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Enhancement of keratinocyte survival and migration elicited by interleukin 24 upregulation in dermal microvascular endothelium upon welding-fume exposure

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Abstract

Occupational exposure to welding fumes constitutes a serious health concern. Although the effects of fumes on the respiratory tract have been investigated, few apparent reports were published on their effects on the skin. The purpose of this study was to investigate the effects of exposure to welding fumes on skin cells, focusing on interleukin-24 (IL-24), a cytokine

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Author contributions

Conceptualization, MaK and YI; methodology, MaK, MT and YI; investigation, MaK, NI, YN, TO, MH, CK, CFAV, MT and YI; visualization, MaK and YI; funding acquisition, MT and YI; project administration, CFAV, MT and YI; writing – original draft, MaK, TN, MiK and YI; writing – review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

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involved in the pathophysiology of skin conditions, such as atopic dermatitis and psoriasis. Treatment with welding fumes increased IL-24 expression and production levels in human dermal microvascular endothelial cells (HDMEC) which were higher than that in normal human epidermal keratinocytes. IL-24 levels in Trolox and deferoxamine markedly suppressed welding fume-induced IL-24 expression in HDMEC, indicating that oxidative stress may be involved in this cytokine expression. IL-24 released from HDMEC protected keratinocytes from welding fume-induced damage and enhanced keratinocyte migration. Serum IL-24 was higher in welding workers than in general subjects and was positively correlated with elevated serum levels of 8-hydroxy-2'-deoxyguanosine, an oxidative stress marker. In summary, welding fumes enhanced IL-24 expression in HDMEC, stimulating keratinocyte survival and migration. IL-24 expression in endothelial cells may act as an adaptive response to welding-fume exposure in the skin.

Keywords

Welding fumes; IL-24; endothelial cells; keratinocytes; oxidative stress

Introduction

Welding fumes are a complex mixture that contain fine and ultrafine particles with a particle size of approximately 0.1 to 1 μm that are solidified in the air from the vapor generated by welding during work such as metal arc welding (Berlinger et 2011). When welding, high temperatures and a consumable electrode are used for joining base metal pieces; this process produces fumes containing oxidized metals that originate from the consumable electrode wire. Furthermore, base metals, shielding gases, paints, and surface coatings may further contribute to fume formation (Riccelli et al. 2020).

The health effects associated with exposure to welding fumes have been investigated with a focus on their action on the respiratory tract. Epidemiological studies demonstrated that, owing to workplace exposure, welders develop respiratory illnesses (Antonini et al. 2003, 2004) including occupational asthma (Hannu et al. 2005), bronchitis (Contreras and Chan-Yeung 1997), and lung cancer (Becker 1999). Transition metals such as iron (Fe) and copper (Cu) found in welding fumes have more than two oxidation states (Raulf et al. 2016). Consequently, these metals might catalyze the conversion of hydrogen peroxidase (H_2O_2) to hydroxyl radicals, which initiate oxidative damage. In addition, metal particles from stainless-steel welding, including chromium (Cr) and manganese (Mn), are highly toxic to mammals and were also detected (Badding et al. 2014). When the lungs are exposed to stainless-steel welding fumes, Cr and NP metal mixtures were shown to induce increased reactive oxygen species (ROS) production and, subsequently, oxidative pulmonary injury (Antonini et al. 1999, 2005). Manganese intoxication following chronic human exposure was reported to initiate a Parkinson's disease (PD)-like syndrome termed manganism (Sriram et al. 2010). It is noteworthy that pulmonary exposure to welding fumes adversely altered reproductive functions via disturbances in the neuroendocrine axis (Krajnak et al. 2017).

The skin forms a protective barrier between the organism and the surrounding environment. The barrier function of the skin primarily depends upon the outermost epidermal layer, the stratum corneum. In human skin, keratinocytes irreversibly exit the cell cycle after mitosis in the basal layer and progressively differentiate across the epidermis toward the stratum corneum (Goleva, Berdyshev, and Leung 2019). Compared with thin epidermis, the dermis is a complex network of cellular and acellular components. Blood vessels, nerves, hair roots, and sweat glands are present in the sublayer structures of the papillary and reticular layers. Hair follicles as well as sweat glands in normal skin are suggested to act as a conduit to nanoparticles (NP) including metals (Tiwari et al. 2022). It is reported that inhaled NP is translocated from the lung to systemic circulation in humans and mice (Miller et al. 2017) probably because NP crosses the alveolo-capillary barrier (Nemmar et al. 2005). Therefore, exposure to NP might affect the dermis as well as epidermis under physiological conditions. In addition, skin barrier function is known to be damaged in conditions including atopic dermatitis, psoriasis, aging, and physical stimuli. Kim et al (2021) reported that skin permeability might increase when it was damaged by NP as evidenced by a decrease in filaggrin expression, which is important for alignment of keratin intermediate filaments, control of keratinocyte shape and maintenance of epidermal texture. Therefore, it is conceivable that ultrafine particles might enhance their harmful effects on skin function in pathophysiological conditions.

Interleukin-24 (IL-24) is a multifunctional cytokine belonging to the IL-10 family. IL-24 was originally identified as a tumor-suppressing protein, melanoma differentiation-associated 7 (MDA-7). The IL-24 signal is transduced through two heterodimeric receptors: the type I IL-20 receptor, which comprises the IL-20 Rα chain (IL-20RA) and IL-20 Rβ chain (IL-20RB), and the type II IL-20 receptor, which is composed of IL-20RB and IL-22 receptor α1 subunit (IL-22RA1) (Mitamura et al. 2020). Several investigators reported the involvement of IL-24 in allergic skin inflammation (Kumari et al. 2013) Mitamura et al. (2018) demonstrated that the IL-13/periostin pathway induces IL-24 production in keratinocytes and plays an important role in barrier dysfunction in atopic dermatitis. The predominant source of IL-24 is considered to be immune cells such as monocytes, macrophages, natural killer (NK) cells, T cells, and B cells (Dabitao et al. 2018; Maarof et al. 2010; Poindexter et al. 2005; Yosef et al. 2013). However, elevated expression of IL-24 was detected in the endothelial cells of synovial blood vessels in patients with rheumatoid arthritis (Kragstrup et al. 2008), suggesting that inflammation triggers IL-24 expression in the endothelium. Oxidative stress is also closely involved in IL-24 expression (Kumari et al. 2013). After binding of IL-24 to its receptor, the signal transduction pathway of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) was activated. Especially, STAT1 and STAT3 are involved in IL-24 mediated-responses including cell differentiation, proliferation, and apoptosis (Zhong, Zhang, and Chong 2022).

Skin may be occupationally exposed to welding fumes. Ultrafine particles and/or chemicals in welding fumes might penetrate the epidermis and dermis under physiological and several pathological conditions and subsequently inhaled welding fumes may translocate from lung to blood to reach dermal microvascular circulation. However, few apparent reports have examined the effects of welding fumes on skin. This study aimed to investigate the effects of

exposure to welding fumes on human dermal microvascular endothelial cells (HDMEC) and keratinocytes with a focus on IL-24 expression.

Materials and methods

Collection of welding fumes

A high-volume air sampler (HV-500 R, Shibata Scientific Technology Ltd., Saitama, Japan) equipped with quartz fiber filters (QR-100, Shibata) was placed on the floor of a welding factory in Kitakyushu, Japan. Welding fumes were collected during working hours from 10:00 a.m. to 12:00 p.m. on April 2022. The welding factory performed only metal inert gas (MIG) welding. Steel flux-cored wires (SM-1F, SF1-EX, SF-1 V, and SF-60 from Nippon Steel Welding and Engineering Co., Ltd., Tokyo, Japan) were used for welding. The filters attached to the welding fumes were cut, mixed with distilled water, and then sonicated to remove the welding fumes. The resulting suspension was passed through the 40 μm nylon filter and subsequently centrifuged at 20,000×*g* for 10 min. The pellets were dried in a desiccator and weighed.

The welding fumes were observed using a scanning electron microscope equipped with energy-dispersive X-ray spectroscopy (SU3500, Hitachi High-Tech Science Corporation, Tokyo, Japan). The welding fume particles were suspended in distilled water by sonication. The suspension was placed on the stage, dried up, followed by electron microscopic observation. Fifteen metals in the welding fumes were measured by IDEA Consultants Inc. (Tokyo, Japan) using inductively coupled plasma−mass spectrometry.

Collection of $PM_{2.5}$

$PM_{2.5}$ used in this study was collected on the rooftop of a building at the Faculty of Science, Fukuoka University in Fukuoka, Japan in 2021, as previously described (Kono et al. 2024). Briefly, $PM_{2.5}$ with an aerodynamic diameter of 2.5 μm was separated using an impactor prior to entry into the cyclone device. Subsequently, the cyclone imparted a centrifugal force on the gas stream within a conical-shaped chamber and created a vortex inside the cyclone body. Fifteen metals in the $PM_{2.5}$ were measured by IDEA Consultants Inc.

