each concentration. Correlation was determined using Pearson’s correlation analysis. Bonferroni test for comparison between pairs was used to calculate statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001, or ns, non-significant; compared to normal HaCaT cells. AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator.

**Results**

**PM2.5 Stimulates AhR to Induce ROS Production in HaCaT Cells**

AhR signaling is associated with exposure to PM2.5 and ROS production [35]. To reaffirm the relationship between PM2.5, AhR stimulation, and ROS production, we performed RNA-seq analysis of whole skin using open-access data from a study by Swindell [36]. The transcriptomic analysis involved in the AhR signaling pathway were identified to predict the association between the AhR-related genes and ROS production. We first analyzed the correlation between AhR and AhR nuclear translocator (ARNT) upon exposure to PM2.5. AhR expression was positively correlated with ARNT expression ($p = 0.012$, $r = 0.506$) (Fig. 1a). To prove the positive correlation of AhR-ARNT gene expression evidenced by transcriptomic analysis, after PM2.5 treatment in HaCaT cells, mRNA expression levels of both genes were measured. The dosage of PM2.5 classified each concentration of PM2.5 in accordance with EPA standards (Online Resource 1). Each concentration of PM2.5 used in the following experiments was set to moderate (PM2.5: 50 µg/mL), unhealthy (PM2.5: 100 µg/mL), hazardous (PM2.5: 200 µg/mL). Similar to the correlation between the two genes presented in Fig. 1a, AhR and ARNT mRNA expression significantly increased as the PM2.5 concentration increased, suggesting the possibility that PM2.5 may act as a ligand for AhR and induce the AhR/ARNT complex formation (Fig. 1b, c). As a sub-mechanism of the AhR/ARNT complex, CYP1AI and Cox-2 play important roles in ROS production in the cytoplasm [37, 38]. The expression of CYP1AI positively correlated with AhR expression ($p = 0.001$, $r = 0.640$; Fig. 1d). Furthermore, the expression of Cox-2 positively correlated with that of AhR ($p = 0.002$, $r = 0.581$; Fig. 1f). The mRNA expression of each of these two genes increased from approximately fivefold to over 27-fold upon exposure to PM2.5 at minimal and maximal concentrations of 50 µg/mL and 200 µg/mL, respectively (Fig. 1e, g). PM2.5 reportedly upregulates ROS production and induces oxidative stress via AhR signaling ($p = 0.008$, $r = 0.522$; Fig. 1h) [37, 39]. To observe mitochondrial ROS accumulation, HaCaT cells were treated with varying concentrations of PM2.5, and the ROS levels were observed using a Mitosox fluorescent dye that specifically targets mitochondria (Fig. 1i). The increase in red fluorescence is related to the oxidation of the Mitosox Red reagent by peroxide in the mitochondria. Therefore, as the concentration of PM2.5 increased, ROS accumulation in the mitochondria was observed to increase substantially.

**PM2.5 Induces Mitochondrial Dysfunction and Promotes Intrinsic Mitochondrial Apoptosis**

Free radicals that have accumulated in the cytoplasm initiate oxidative damage mechanisms in various organelles, including mitochondria [31, 40]. Accumulation of cytoplasmic ROS in HaCaT cells causes mitochondrial dysfunction, increasing the expression of the mitochondrial apoptosis factor, Bax, and decreasing the expression of apoptosis inhibitor, Bcl-2 [41, 42]. Thus, the increase in the ratio of Bax/Bcl-2 via ROS is defined as a direct marker of mitochondrial damage. Using correlation analysis, Bax was positively correlated with the expression of Cox-2 ($p < 0.001$, $r = 0.785$; Fig. 2a). After treatment with 100 µg/mL PM2.5, the mRNA level of Bax was significantly increased compared to that of the control HaCaT cells and the positive control polyinosinic:polycytidylic acid (poly I:C group) (Fig. 2b). Meanwhile, the expression of Bcl-2 showed significant negative correlation with Cox-2 expression ($p < 0.001$, $r = 0.512$) in the transcriptomic analysis (Fig. 2c). The mRNA levels of Bcl-2 also showed an inverse trend compared to that of Bax. Although not significant, the reduction in Bcl-2 mRNA levels was remarkable compared to that of the control HaCaT cells (Fig. 2d). Also, the hallmark genes of oxidative stress induced by PM2.5 showed a significant positive correlation with the Kyoto Encyclopedia of Genes and Genomes (KEGG) category, apoptosis ($p < 0.001$, $r = 0.769$; Fig. 2e). Compared to the normal HaCaT cells and poly I:C-treated group, the PM2.5-treated group was evaluated as a factor that significantly increased mitochondrial dysfunction (Fig. 2f). Furthermore, the intercellular density of the control HaCaT cells and the PM2.5-treated cells (100 µg/mL) was compared and analyzed. Control HaCaT cells showed high intercellular density and evidence of persistent mitosis, whereas, in the
Oxidative stress caused by ROS accumulation in the cytoplasm is closely related to apoptosis. (a) Correlation analysis of Cox-2 with Bax expression reveals their positive correlation at the mRNA level. (b) Measurement of the mRNA level of Bax relative to 18S rRNA. (c) Correlation analysis of Cox-2 with Bcl-2 expression reveals their negative correlation at the mRNA level. (d) Measurement of the mRNA level of Bcl-2 relative to 18S rRNA. (e) Correlation analysis of hallmark genes of oxidative stress with KEGG-apoptosis reveals their positive correlation. (f) Measurement of the relative Bax/Bcl-2 ratio for mitochondrial-dependent cell death after PM2.5 treatment.