Cell culture

Normal human epidermal keratinocytes (NHEK) were purchased from Kurabo Industries Ltd. (Osaka, Japan) and cultured in HuMedia-KG2 (Kurabo). HDMEC were obtained from Takara Bio Inc. (Shiga, Japan) and cultured in the endothelial cell growth medium MV 2 (Takara Bio). Lung microvascular endothelial cells, HULEC-5a, were obtained from the American Type Culture Collection (Gaithersburg, MD, USA) and cultured in MCDB131 medium supplemented with 10 ng/ml epidermal growth factor, 1 μg/ml hydrocortisone, and 10% FBS. Normal human dermal fibroblasts (NHDF) were purchased from Kurabo and were cultured with DMEM supplemented with 10% FBS. Human hepatocellular carcinoma, HepG2 cells were obtained from the American Type Culture Collection and were cultured with DMEM supplemented with 10% FBS. The human keratinocyte cell-line HaCaT was obtained from the Cell Lines Service GmbH (Eppelheim, Germany) and cultured in DMEM supplemented with 10% FBS. The welding fume particles or $PM_{2.5}$ were suspended in

distilled water by sonication at 100-fold higher than the final concentration and then added to cultured cells. The fumes or $PM_{2.5}$ were exposed to cells at concentrations of 1, 10, or 100 μg/ml for 6 or 24 hr.

Total RNA extraction and qPCR

The mRNA levels were determined according to a previously described protocol (Ishihara et al. 2022). The primer sequences are listed in Table 1. The mRNA levels were normalized to the level of the housekeeping gene β-actin, and the values for the treated samples were divided by that for the untreated samples to calculate the relative mRNA levels.

IL-24 ELISA

IL-24 in the cell culture medium or human serum was quantified using a Human Interleukin-24 ELISA Kit (Millipore Sigma, Burlington, MA, USA) according to the manufacturer's instructions.

Simultaneous analysis of cytokines in media

Cytokines in the culture media were measured using the ProcartaPlex Human Cytokine Panel (Thermo Fisher, Waltham, MA, USA) according to our previous report (Kono et al. 2023). The concentrations of 17 cytokines, IL-1α (0.122), IL-1β (0.445), IL-2 (1.585), IL-4 (3.011), IL-5 (2.280), IL-7 (0.156), IL12p70 (2.235), IL13 (0.799), IL-15 (2.635), IL18 (2.767), IL-31 (3.624), IL-1RA (9.150), TNFα (1.353), TNFβ (1.792), GM-CSF (2.864), IFN-α (0.161) and IFN-γ (9.330), were determined using standard curves. The limits of detection (LOD) (ng/ml) for each cytokine in this system are indicated in parentheses.

Measurement of intracellular ROS levels

The ROS levels were determined using H_2DCFDA as a fluorescent probe (Ishihara et al. 2016). The cells were incubated with 10 μM H_2DCFDA for 10 min under culture conditions. The fluorescence of detached cells was measured using a CytoFLEX S flow cytometer (Beckman Coulter K.K., Tokyo, Japan). Over 10,000 events were monitored and analyzed.

Measurement of lipid peroxides

The levels of thiobarbituric acid-reactive substances (TBARS) were measured as previously described (Ishihara et al. 2012). Briefly, the cells were homogenized in a 1.15% KCl solution. The homogenates were mixed with 7 mM sodium dodecyl sulfate, 16 mM thiobarbituric acid, and 340 μM dibutylhydroxytoluene in acetic acid buffer (pH 3.5). The mixture was incubated at 100°C for 60 min, and TBARS was extracted using 1-butanol-pyridine (15:1) solution. The absorbance of the TBARS was measured at 540 nm. 1,1,3,3-Tetraethoxypropane was used as a standard for determining the amount of TBARS.

Semi-quantitative RT-PCR

Total RNA was isolated from cultured cells using the High Pure RNA Isolation Kit (Roche Life Sciences, Basel, Switzerland). The synthesized cDNA was amplified using GoTaq DNA Polymerase (Promega Corp., Madison, WI, USA) (Ishihara and Shimamoto 2012). The primer pairs used are listed in Table 1. The amplified products were separated by

electrophoresis on 1.5% agarose gel. The bands were visualized by ethidium bromide staining.

Preparation of conditioned medium

HDMEC were cultured in the presence or absence of 10 μg/ml welding fume for 24 hr. Culture supernatants were collected and centrifuged to remove cellular debris and welding fumes for use as conditioned medium. To inhibit an action of IL-24 in the conditioned medium, anti-IL-24 antibody (12064–1-AP, Proteintech, Rosemont, IL, USA) at a final concentration of 0.5 μg/ml was added. Normal rabbit IgG (Millipore Sigma) was used as a control.

Measurement of cell viability

Cell viability was evaluated using the Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions.

Determination of caspase-3 activity

Caspase-3 activity was determined by a Caspase-Glo 3/7 Assay (Promega, Madison, WI), according to our previous report (Ishihara, Shiba, and Shimamoto 2005). The luminescence was measured by GloMax 20/20 Luminometer (Promega). This kit measures the combined activity of caspase-3 and caspase-7. However, because caspase-3 is a major caspase which executes apoptosis within the caspase family, results are presented as caspase-3 activity in this study.

Scratch-wound healing assay

HaCaT cells were seeded into plastic six-well plates (Falcon plates, Corning, NY, USA) and cultured until the wells are approximately 95% confluent. Mitomycin C at a final concentration of 5 μg/ml was added to each well and the cells were then cultured for 2 hr. Uniform scratch wounds were scraped using a sterile pipette tip (10 μl volume). Each well was washed with PBS to remove floating cells, and then normal media (vehicle), conditioned media from untreated HDMEC or conditioned media from fume-treated HCMEC were added to each well. Anti-IL-24 antibody or control rabbit IgG was mixed with the media. The cells were cultured for 48 hr with observation of each scratch under a microscope. The average wound area was determined using the ImageJ software (Bethesda, MD, USA).

Human serum samples

Sera from 10 welders were collected in Kitakyushu, Japan, in 2022 who were engaged in welding work indoors. Sera from 10 general subjects (unexposed during work) living in South Kyushu were collected in 2014. After the blood samples were collected, the blood was allowed to clot for approximately 1 hr and then centrifuged at 2,500 ×g for 30 min to separate the serum portion of the blood. The sera were transferred to clean tubes and stored at −80°C until use. This study was approved by the Ethics Committee of Hiroshima University (E2020–2181–02 and E2018–1440–05) and the Institutional Ethics Committee of

the University of Occupational and Environmental Health (R2–011 and UOEHCRB20–197, respectively).

Determination of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in serum

The levels of 8-OHdG, a marker of oxidative stress, were measured using a highly sensitive 8-OHdG ELISA kit (Japan Institute for the Control of Aging, Nikken SEIL Co. Ltd., Shizuoka, Japan) according to the manufacturer's instructions.

Statistics

All data are presented as the mean ± standard deviation (S.D.). All data were analyzed using GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA). Student's t-test was used for determining significant differences between the means of independent 2 groups, one-way ANOVA with Dunnett's corrected multiple-comparison tests were used between control and other treatment groups and two-way ANOVA with Tukey's corrected multiple-comparison tests were used among all experimental groups. Mann – Whitney test and Spearman's rank correlation coefficient were used for human study. The p-values less than 0.05 were considered to indicate statistical significance and indicated in each figure.

Results

Increased expression of IL-24 in HDMEC exposed to welding fumes

Welding fumes were collected from the air at a welding factory. Images captured using a scanning electron microscope showed several ultrafine particles less than 1 μm, as well as several fibers with sizes in the order of micrometers (Figure 1). Energy-dispersive X-ray spectroscopy indicated that particles included Fe and Mn, which were used for welding (Figure 1). Mass spectrometry analysis revealed that the collected welding fumes contained at least 15 metals including zinc, titanium, Mn and a large amount of Fe (Table 2).

Subsequently, HDMEC were treated with welding fume to examine cellular damage. NHEK and HULEC were also used in this study as epidermal keratinocytes might be one of the primary targets of fumes and because pulmonary microvascular endothelial cells have a possibility to be exposed to inhaled fumes. Although exposure to welding fumes at a concentration of 10 or 100 μg/ml for 6 hr did not markedly initiate any cytotoxicity in NHEK, HDMEC, and HULEC, treatment with 100 μg/ml welding fumes for 24 hr induced cellular damage in the 3 types of cultured cells (Figure 2(a–c)). To characterize a type of cell death induced by welding fume exposure, caspase-3 activity was measured. When NHEK, HDMEC, and HULEC were treated with 100 μg/ml welding fume for 24 hr, no significant changes in caspase-3 activity were observed (Figure 2(d)), suggesting that the necrosis-like cell death was induced by exposure to welding fume. Treatment of HepG2 cells with TNFα and actinomycin D, an apoptosis-inducing stimulation, which is well known to produce classical apoptosis with caspase-3 activation (Cuevas et al. 2009), and markedly elevated caspase-3 activity in this study (Figure 2(e)). Therefore, it is confirmed that the method to measure caspase-3 activity was reliable. Taken together, evidence indicates that high concentration of welding fumes induced caspase-independent necrotic cell death in several types of cell

When NHEK and HDMEC were treated with several concentrations of welding fumes for 6 hr, IL-24 mRNA levels increased in a concentration-dependent manner in both cell types (Figure 3(a,b)). Because welding fumes at a concentration of 10 μg/ml significantly upregulated IL-24 expression in HDMEC but not in NHEK (Figure 3(a,b)), HDMEC are considered to express higher potency to induce IL-24 in response to welding fumes than NHEK. In addition, HULEC responded to the treatment with 10 μg/ml welding fumes (Figure 3(c)), suggesting that endothelial cells may be the main target for IL-24 production in response to fume exposure. IL-24 was released from HDMEC treated with welding fumes (Figure 3(d)). Metal particles are reported to induce inflammatory reaction characterized by the upregulation of several cytokines such as IL-6 and IL-8 in keratinocytes as well as endothelial cells (Murray et al. 2013; Xia et al. 2023; Zhu et al. 2011). Kono et al (2023) also reported that the expressions of IL-6 and IL-8 in human keratinocytes were enhanced by $PM_{2.5}$ exposure. Thus, other than IL-24 cytokine expression, especially focusing on IL-6 and IL-8 were subsequently determined. The expressions of IL-6 and IL-8 were significantly induced by exposure to 100 or 10 μg/ml welding fumes, respectively, in HDMEC (Figure 3(e,f)). IL-6 and IL-8 were significantly elevated by welding fume exposure in HULEC (Figure 3(g,h)). When the expression of 17 cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-7, IL12p70, IL13, IL-15, IL18, IL-31, IL-1RA, TNFα, TNFβ, GM-CSF, IFN-α and IFN-γ) in HDMEC treated with 10 μg/ml welding fumes for 24 hr was measured by Multiplex assay, all cytokines detected were not detected in both untreated and welding fume-treated culture media. Therefore, IL-24 upregulation is considered to be one of the major responses to welding fume exposure in endothelial cells, including HDMEC.

As a control of outdoor environments, $PM_{2.5}$ was collected using a cyclone sampler in Fukuoka city, Japan, where is approximately 50 km far from Kitakyushu city, and then compared the metal content in $PM_{2.5}$ to that of welding fumes. These $PM_{2.5}$ effects were further investigated on cell viability and IL-24 expression in HDMEC. The amounts of Fe and Mn in welding fumes were markedly higher than those in $PM_{2.5}$ (Table 2). Exposure to $PM_{2.5}$ up to 100 μg/ml did not markedly induce cell death in HDMEC and exerted no effect on IL-24 expression (Figure 4(a,b)). These results suggest that the outdoor environment did not interfere in the expression of IL-24 and cell death induced by welding fume exposure.

Because the welding fumes collected in this study included many transition metals, such as Fe, which generate ROS via Fenton and Haber–Weiss reactions, the effects of oxidative stress were subsequently examined on welding fume-induced IL-24 expression in HDMEC. When HDMEC were treated with several concentrations of welding fumes for 1 hr, intracellular ROS levels increased in a concentration-dependent manner (Figure 5(a)). Treatment with 10 μg/ml welding fumes significantly elevated ROS levels (Figure 5(a)). TBARS levels were also significantly increased by treatment with 10 μg/ml welding fumes (Figure 5(b)) Hemeoxygenase-1 (HO-1) is known to be upregulated by Nrf-2 signaling and thus its expression levels are employed as an oxidative stress marker (Zhang et al. 2019). Treatment of HDMEC with welding fume concentration-dependently enhanced HO-1 expression and 10 μg/ml welding fumes exposure significantly induced HO-1 expression (Figure 5(c)). These results indicated that welding fume exposure induced oxidative stress in HDMEC. The antioxidant trolox and the Fe chelator deferoxamine (DFO) significantly suppressed mRNA and protein expression of IL-24 initiated by welding-fume treatment

(Figure 5(d,e)). Treatment with trolox or DFO alone did not exert any marked effect on IL-24 expression (Figure 5(d,e)). Therefore, oxidative stress induced by welding fumes may be involved in IL-24 expression in HDMEC

Stimulation of keratinocyte survival by IL-24 released from welding-fume-exposed HDMEC

The receptor expression in NHEK, HDMEC, NHDF, and HaCaT, was determined to establish the main target associated with IL-24 in the skin. IL-20R1 was expressed in all four cell types, whereas the NHDF did not express IL-22RA1 (Figure 6). Notably, IL-20R2 expression was lower in HDMEC and NHDF than in NHEK (Figure 6). HaCaT cells markedly expressed IL-20R2, similar to NHEK (Figure 6). Data suggested that keratinocytes are a major target of IL-24 in the skin.

The effects of HDMEC-derived IL-24 were examined on keratinocyte survival and migration. When NHEK were treated with welding fumes, a concentration-dependent decrease in cell viability was observed in the presence of conditioned media isolated from untreated HDMEC (Figure 7). Treatment with conditioned media from welding-fume-treated HDMEC cultures significantly suppressed cellular injury induced by welding fumes at concentrations of 100 or 300 μg/ml (Figure 7). Interestingly, upon the addition of an anti-IL24 antibody to the conditioned media for neutralizing IL-24 action, the protective effects of conditioned media from the welding-fume-treated HDMEC culture were abolished (Figure 7). Control IgG showed no significant effect on cell viability (Figure 7). Therefore, IL-24 derived from HDMEC suppressed keratinocyte damage induced by exposure to high concentrations of welding fumes.

Subsequently, the scratch-wound healing assay was performed. This assay requires a large number of cells. The proliferation and migration rates of NHEK are predominantly depending upon the lot of NHEK. HaCaT expressed IL-20R1, IL-20R2, and IL-22RA1 similar to NHEK. In this regard, HaCaT was used for the scratch-wound healing assay. There was no significant difference between scratch areas of vehicle treated cell culture and these of vehicle plus rabbit IgG treated cell culture (Figure 8(a–d)). Therefore, rabbit IgG was considered not to markedly affect cellular migration. Scratch areas in the welding fume-treated CM plus rabbit IgG group were smaller than those of untreated CM + rabbit IgG group, data indicated that welding fume-treated CM enhanced HaCaT migration (Figure 8(a–d)). Because the addition of IL-24 to cell culture instead of rabbit IgG significantly suppressed cellular migration, it is conceivable that IL-24 is involved in the potentiation of cellular migration (Figure 8(a–d)). Collectively, IL-24 released from HDMEC stimulated by welding fumes enhanced keratinocyte survival and migration.

Relationships between serum IL-24 expression and oxidative stress in welding workers

The serum IL-24 concentration in welding workers was significantly higher than that in the general subjects (Figure 9(a)). The serum levels of 8-OHdG in welding workers were significantly higher than that in the general subjects, indicating that welding workers were exposed to oxidative stress (Figure 9(b)). The Spearman rank correlation coefficient between the amounts of serum IL-24 and 8-OHdG in welding workers was 0.75 (Figure 9(c)), indicating that oxidative stress was correlated with IL-24 expression.

Discussion

A large number of round-shaped particles with a diameter of 1 μm or less were observed in particulates collected at a welding factory. The collected particulates contained high amounts of metals used in welding, including Fe, Mn, zinc, and titanium, compared with $PM_{2.5}$ collected at urban area in Japan (Kono et al. 2023). When respirable convention concentration during welding work was investigated using a personal sampler, it was found to be extremely high at 7,600 μg/m^3 (average of 5 welders). The average $PM_{2.5}$ concentration in Fukuoka on the same day as when particulates were collected at a welding factory was 7 μg/m^3. Further, there are no major emissions other than welding in the factory. Therefore, particulates collected inside the welding factory are considered to be predominantly welding fumes.

In this study, the cells were treated with welding fumes at concentrations of 1 to 100 μg/ml. Considering that respirable convention concentration during welding work was 7,600 μg/m^3, the deposition rate was calculated using the dry deposition velocity, 0.01 cm/s (a representing value of submicron particle flux toward smooth surface under calm wind conditions reported by Petroff and Zhang (2010), as follows:

$$7,600\ \ \mu g/\mathrm{m}^2 \times 0.01\ \ \mathrm{cm/s} = 7.6 \times 10^{-5}\ \ \mu g/\mathrm{cm}^2/s$$

Assuming an 8-hour welding work per day, the deposition amount per skin area per day was calculated as follows:

$$7.6 \times 10^{-5} \mu g/\mathrm{cm}^2/\mathrm{s} \times 28,800\mathrm{s} = 2.2\ \ \mu g/\mathrm{cm}^2/\mathrm{day}$$

Cells were seeded in six-well culture plates (bottom area: 9.6 cm^2) with 2 ml culture media and cultured until almost confluent, followed by treatment with welding fumes. Metal particles in welding fumes have a high density and are expected to settle. Based upon these conditions and hypothesis, when cells are treated with 10 μg/ml welding fume, the deposition amount of welding fumes per unit area was calculated as 2.1 μg/cm^2. Therefore, a concentration of 10 μg/ml may be estimated to be equivalent to the skin exposure experienced during a day in the welding facility, although exact estimation of welding fume exposure is a future issue.