(g) Diff-quick staining for observing cytological features of 100 μg/mL PM2.5-treated group compared to control HaCaT cells (normal HaCaT: red circle, mitosis progression; PM2.5-treated-HaCaT: red arrows, nuclear degeneration; red circle, apoptosis). Scale bar, 50 μm. The mRNA level of Bax and Bcl-2 relative to 18S rRNA was measured after 12 h of PM2.5 treatment for each concentration. Correlation was determined using Pearson’s correlation analysis. Bonferroni test for comparison between pairs was used to calculate statistical significance. *p<0.05, **p<0.01, ***p<0.001, ns, non-significant; compared to normal HaCaT cells.
PM$_{2.5}$-treated cells, the intercellular density was low, and indicators of nuclear degeneration as well as apoptosis were evident (Fig. 2g). As a result, an increased Bax/Bcl-2 ratio in HaCaT cells may indicate the onset of intrinsic mitochondrial apoptosis upon exposure to PM$_{2.5}$ [43].

**Staphylococcus epidermidis** WF2R11 Supernatant (Se Solution) Reduces the mRNA Levels of Inflammatory Cytokines Induced via PM$_{2.5}$

The AhR signaling pathway plays an important role in controlling the innate and adaptive immune response [44, 45]. The correlation between hallmark genes related to the immune response was analyzed according to the expression level of the AhR ($p < 0.001$, $r = 0.817$; Fig. 3a). AhR signaling is also closely related to the induction of oxidative stress, as shown in previous results. Similarly, an increase in oxidative stress led to an increase in the immune response ($p < 0.001$, $r = 0.633$; Online Resource 2a). To confirm these associations, the levels of immune cytokines were measured by treating HaCaT cells with PM$_{2.5}$ at variable concentrations of 50, 100, and 200 µg/mL for 1, 2, and 4 h, respectively (Online Resource 1b–e). Subsequently, six skin-derived microbes that could significantly reduce the secretion of inflammatory cytokines induced via PM$_{2.5}$ were screened (Online Resource 3). For screening, each concentration was fixed with a 10% concentration of the modified supernatant (Online Resource 1f). IL-1β, IL-6, IL-8, and TNF-α were selected as major screening factors that could contribute to the immune response of keratinocytes. Compared to the levels of four cytokine mRNAs in the untreated HaCaT cells, cytokine secretion was significantly reduced in cells treated with the supernatant derived specifically from the *S. epidermidis* WF2R11 culture medium (Online Resource 4a-d). The mRNA levels of inflammatory cytokines IL-6, IL-8, IL-1β, and TNF-α, were significantly reduced in the groups treated with Se solution compared to the respective PM$_{2.5}$ concentrations (50, 100, 200 µg/mL)-treated groups (Fig. 3b–e). Also, upon comparing IL-1β, IL-6, IL-8, and interferon-gamma (IFN-γ) at the protein level, treatment with *S. epidermidis* WF2R11 supernatant significantly reduced protein abundance of all the cytokines mentioned above (Online Resource 4e-h). Taken together, *S. epidermidis* WF2R11 is a skin-bacterium that can significantly reduce the secretion of inflammatory cytokines induced by PM$_{2.5}$.