Lipopolysaccharides and several cytokines induce IL-24 expression (Wolk et al. 2002). T-cell and B-cell receptor signaling pathways are involved in IL-24 expression in T helper 2 lymphocytes and B lymphocytes, respectively (Maarof et al. 2010; Sahoo et al. 2011). IL-1β stimulation reportedly induces IL-24 expression in keratinocytes (Andoh et al. 2009), suggesting that dermal inflammation might enhance IL-24 expression. Kumari et al (2013) demonstrated the autocrine action of IL-24; tumor necrosis factor α induces IL-24 expression in keratinocytes via the ROS-ERK pathway, and the released IL-24 acts on keratinocytes themselves, leading to psoriasis-like skin inflammation. Our findings demonstrated that the rise in IL-24 expression induced by welding fumes was greater in HDMEC than in NHEK. Regarding IL-24 receptor expression, keratinocytes are major

targets of IL-24 in the skin. Thus, IL-24 released from HDMEC might act on keratinocytes in a paracrine manner during welding fume exposure.

IL-24 expression was initially reported to be dependent upon the C/EBP-β and c-Jun transcription factors. These transcriptional regulators bind directly to the IL-24 promoter to enhance gene expression (Madireddi, Dent, and Fisher 2000). In keratinocytes, the p38 MAPK signaling pathway stabilizes IL-24 mRNA and upregulates IL-24 protein expression (Otkjaer et al. 2010). Because treatment with welding fumes elicited an elevation in ROS production and trolox and DFO suppressed IL-24 expression in HDMEC, oxidative stress is considered to be involved in IL-24 upregulation. In experiments using blood samples from welders, although the origin of the increased 8-OHdG remains unclear, the significant correlation between the concentration of 8-OHdG, a marker of oxidative stress, and the expression level of IL-24 suggests involvement of oxidative stress in the elevated levels of IL-24 in welders. Kawada et al (2018) also reported that Fe treatment upregulated IL-24 expression in human aortic vascular smooth muscle cells. Transition metals such as Fe and Mn are known to generate ROS through the Fenton/Haber-Weiss reactions. Therefore, it is possible that the high Fe content present in welding fumes enhanced IL-24 expression through oxidative stress. C/EBP-β is activated by ATF4 signaling, which is induced by treatment with H_2O_2 (Jin et al. 2009). The MAP kinase pathway is affected by redox regulation (Son et al. 2013; Torres and Forman 2003). Collectively, the data suggested that oxidative stress initiated by welding fume exposure might regulate C/EBP-β and MAP kinase pathway. Further studies are needed to determine the molecular mechanisms by which welding fumes increase IL-24 expression via oxidative stress in endothelial cells.

Poindexter et al (2010) reported that IL-24 expression was significantly enhanced in keratinocytes during wound repair, suppressing TGFα-induced migration and proliferation. In contrast, IL-24 expressed in skin stromal cells contributes to keratinocyte proliferation (Xu et al. 2021), suggesting that IL-24 response might depend upon the cell types that produce IL-24. In the present study, IL-24 released from HDMEC potentiated keratinocyte survival and enhanced cell migration. As keratinocyte migration plays an important role in wound repair, IL-24 released by exposure to welding fumes may enhance the healing of welding-fume-induced skin lesions; IL-24 induction in vascular endothelial cells may serve as an adaptive response to welding fumes. In contrast, IL-24 is also involved in skin pathophysiology. IL-24 and IL-20 produced in IL-31-stimulated keratinocytes cooperatively downregulate the expression of filaggrin, which is essential for both the terminal differentiation of keratinocytes and the barrier function of the skin (Cornelissen et al. 2012). IL-24 expression was found to be elevated in a mouse model of allergic contact dermatitis initiated by painting the skin with 2,4-dinitrofluorobenzene (Kunz et al. 2006). IL-24 transgenic mice exhibit skin inflammation and psoriasis-like alterations (He and Liang 2010). Notably, IL-24 is upregulated in psoriatic epidermis in humans (Kumari et al. 2013). Keratinocyte overgrowth was detected in psoriatic skin. In addition, welders displayed keratosis-like symptoms compared with general subjects who were not exposed to welding fumes during work and lived in South Kyushu far enough away from the welding factory (our unpublished observation). Therefore, IL-24-induced uncontrolled and excessive proliferation of keratinocytes may be involved in skin pathology.

It is of interest that Wang et al (2016) reported that IL-24 protected human umbilical vein endothelial cells from H_2O_2 -induced cellular damage. IL-24 reportedly upregulates the expression of antioxidant enzymes such as glutathione peroxidase and catalase in vascular smooth muscle cells (Lee et al. 2012). These functions are considered to be protective against cardiovascular disease. The effect of IL-24 on ROS tolerance needs to be investigated in keratinocytes and endothelial cells to understand the physiological role of IL-24 in maintaining skin homeostasis.

Ultraviolet (UV) light is generated during the welding process, and under conditions where welding fumes are exposed to the skin, the skin may also be exposed to UV light. Oxidative stress induced by UV irradiation is known to activate the JAK/STAT signaling pathway (Charras et al. 2019; de Jager, Cockrell, and Du Plessis 2017). UV irradiation was found to activate the transcription factor, AP-1 (Li et al. 2010). Considering that the JAK/STAT pathway as well as AP-1 are involved in IL-24 expression (Imaeda et al. 2011; Smith et al. 2023), UV irradiation accompanied by welding process may thus affect IL-24 expression. However, it would be necessary to investigate whether the endothelial cells in the papillary and reticular layers of the skin might be affected by UV irradiation.

The skin provides an effective barrier between the organism and external environments to protect the body from invasion of pathogens, chemical exposure, and physical impacts. Atmospheric particulates such as $PM_{2.5}$ and welding fumes cannot penetrate healthy skin because of an effective barrier function. However, hair follicles as well as sweat glands in normal skin are suggested to act as a conduit to NP including metals (Tiwari et al. 2022), indicating the possibility that welding fume particles might reach dermal microvascular endothelial cells via these pathways. In addition, $PM_{2.5}$ exposure reportedly attenuated skin barrier function by decreased expression of filaggrin (Kim et al. 2021). Persistent skin inflammation such as atopic dermatitis also initiates attenuation of the skin barrier (Egawa and Kabashima 2018). Therefore, there is a possibility that welding fumes may penetrate the skin barrier in several pathophysiological conditions. In addition, Miller et al (2017) reported that inhaled NP were detected in the blood in human and mice, suggesting their translocation from lung to blood. Because the alveolo–capillary barrier became loose in the presence of histamine (Nemmar et al. 2005), lung inflammation might increase the permeability of welding fumes. Importantly, Tsuji et al (2023) reported that approximately 40% of welding workers failed the mask fit test. Collectively, dermal microvascular endothelium might be exposed to welding fumes via several pathways. The kinetics of welding fumes exposed to the skin as well as the respiratory tract require further investigation.

Conclusions

Dermal endothelial cells are upregulated and released IL-24 upon exposure to welding fumes. IL-24 was found to exhibit protective effects following acute exposure to welding fume by suppressing keratinocyte damage and promoting keratinocyte migration (wound repair). However, excessive proliferation and migration of keratinocytes might induce hyperkeratosis. Therefore, long-term upregulation of IL-24 expression may lead to epidermal changes that may contribute to keratosis. In this regard, the action of IL-24 is

postulated to differ between acute and chronic phases. Evaluating the endothelial response to welding fumes may be important for understanding not only skin damage but also pathophysiology of keratosis induced by welding fumes.

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Data availability statement

All data generated or analyzed in this study are included in this published article.

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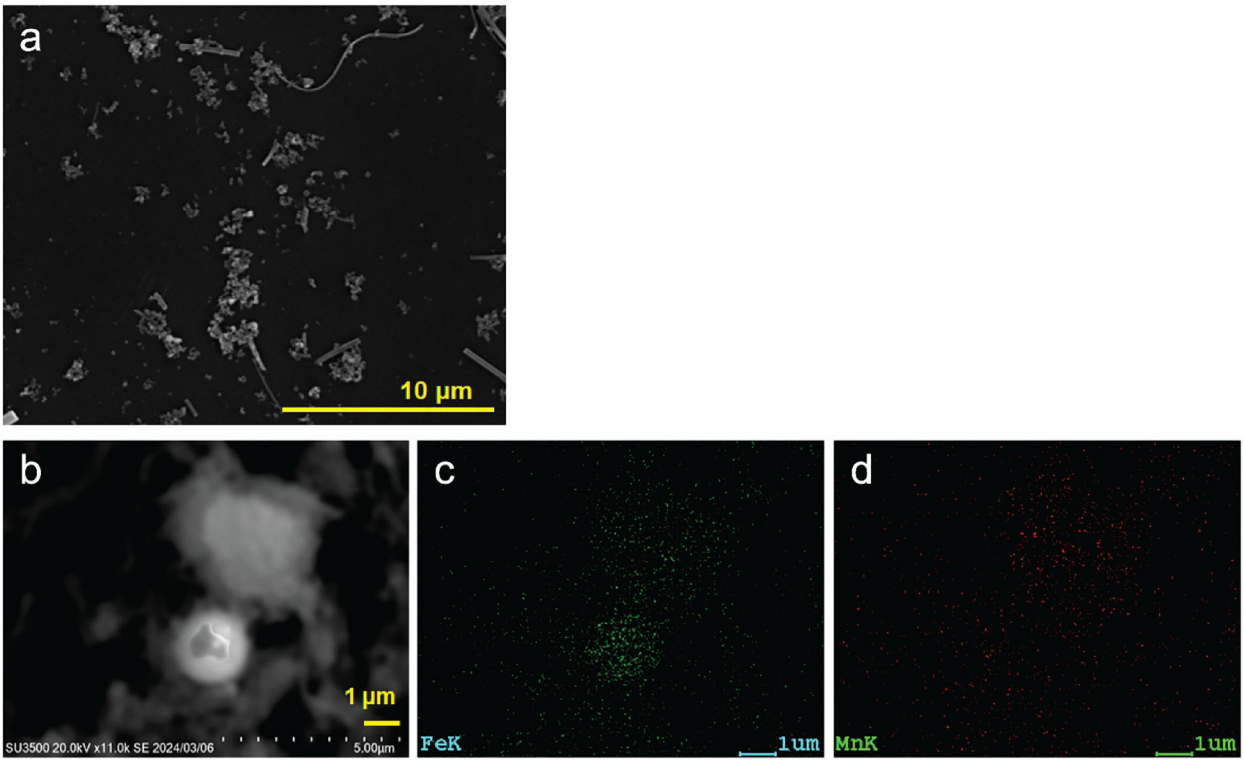

FIGURE 1.

Image of the welding fume particulates under a scanning electron microscope with energy-dispersive X-ray spectroscopy. Welding fume particulates were observed using a scanning electron microscope (A and B). Elemental mapping showed the distribution of prominent Fe (C) and Mn (D) in welding fume particles.

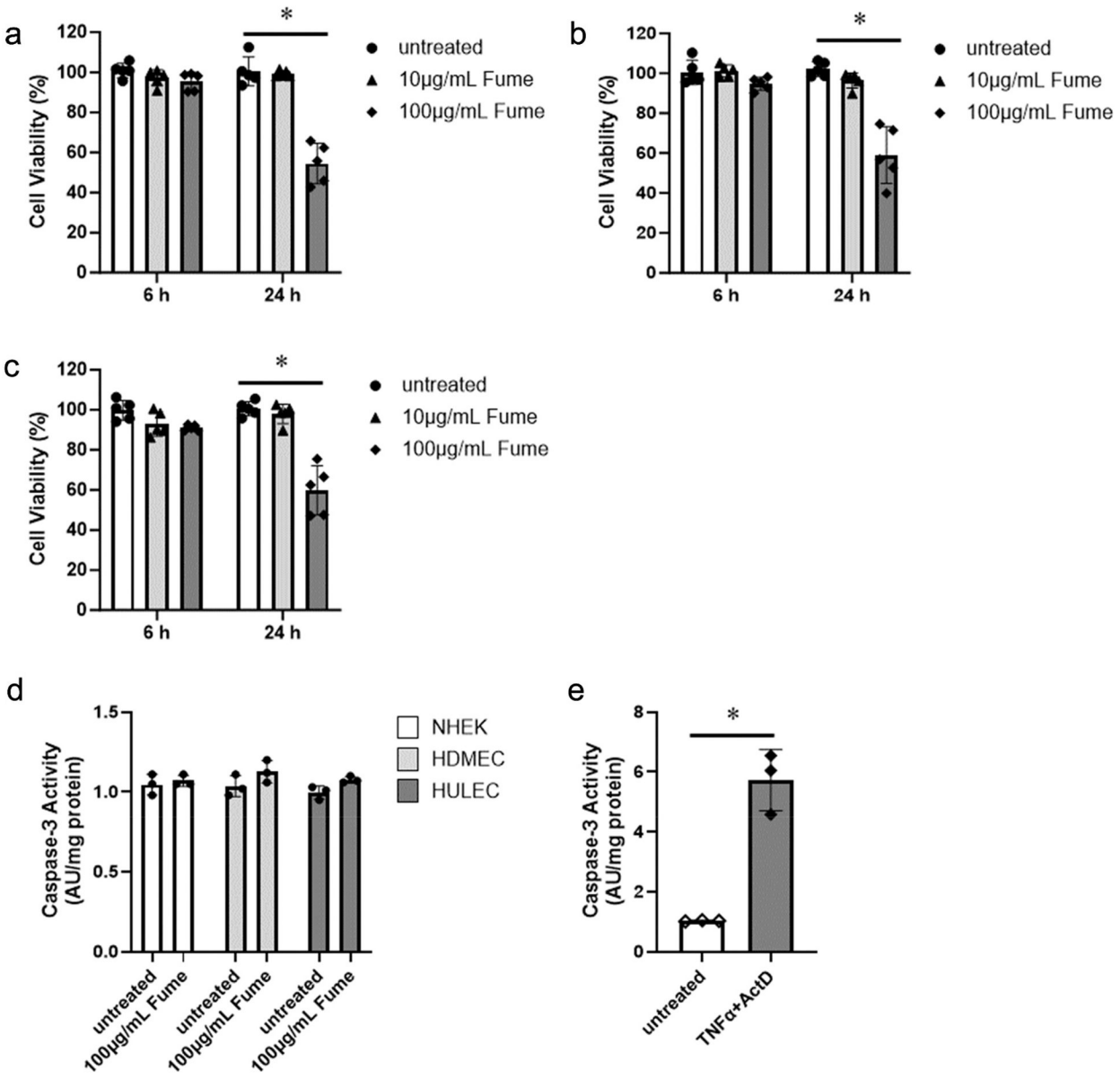

FIGURE 2.

Effects of welding fume exposure on cellular viability. NHEK (a), HDMEC (b), and HULEC (c) were treated with various concentrations of welding fumes for 6 or 24 hr, followed by measurement of cellular viability. The values are presented as the mean ± S.D. (n = 5 in each group). Data were analyzed using two-way ANOVA followed by Tukey's corrected multiple comparison tests. (d) Caspase-3 activity was measured in NHEK, HDMEC, and HULEC treated with 100 μg/ml welding fume for 24 hr. The values are presented as the mean ± S.D. (n = 3 in each group). (e) HepG2G2 cells were treated with 50 ng/ml TNFα and 50 ng/ml actinomycin D (ActD) for 24 hr and then caspase-3 activity was measured. This experiment was used as a positive control of caspase-3 activation. The values are presented as the mean ± S.D. (n = 3 in each group). Data were analyzed using ANOVA followed by student's t-test. $*p < 0.05$.

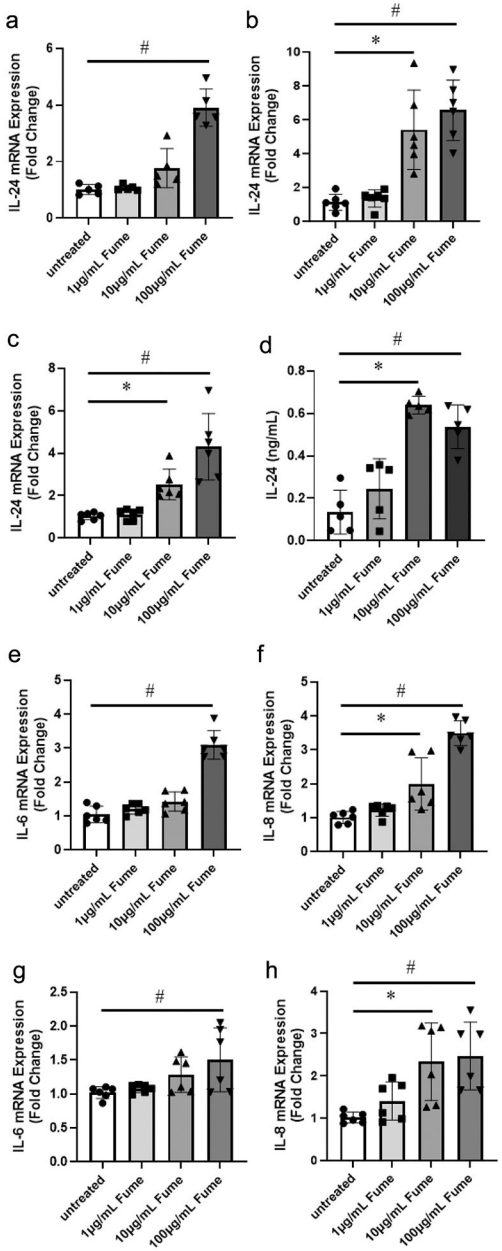

FIGURE 3.