**Se Solution Suppresses the Accumulation of ROS in HaCaT Cells**

To determine whether the Se solution downregulates AhR signaling and reduces ROS production, HaCaT cells were treated with different concentrations of PM$_{2.5}$ (50, 100, and 200 µg/mL) and inoculated with Se solution. The treatment with Se solution reduced the mRNA level of AhR by more than 30% at each PM$_{2.5}$ concentration group (Fig. 4a). Regardless of the concentration of PM$_{2.5}$ treatment, the level of ARNT mRNA was similar to that of the control group after treatment with the Se solution (Fig. 4b). As expected, the expression of CYP1A1 was significantly reduced when inoculated with the Se solution than with only PM$_{2.5}$ (Fig. 4c). In addition, activation of AhR may contribute to the upregulation of Cox-2 expression involved in ROS generation in the cytoplasm [44]. The expression of Cox-2 was significantly increased when PM$_{2.5}$ was treated with 100 µg/ml or more. Moreover, when the Se solution was inoculated to the PM$_{2.5}$-treated group of the same concentration, the expression level of Cox-2 was significantly reduced (Fig. 4d). Furthermore, JC-1 dye was used to measure the change in mitochondrial membrane potential due to ROS accumulation upon exposure to PM$_{2.5}$. HaCaT cells stained with JC-1 showed a gradual loss of red J-aggregate fluorescence and cytoplasmic diffusion of green monomeric fluorescence after exposure to accumulated ROS in the cytoplasm. We confirmed that the cytoplasmic diffusion of green monomer fluorescence was significantly reduced when treated with the Se solution of each PM$_{2.5}$ treatment group (Fig. 4e). Based on previous results, we hypothesized that *S.epidermidis* WF2R11 is caused by the inhibition of AhR signaling pathway due to the decrease in intracellular ROS accumulation via PM$_{2.5}$. Therefore, to investigate the correlation between the *S. epidermidis* WF2R11 and the progression of the AhR signaling pathway, the AhR and the CYP1A1 genes were silenced (ΔAhR and ΔCYP1A1) in HaCaT cells using siRNA.

First, siAhR treatment reduced the mRNA expression levels of AhR, ARNT, and CYP1A1 genes to less than half in all other groups compared to the si-control group respectively. However, the Cox-2 showed a lower mRNA expression level compared to other genes when treated with siAhR. In addition, the expression level of Cox-2 in the group treated with siAhR and PM$_{2.5}$ was almost similar to that of the si-control group. The mRNA expression was significantly reduced in the Se solution treatment group compared to the PM$_{2.5}$ treatment group (Fig. 4f). Next, we determined whether *S. epidermidis* WF2R11 inhibits AhR alone or regulate sub-mechanisms by silencing the CYP1A1 gene. When CYP1A1 was silenced, the mRNA expression levels of AhR, ARNT, and Cox-2 showed a similar trend to the previous results (Fig. 4a, b, d). However, the expression of CYP1A1 gene was significantly lower than that of si-control mRNA in either the PM$_{2.5}$ treatment group or the PM$_{2.5}$ and Se solution treatment group (Fig. 4g). We also observed that the mRNA expression level of Cox-2 is independent of CYP1A1 silencing and that the expression of Cox-2 gene is not directly regulated by CYP1A1 gene expression. Therefore, the alleviation of PM$_{2.5}$-derived ROS accumulation...
via the AhR signaling pathway of *S. epidermidis* WF2R11 would directly inhibit AhR and down-regulate the sub-gene *CYP1A1*. In addition, we assumed that the regulation of Cox-2 is correlated with other mechanisms according to AhR activity or is affected by the increased intracellular ROS due to increased *CYP1A1* expression.
**Staphylococcus epidermidis** WF2R11 Inhibits Mitochondrial Apoptosis Induced by Accumulated Intracellular ROS