IL-24 upregulation in vascular endothelial cells exposed to welding fumes. NHEK (a), HDMEC (b), and HULEC (c) were treated with various concentrations of welding fumes for 6 hr. Total RNA was extracted, and IL-24 expression was evaluated by real-time PCR. The values are presented as the mean ± S.D. (A: n = 5 in each group, B and C: n = 6 in each group). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. D. HDMEC were treated with welding fumes for 24 hr. IL-24 in the medium was detected using ELISA. The values are presented as the mean ± S.D. (n = 5 in each group). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. (E and F) HDMEC were treated with welding fumes for 6 hr, and the expressions of IL-6 (E) and IL-8 (F) mRNAs were measured using real-time PCR. The

values are presented as the mean ± S.D. (n = 6 in each group). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. (G and H) HULEC were treated with welding fumes for 6 hr, and the expressions of IL-6 (G) and IL-8 (H) mRNAs were measured using real-time PCR. The values are presented as the mean ± S.D. (n = 6 in each group). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. *$p < 0.05$, $^{\#}p < 0.05$.

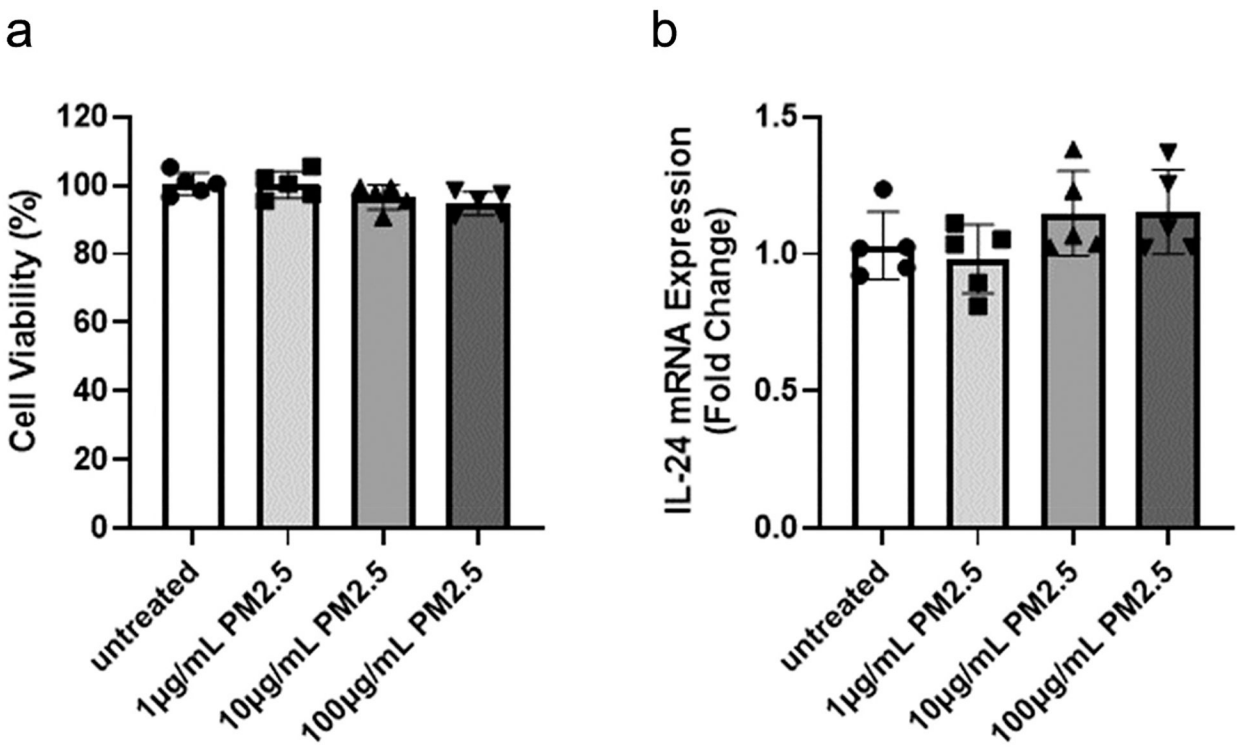

FIGURE 4.

Effects of $PM_{2.5}$ collected at Fukuoka, Japan on cell viability and IL-24 expression in HDMEC. (a) HDMEC were treated with various concentrations of $PM_{2.5}$ collected at Fukuoka, Japan for 24 hr, followed by measurement of cellular viability. The values are presented as the mean ± S.D. (n = 5 in each group). (b) HDMEC were treated with $PM_{2.5}$ for 6 hr and then IL-24 expression was evaluated by real-time PCR. The values are presented as the mean ± S.D. (n = 5 in each group).

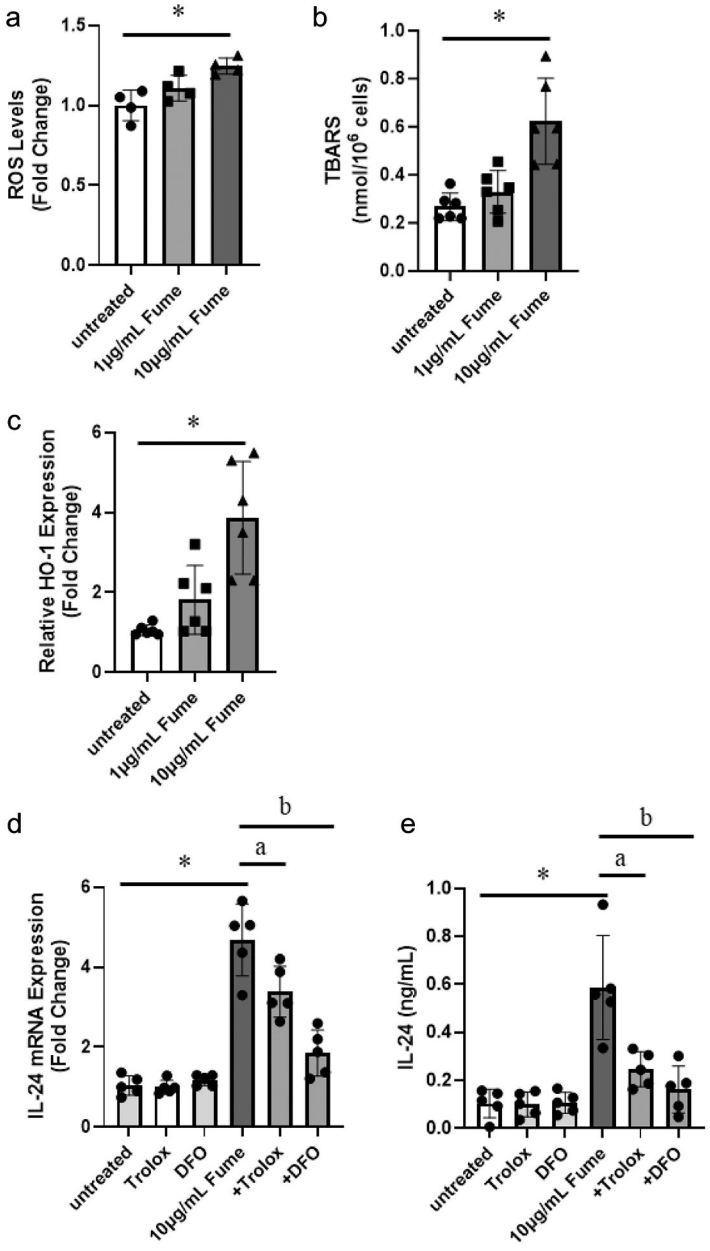

FIGURE 5.

Involvement of oxidative stress in IL-24 induction by welding-fume exposure. (a) HDMEC were treated with 1 and 10 μg/ml welding fumes for 1 hr. H_2DFC-DA was added to the cell culture, and the related fluorescence was detected by flow cytometry. Fluorescence was quantified and compared among three groups. The values are presented as the mean ± S.D. (n = 4 in each group). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. (b) HDMEC were treated with 1 and 10 μg/ml welding fumes for 24 hr, and thiobarbituric acid-reactive substances (TBARS) were measured as an index of lipid peroxidation. The values are presented as the mean ± S.D. (n = 6 in each group). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. (c) HDMEC were treated with 1 and 10 μg/ml welding fumes for 6 hr, and then

mRNA expression of HO-1 was evaluated. The values are presented as the mean ± S.D. (n = 6 in each group). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. (d and e) HDMEC were pretreated with 100 μM trolox or 500 μM deferoxamine (DFO) for 20 min and subsequently treated with 10 μg/ml welding fume for 6 hr (D) and 24 hr (E), respectively. (D) RNA was extracted, and IL-24 mRNA was measured by real-time PCR. (E) IL-24 in the culture medium was quantified by ELISA. The values are presented as the mean ± S.D. (n = 5 in each group). The data were analyzed using two-way ANOVA followed by Tukey's corrected multiple comparison tests. *$p < 0.05$, [a]$p < 0.05$, [b]$p < 0.05$.

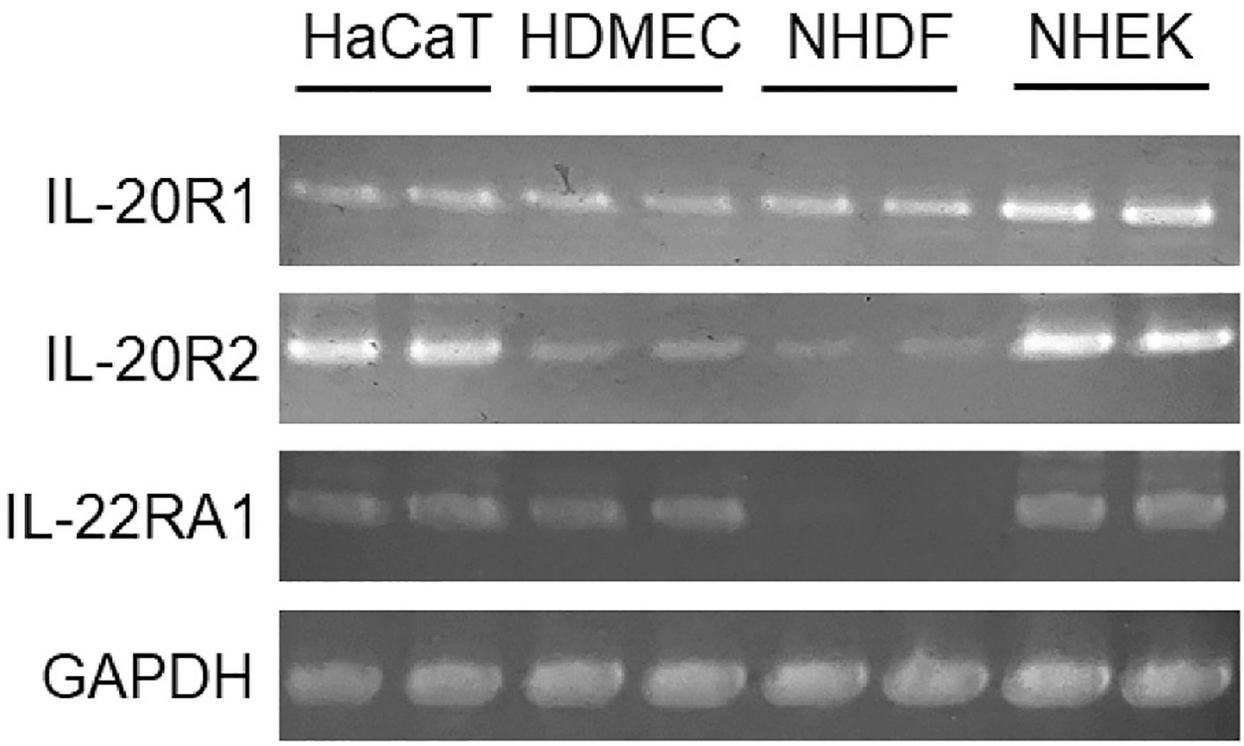

FIGURE 6.

Expression of IL-24 receptors in several skin cells. IL-20R1, IL-20R2, IL-22R1, and GAPDH mRNA levels in HaCaT, HDMEC, NHDF, and NHEK were detected using semi-quantitative reverse transcription PCR. Data are presented in duplicate.

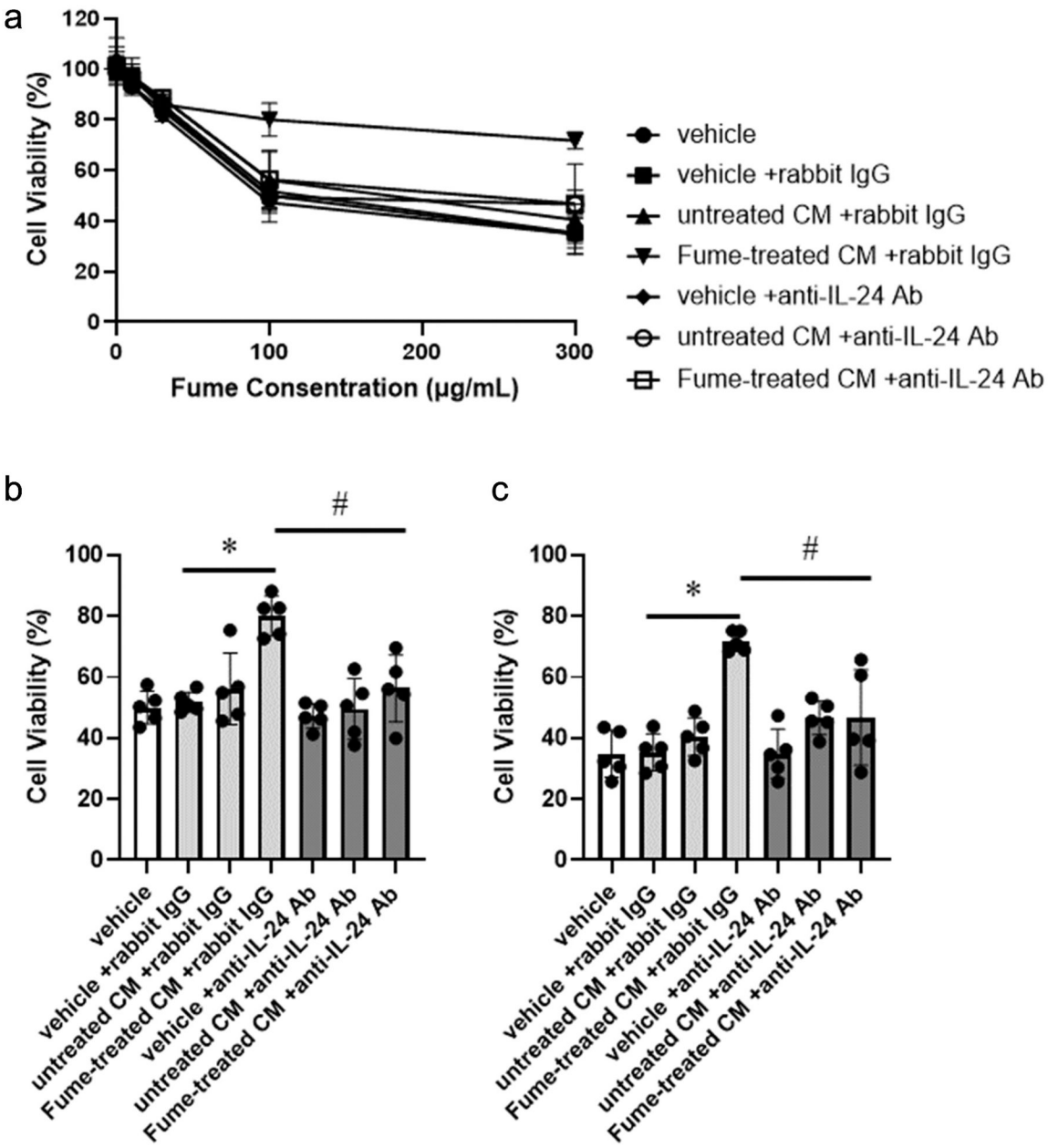

FIGURE 7.

Changes in cell viability affected by IL-24 released from fume-treated HDMEC. NHEK were pretreated with conditioned medium isolated from untreated and 10 μg/ml fume-treated HDMEC for 24 hr. Anti-IL-24 antibody or control rabbit IgG was added to the medium. Cells were treated with various concentrations of welding fumes (0, 10, 30, 100 and 300 μg/mL) for 24 hr, and cell viability was measured using cell counting kit-8. Panel a showed the concentration dependency in cell viability, while panels B and C represented the results extracted from treatments with (B) 100 μg/mL and (C) 300 μg/ml welding fume, respectively. The values are presented as the mean ± S.D. (n = 5 in each group). The data were analyzed using two-way ANOVA with Tukey's corrected multiple comparison tests. *$p < 0.05$, $^{\#}p < 0.05$.