To investigate the mitochondrial apoptosis mechanism of PM2.5 mediated by AhR signaling, we assessed the phosphorylation levels of stress-activated protein kinases, such as c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase 1/2 (ERK), and p38 mitogen-activated protein kinase (p38 kinase). After treatment with either PM2.5 (100μg/ml) or PM2.5 (100 μg/ml) and Se solution for 12 h, the phosphorylation level of protein kinase was determined (Fig. 5a). Treatment with PM2.5 and Se solution simultaneously decreased both the expression of protein kinases and the degree of phosphorylation compared to PM2.5 only treatment. Furthermore, in the same conditions, the Bax protein expression decreased while the Bcl-2 protein expression increased in the group treated with the PM2.5 and Se solution compared to that treated with only PM2.5. We quantified the phosphorylation levels of each mitogen-activated protein kinase (MAPK), JNK, ERK, and p38 protein and calculated the Bax/Bcl-2 ratio for correlation with mitochondrial apoptosis mechanisms (Fig. 5b). Although the Bax/Bcl-2 ratio was significantly decreased in normal HaCaT cells compared to the control group (β-actin), the phosphorylation levels of p38 and JNK kinase and the Bax/Bcl-2 ratio were significantly increased following PM2.5 treatment.

In addition, Se solution had no effect on MAPKs phosphorylation or Bax/Bcl-2 ratio. Moreover, compared to other skin microbiota, *S.epidermidis* WF2R11 contributed to the observed decrease in mitochondrial Bax expression, which in turn decreased the Bax/Bcl-2 ratio caused by PM2.5 treatment (Online Resource 5a–c). However, in the group treated with PM2.5 and Se solution simultaneously, the change in the Bax/Bcl-2 ratio and the degree of phosphorylation of MAPKs were not significant compared to the control group. These results suggest that *S.epidermidis* WF2R11 decreased the Bax/Bcl-2 ratio in PM2.5-treated cells at each concentration, thereby significantly reducing mitochondrial-dependent apoptosis (Fig. 5c).

Next, we determined whether PM2.5 enables mitochondrial apoptosis via AhR signaling pathway by partially blocking AhR signaling (Fig. 5d). The Bax/Bcl-2 ratio was almost similar in the siAhR-treated group (gray color) regardless of PM2.5 or PM2.5 and Se solution treatment. However, the siCYP1A1 treatment group (pattern) showed a significant decrease in the Bax/Bcl-2 ratio compared to the si-control group following PM2.5 treatment, and a more significant decrease compared to the PM2.5 alone treatment group when Se solution was added. Furthermore, when AhR signaling was blocked by treatment with both siAhR and siCYP1A1, the Bax/Bcl-2 ratio was significantly reduced regardless of PM2.5 and Se solution treatment. Our results suggest that CYP1A1 is an important gene that mediates mitochondrial apoptosis signaling induced by PM2.5-induced intracellular ROS accumulation. In addition, *S.epidermidis* WF2R11 directly inhibits AhR to regulate the sub-mechanism of CYP1A1-induced sub-mechanisms. To further show that ROS accumulated in HaCaT cells via AhR signaling pathway induced mitochondrial apoptosis, we analyzed apoptosis by treating HaCaT cells with PM2.5 and PM2.5 and Se solution in siAhR-treated and untreated groups, respectively. We labeled cells with Annexin V-PI and observed the percentage of apoptotic cells using flow cytometry (Fig. 5e) and confirmed that PM2.5 alone treatment group significantly increased apoptosis compared to the control group. In addition, PM2.5 treatment group to which the Se solution was added showed significant decrease in apoptosis compared to the untreated group. Meanwhile, we treated siAhR-treated cells with PM2.5 and analyzed the ratio of apoptosis with and without Se solution. After siAhR treatment, no significant increase in apoptosis was observed in either group treated with PM2.5 regardless of Se solution treatment. In the PM2.5-treated group not treated with siAhR, treatment with Se solution shifted the histogram peak from apoptosis state to live state. However, in the PM2.5-treated group with siAhR, the histogram peaks of the two groups overlapped regardless of the Se solution. A significant positive correlation was also observed between the expression of Cox-2 and the Fas Associated via death domain (FADD) gene (p < 0.01, r = 0.496; Online Resource 5d). In addition to FADD, the TNFRSF1A associated via death domain (TRADD) gene showed a significant positive correlation with CYP1A1 (p < 0.01, r = 0.443; Online Resource 5e).

Ultimately, ROS accumulation via AhR signaling activation could increase TRADD gene expression due to mitochondrial-dependent apoptosis through TNF-α signaling (p < 0.05, r = 0.455; Online Resource 5f).
Staphylococcus epidermidis WF2R11 Potentially Affects the Proliferation of HaCaT Cells by Activating Anti-oxidant Activity

PM$_{2.5}$ is also known to induce DNA damage and apoptosis as well as arrest cell cycle G2/M transition due to mitochondrial dysfunction [46, 47]. The NF-E2-related factor 2 (Nrf2) and antioxidant response element (ARE) pathway are known to be involved in adaptation to oxidative stress through the upregulation of antioxidant activity and the expression of genes such as NAD(P)H-quinone dehydrogenase 1 (NQO1) and heme oxygenase-1 (HO-1).
rRNA was measured after 12 h of CYP1A1, Cox-2 treatment to calculate statistical significance. 

Comparison between pairs was used compared to each si-control group and Se solution co-treatment. 

**ns**, non-significant; comparison between PM2.5 alone treatment group, the Ki-67 index was approximately 50% (Online Resource 6e). These results showed changes in Nrf2 expression and confirmed the association of the Nrf2 signaling pathway by upregulation of AhR signaling during PM2.5 treatment. Moreover, we confirmed the involvement of *S. epidermidis* WF2R11 in HaCaT cell proliferation by participating in AhR-Nrf2 signaling. Thus, we next silenced AhR and CYP1A1 and observed Nrf2 gene expression following treatment with PM2.5 (100 μg/ml), Se solution, and PM2.5 (100 μg/ml) and Se solution. The expression of Nrf2 was significantly decreased in the AhR silenced group (gray color) regardless of Se solution treatment with PM2.5 compared to the control. However, in the group (pattern) in which CYP1A1 was silenced, the expression of Nrf2 was increased more than threefold compared to the control when PM2.5 was treated. Meanwhile, when comparing the PM2.5-treated and the Se solution treated groups, the expression of Nrf2 was significantly reduced in the Se solution-treated group, although the group in which both AhR and CYP1A1 were silenced (black color) showed almost no expression of Nrf2 regardless of PM2.5 and Se solution treatment (Fig. 5f). Taken together, these results suggest that *S. epidermidis* WF2R11 effectively inhibited ROS production through AhR signaling and caused a decrease in apoptosis.

In this process, the Nrf-2 signaling pathway, a part of AhR signaling, was directly/indirectly upregulated based on AhR signaling and ROS generation, affecting the regulation of cell proliferation as well as mitochondrial-dependent cell death (Fig. 6).