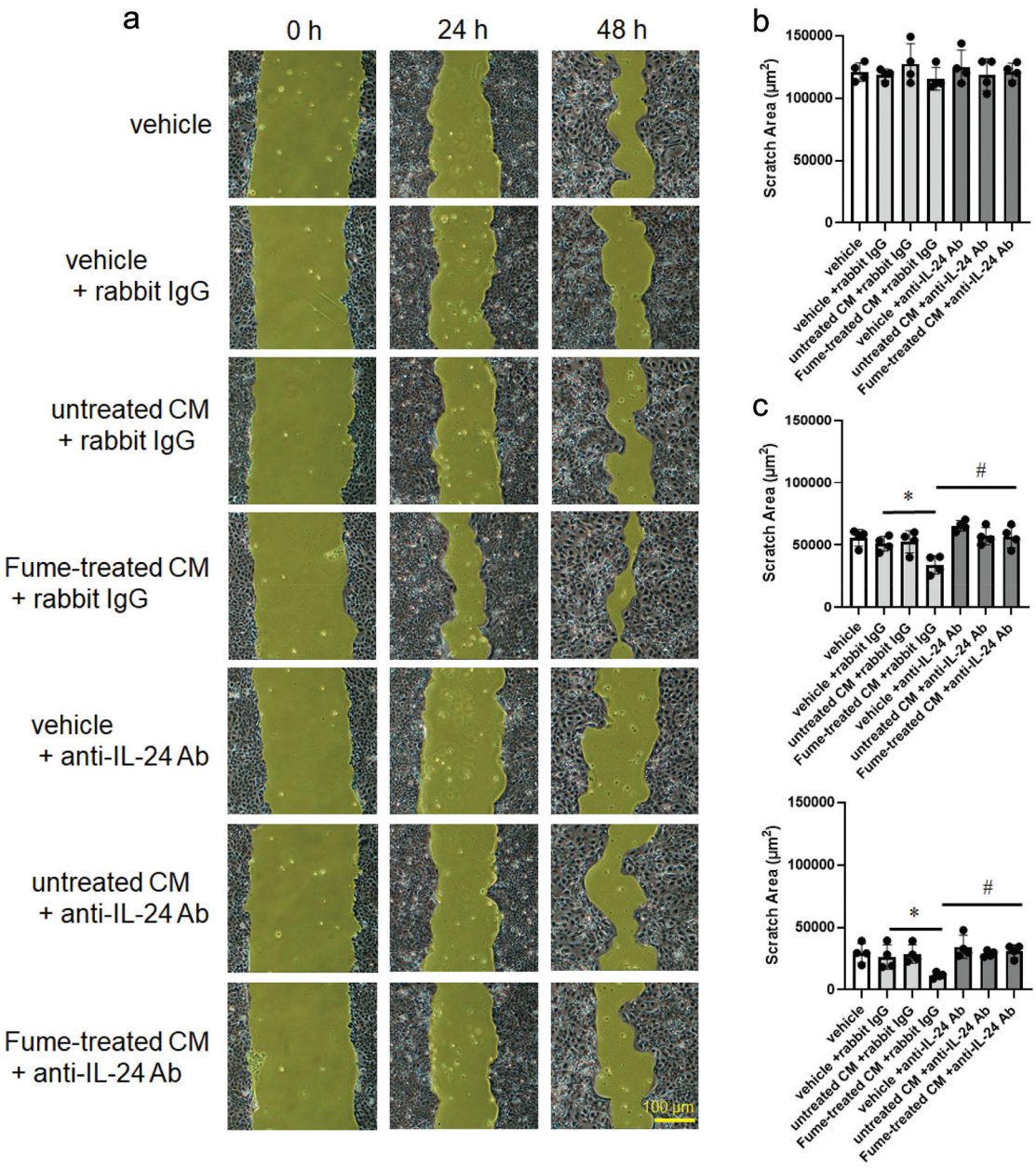

FIGURE 8.

Enhancement of keratinocyte proliferation by IL-24 released from fume-treated HDMEC. HaCaT cells were treated with conditioned medium isolated from untreated or 10 μg/ml fume-treated HDMEC. Anti-IL-24 antibody or control rabbit IgG was added to the media. The cells were then cultured for 48 hr with observation of each scratch under a microscope and time dependent changes in the area of scratch were calculated. Panel a showed representative images of scratch 0, 24 or 48 hr after incubation. Panel B to D showed the areas of scratch calculated at (B) 0, (C) 24 and (D) 48 hr. The values are presented as the mean ± S.D. (n = 4 in each group). Data were analyzed using two-way ANOVA followed by Tukey's corrected multiple comparison tests. *$p < 0.05$, #$p < 0.05$.

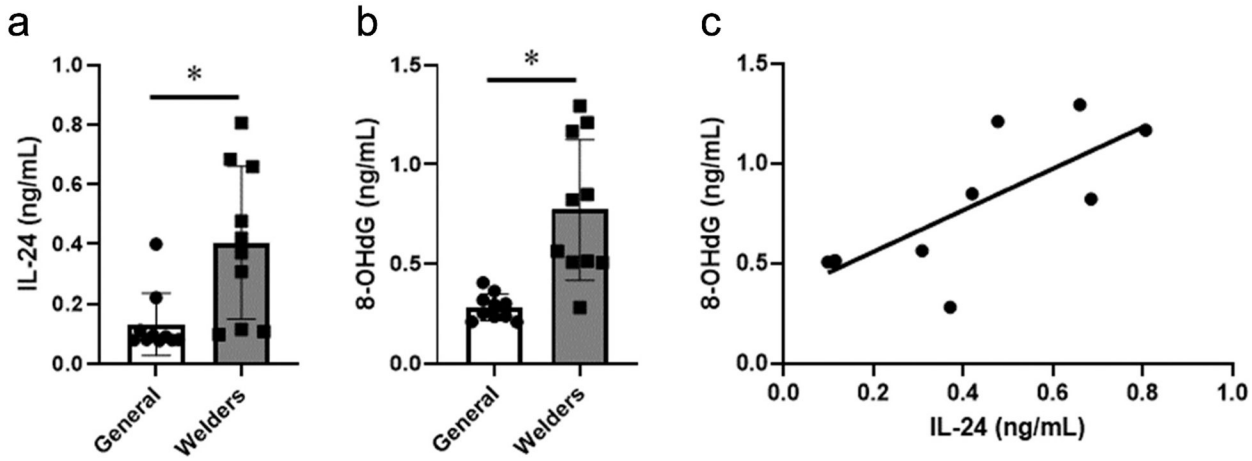

FIGURE 9.

Increased IL-24 expression and oxidative stress in the blood of welders. (a) Serum IL-24 levels in general subjects and welders were measured using ELISA. The values are presented as the mean ± S.D. (n = 10 in each group). The data were analyzed using the Mann–Whitney test. (b) Serum 8-OHdG levels were detected using ELISA. The values are presented as the mean ± S.D. (n = 10 in each group). Data were analyzed using one-way ANOVA followed by Mann–Whitney test *$p < 0.05$. (c) The correlation between serum IL-24 and 8-OHdG levels was determined. Spearman rank correlation coefficient was 0.75.

TABLE 1.

Primers Used in This Study

Name	Sequence (5’−3’)
Human IL-6 Forward	GAAGGCAGCAGGCAACAC
Human IL-6 Reverse	CAGGAGCCCAGCTATGACT
Human IL-8 Forward	GAAGTTTTTGAAGAGGGCTGAGA
Human IL-8 Reverse	TTTGCTTGAAGTTTCACTGGCA
Human IL-24 Forward	TGGACTTTAGCCAGACCCTTC
Human IL-24 Reverse	AAGGGAGCACAACCATCTGC
Human HO-1 Forward	CTGCGTTCCTGCTCAACATC
Human HO-1 Reverse	GCAGAATCTTGCACTTTGTTGC
Human β-actin Forward	ACAGAGCCTCGCCTTTGC
Human β-actin Reverse	ATCATCCATGGTGAGCTGGC
Human IL-20R1 Forward	ACTGTGCAGTATTTCATATATGGGC
Human IL-20R1 Reverse	TTCTCAGAAGGCTGAGCACG
Human IL-20R2 Forward	AACAATTCAGGCTTCGCTGC
Human IL-20R2 Reverse	CTCTCGTACTCCCCCTGGTA
Human IL-22RA1 Forward	GACGTACGGAGAGAGGGACT
Human IL-22RA1 Reverse	GCTTCCCTCCAAGGTGCATT
Human GAPDH Forward	ACCACAGTCCATGCCATCAC
Human GAPDH Reverse	CAGGAAATGAGCTTGACAAA

TABLE 2.

Metal Concentrations of the Welding Fumes and $PM_{2.5}$

	Concentration (ppmw)	
	Welding fumes	**$PM_{2.5}$**
Mg	25,000	9,890
Al	22,000	79,300
K	7,800	9,870
Ca	46,000	24,300
Ti	20,000	1,270
V	87	58.4
Cr	400	420
Mn	68,000	476
Fe	220,000	15,800
Co	15	11
Ni	1,200	215
Cu	3,800	158
Zn	27,000	1,080
Cd	2.4	5.2
Pb	200	138