**Discussion**

This study demonstrated the effect of PM2.5 exposure on the AhR pathway and mitochondrial dysfunction-dependent apoptosis. To the best of our knowledge, this is the first study to analyze how a member of skin microbiota can downregulate the AhR pathway, which leads to the inhibition of skin inflammation and ROS production. We specifically investigated the effect of *S. epidermidis* WF2R11 on the progression of the AhR pathway. The main mechanism by which PM2.5 induces cellular oxidative stress is through the generation of ROS in skin keratinocytes [51]. PM2.5 directly increases the level of ROS production and free radicals on the surface of skin keratinocytes, indicating potent redox activity in PAHs [52, 53]. In addition, oxy-PAH reportedly promotes the oxidation of nucleic acids, proteins, and lipids more than PAH, which can cause severe redox stress in cells and tissues [54, 55]. Water-soluble PAHs such as BaP, unlike other organic compounds, tend to oxidize rapidly [56], and oxy-PAHs show increased cell permeability and AhR reactivity [26]. Initiation of AhR-mediated signaling by PAHs or oxy-PAHs is first transferred to the nucleus upon binding of the PM2.5-binding AhR complexes to ARNT [26, 57]. It was evident that, when PM2.5 acted as a ligand for AhR, both the AhR and ARNT mRNA levels increased. Our results demonstrated that although *S. epidermidis* WF2R11 did not...
directly affect AhR, it reduced AhR and ARNT mRNA levels. It was initially speculated that the reduced expression of AhR and ARNT is caused by the metabolites secreted by S. epidermidis WF2R11, thereby affecting the formation of AhR/ARNT complexes. However, when AhR was knocked down in HaCaT cells, ARNT and CYP1A1 expression was inhibited regardless of treatment with PM$_{2.5}$ or the S. epidermidis WF2R11 supernatant. Although previous hypotheses have speculated that the S. epidermidis WF2R11 metabolites inhibit the formation of the AhR/ARNT complex, this finding demonstrated that AhR activation to PAH is a prerequisite for ARNT and CYP1A1 expression. We therefore suggest that the S. epidermidis WF2R11 metabolites may operate by suppressing AhR activity on PAHs or act as a competitive ligand inhibitor, rather than directly inhibiting the formation of the AhR/ARNT complexes. AhR/ARNT heterodimers bind to xenobiotic responsive elements and activate the transcription of sub-target genes such as CYP1 [58]. CYP1 is a key factor capable of promoting ROS accumulation in the cytoplasm, concomitantly causing oxidative stress [59, 60]. In addition, CYP enzymes induce ROS accumulation in the cytoplasm by damaging keratinocytes and altering DNA formation [25, 37, 61]. The mRNA level of CYP1A1 was significantly increased after PM$_{2.5}$ treatment, while S. epidermidis WF2R11 was involved in the inhibition of AhR activity, thereby reducing the expression of CYP1A1. Thus, the AhR/ARNT complex affects the activity of the CYP1 enzyme, thereby increasing the expression of CYP1A1 [39, 62]. In addition, cytoplasmic ROS production by Cox-2 overexpression in HaCaT cells critically exerts oxidative stress on various organelles, including mitochondria [59, 63]. Signaling due to AhR activity was associated with an increase in CYP1A1 and in Cox-2 expression [64]. In particular, when AhR was silenced, the expression of Cox-2 was significantly reduced, which was restored following PM$_{2.5}$ treatment to the same level as that of si-control. However, despite silencing CYP1A1, signal transduction of AhR caused an increase in the expression of Cox-2, which suggests that the expression of Cox-2 is determined somewhat independently of the sub-mechanism of CYP1A1, and its expression may be regulated by AhR activity. Moreover, PAH and oxy-PAH result in differential ROS generation and Ca$^{2+}$ perturbation and promote electrophysiological instability, which increases mitochondrial inner membrane permeability. Furthermore, an increase in ROS not only produce a cytotoxic effect, but also activate MAPK pathways, including JNK, ERK, and p38 MAPK, which ultimately lead to mitochondrial stress and excessive free radical accumulation, resulting in mitochondrial dysfunction and apoptosis (i.e., the intrinsic pathway of apoptosis) [65–69]. We visualized mitochondrial superoxide and membrane potential after PM$_{2.5}$ and Se solution treatment via fluorescent staining (Mito-Sox, JC-1), suggesting that the metabolite of S. epidermidis WF2R11 is effective in suppressing PM$_{2.5}$-induced oxidative stress. Furthermore, we demonstrated that the metabolites of S. epidermidis WF2R11 can significantly reduce phosphorylation of p38 kinase and JNK among ROS-activated MAPK subgroups. Bax is phosphorylated by stress-activated p38 kinase and/or JNK and phosphorylation of Bax leads to mitochondrial translocation prior to apoptosis. The mitochondrial translocation of pro-apoptotic Bax can act as apoptosis stimulators or conditions that induce mitochondrial apoptosis [70, 71]. PM$_{2.5}$-induced apoptosis upregulates Bax and downregulates Bcl-2 [68, 72–74]; therefore, a decrease in the Bax/Bcl-2 ratio suggests a decrease in mitochondrial dysfunction-dependent apoptosis in the S. epidermidis WF2R11-treated group. Although the mechanism by which increased ROS can activate ERK, JNK and p38 MAPK remain unclear [75], we found that groups treated with S. epidermidis WF2R11 did not show any significant difference in the regulation of Bcl-2 but exhibited downregulated Bax expression. In addition, since the PM$_{2.5}$-derived ROS-induced mitochondrial apoptosis mechanism is increased through the AhR signaling pathway, the change in the Bax/Bcl-2 ratio was confirmed after suppressing the AhR or CYP1A1 gene, or both. We confirmed that the key gene for PM$_{2.5}$-derived ROS increase was the expression of CYP1A1, while S. epidermidis WF2R11 suppressed AhR, the upper gene of CYP1A1, to reduce PM$_{2.5}$-derived ROS. Transcriptome analysis showed that the AhR signaling pathway not only increased the expression of CYP1A1 but...
also contributed to the activation of TNF-α signaling to induce mitochondrial apoptosis, or upregulate Cox-2, thereby contributing to the activity of p38 and JNK MAPks to enable translocation of mitochondrial Bax. Taken together, excessive oxidative stress due to the AhR pathway specifically affects the expression level of Bax via phosphorylation of MAPks and may be a major contributor to mitochondrial dysfunction [76–78]. The reduction of oxidative stress, resulting from inhibition of AhR pathway progression, may have an inhibitory effect on the upregulation of Bax, which suggests AhR as an important receptor that leads to apoptosis by causing mitochondrial dysfunction. Furthermore, the metabolites of Staphylococcus epidermidis WF2R11 can inhibit the progression of ROS-mediated damage by reducing AhR activity. Endogenous ROS can also cause structural damage and cell degradation, leading to apoptosis in HaCaT cells [33]. Previous studies showed that mitochondrial dysfunction caused by PM$_{2.5}$ triggers an inflammatory cascade [34]. Activated Toll-like receptors initiate the NF-κB pathway and secrete inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-8, and TNF-α [79]. The secretion of these cytokines promotes the rapid activity of inflammatory reactions. Activated NF-κB initiates an inflammatory cascade, which causes the skin barrier to collapse, aggravating skin inflammation and leading to apoptosis [17, 80, 81]. The finding of this study suggests that AhR regulation of microbial metabolites is expected to help alleviate these inflammatory responses.

Finally, we confirmed whether Staphylococcus epidermidis WF2R11 effectively reduced apoptosis and inflammatory responses from PM$_{2.5}$-induced oxidative stress via AhR-mediated Nrf2 signaling. Previous studies have suggested two different mechanisms for the activation of Nrf2 by AhR: direct transcriptional activation of Nrf2 following AhR signaling activation or ROS generation by CYP1A1 induction [82, 83]. Among these two hypotheses, we investigated the pathway by which metabolites of Staphylococcus epidermidis WF2R11 influence initiation of Nrf2 signaling. AhR silencing significantly reduced the expression level of Nrf2 and sub-genes such as NQO1 and HO-1 regardless of PM$_{2.5}$ or Staphylococcus epidermidis WF2R11 treatment. However, when CYP1A1 was silenced, while treating with PM$_{2.5}$, the expression of Nrf2 gene was significantly increased, whereas when PM$_{2.5}$ and Staphylococcus epidermidis WF2R11 were co-treated, the expression of Nrf2 was significantly decreased.

These results indicate that AhR, not CYP1A1, has direct transcriptional activation function for Nrf2 signal and that two other pathways of AhR may be activated simultaneously, one to increase cellular ROS and the other to trigger an antioxidant response. Furthermore, the decrease in expression Nrf2 following inhibition of AhR may possibly reduce the activation signal for other ROS generation mechanisms involved in the AhR signaling pathway. Conversely, we consider that the reason for the increase in Nrf2 during PM$_{2.5}$ treatment despite the silencing of
CYP1A1 is probably attributed to ROS generation associated with the increased expression of Cox-2 based on the activity of AhR. Thus, the direction of pathway progression of AhR will depend on either the ligand, intracellular ROS accumulation, or additional factors, which will determine the agonist or compensatory antioxidant AhR pathway.

Taken together, these findings provide new insights into the potential application of skin microbiome interventions in clinical practice. However, this study could not comprehensively explore the mechanism by which the metabolites of S. epidermidis WF2R11 can inhibit the activation of AhR. Therefore, further research is required to study the effects of metabolites secreted by the skin microbiome on the AhR signaling pathway. We will also investigate the association of AhR-Trp metabolism and explore the functional significance of microbial Trp metabolites in skin inflammation. Future studies should harbor the prospect of overcoming these limitations and treating skin diseases caused by PM$_{2.5}$ exposure.

**Supplementary Information**  The online version contains supplementary material available at https://doi.org/10.1007/s12602-022-09922-8.

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**Author Contribution**  Eulgi Lee performed qPCR and in vitro cell assays, analyzed the RNA sequences, prepared the figures, and wrote the manuscript. Shinyoung Park isolated whole skin microbiomes and the product of the supernatants. Myung-Giun Noh analyzed the immunohistochemistry results. Hyeok Ahn, Yunjae Kim, Hyun Kim, Gihyeon Kim, and Jae-sung Yeon performed in vitro cell assays and qPCR. Hansoo Park designed and supervised all experiments and analyses.

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**Availability of Data and Material**  All processed gene expression data used in this study were procured from the Gene Expression Omnibus under accession number GSE107871.

**Declarations**

**Ethics Approval**  This study was approved by the Institutional Review Board (IRB: P01-201605-31-003) of Korea National Institute for Biomedical Policy (KONIBP). The study was performed in accordance with the ethical standards as laid down in the declaration of Helsinki and also all study protocols adhered to relevant ethical guidelines.

**Consent to Participate**  All participants provided written informed consent before enrolment.

**Conflict of Interest**  The authors declare no competing interests.

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