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Transcriptome analysis of airborne $PM_{2.5}$-induced detrimental effects on human keratinocytes

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HIGHLIGHTS

- RNA-Seq analysis revealed that PM2.5 showed harmful effects on keratinocytes.
- PM2.5 caused epidermal dysfunctions by altering the expression of barrier markers.
- PM2.5 caused significant changes of psoriatic skin disease-related genes.

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ABSTRACT

Ambient air pollution is becoming more severe worldwide, posing a serious threat to human health. Fine airborne particles of particulate matter ($PM_{2.5}$) show higher cytotoxicity than other coarse fractions. Indeed, $PM_{2.5}$ induces cardiovascular or respiratory damage; however, few studies have evaluated the detrimental effect of $PM_{2.5}$ to normal human skin. We used a next-generation sequencing-based (RNA-Seq) method with transcriptome and Gene Ontology (GO) enrichment analysis to determine the harmful influences of $PM_{2.5}$ on human normal epidermal keratinocytes. DAVID analysis showed that the most significantly enriched GO terms were associated with epidermis-related biological processes such as "epidermis development (GO: 0008544)" and "keratinocyte differentiation (GO: 0030216)", suggesting that $PM_{2.5}$ has some deleterious effects to the human epidermis. In addition, Ingenuity Pathway Analysis predicted inflammation-related signaling as one of the major $PM_{2.5}$-induced signaling pathways, and pro-inflammatory cytokines as upstream regulators with symptoms similar to psoriasis as downstream effects.

$PM_{2.5}$ caused considerable changes in the expression of pro-inflammatory cytokines and psoriatic skin disease-related genes, might lead to epidermal dysfunctions. Our results might help to understand the mechanism of air pollution-induced skin barrier perturbation and contribute to the development of a new strategy for the prevention or recovery of the consequent damage.

Abbreviations: ADSP, Asian dust storm particles; CYP1A1, cytochrome P450, family 1, subfamily A, polypeptide 1; CYP1B1, cytochrome P450, family 1, subfamily B, polypeptide 1; DEG, differentially expressed gene; EDS, energy dispersive X-ray spectrometry; ELISA, enzyme-linked immunosorbent assay; ICP-MS, inductively coupled plasma mass spectrometry; IL, interleukin; MDS, multidimensional scaling; NHEK, normal human epidermal keratinocyte; PM, particulate matter; ROS, reactive oxygen species; SEM, scanning electron microscopy; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; SC, stratum corneum; SG, stratum crnulosum; SS, stratum spinosum; SB, stratum basale; IPA, ingenuity pathway analysis; AVR, average; SD, standard deviation.

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1. Introduction

Atmospheric pollutants cause serious health problems in both developed and developing countries. The World Health Organization recently reported that the premature death of 3.7 million people annually worldwide was linked to air pollution (Krutmann et al., 2014). There are several types of particulate air pollutants, including Asian dust storm particles (ADSP) and fine particulate matter (PM), with the latter comprising a coarse fraction (PM_{10}) and fine fraction ($PM_{2.5}$) with an aerodynamic diameter of $<10\ \mu m$ or $2.5\ \mu m$, respectively (Choi et al., 2004).

Several studies have identified an increased risk of cardiovascular and respiratory damage or neurotoxicity due to airborne particles (Buka et al., 2006; Chuang et al., 2007; Gillespie et al., 2013; Lei et al., 2004). However, there have been limited reports on the effects of airborne particles to the normal skin (Magnani et al., 2016). The epidermis is the outermost layer of the skin and is the first line of host defense from a variety of environmental risk factors. Therefore, it is important to elucidate the diverse xenobiotic responses of the epidermis to external stimuli such as air pollutants. Long-term exposure of airborne particles was found to induce oxidative stress, resulting in extrinsic skin aging such as wrinkles and pigment spots (Vierkotter et al., 2010). Moreover, the prevalence of allergic disease in developing countries is on the rise, and childhood allergic eczema is significantly associated with augmented air pollution. Many studies have shown that the exacerbation of asthma and other allergic diseases is strongly related to air pollution (Chang et al., 2006; Hwang et al., 2006; Nikasinovic et al., 2006), and that airborne particles could develop and aggravate the symptoms of skin diseases such as atopic dermatitis (Ahn, 2014; Gehring et al., 2010). However, the underlying biological mechanisms for this aberration remain to be elucidated.

ADSP consist of complex mixtures such as crustal-derived elements and biological substances (Choi et al., 2011; Ichinose et al., 2009), whereas PMs are composed of mixtures of several molecules, including toxic heavy metals, ionic elements, and polycyclic aromatic hydrocarbons, which form the primary hazardous components of air pollutants. In particular, depending on their size, ambient PMs are capable of penetrating through the stratum corneum and hair follicles (Baroli et al., 2007; Lademann et al., 2004; Vierkotter et al., 2010). These ambient particles could then make contact with the cells in the deeper layers of the epidermis and release their surface-bound molecules to initiate the development of a skin disorder. Furthermore, the size of PM particles was shown to be inversely related to their toxicity, in which ultrafine particles produced more pro-inflammatory responses than larger particles (Gillespie et al., 2013). Moreover, the chemical components and physical characteristics of $PM_{2.5}$ differ from those of PM_{10} (Monn and Becker, 1999). The smaller-sized particles can reach deeper sites of lung epithelial cells (Choi et al., 2004), and transepidermally penetrate into the skin (Lademann et al., 2004).

In this study, we conducted a transcriptome analysis of $PM_{2.5}$-treated normal human epidermal keratinocytes (NHEKs), and identified significant effects on genes related to epidermal development and keratinocyte differentiation. We further analyzed the transcriptome analysis dataset to determine the cause of the observed $PM_{2.5}$-induced changes in NHEKs through identification of the Ingenuity Pathway Analysis (IPA) upstream regulators and consequent downstream effects.

2. Materials and methods

2.1. Preparations of airborne particles

$PM_{2.5}$ was collected on a Teflon filter (Zefluor, Pall Life Science, Ann Arbor, MI, USA) with a low-volume air sampler consisting of a cyclone (2.5-μm size cut, URG-2000-30EH), two upstream denuders (annular, URG-2000-30 × 242-3CSS), Teflon filter (ZefluorTM 2.0 μm Pall lifesciences, Mexico), backup filter, and backup denuder in series (Kim, 2015). The collection started at around 10:00 a.m., replacing the filters every 24 h, at a flow rate of 16.7 L/min. The sampling region was located 35 km southeast of downtown Seoul, Korea (37.34°N, 127.27°E, 167 m above sea level). Detailed information of the location and collection methods is provided in our previous report (Kim, 2015). The filter was sonicated in ethanol for 30 min. And then, the EtOH was evaporated, and $PM_{2.5}$ was resuspended with deionized water. ADSP was also sampled from the roof of a Korea University science building, located in the northeastern region of Seoul, Korea (37.35°N, 127.01°E). The particulates were collected using a high-capacity air collector with a quartz filter, as described previously (Choi et al., 2011).

2.2. Elemental and ionic analysis of airborne particles

The sampled airborne particles were used for elemental and ionic analysis. Elemental analysis and identification of the metal compositions of airborne particulates were carried out on an inductively coupled plasma mass spectrometry (ICP-MS) instrument (7700X, Agilent Technologies, USA), and 25 elements were detected. A Metrohm ion chromatograph (Metrohm, Switzerland) was used to analyze the ionic components. Scanning electron microscopy (SEM) with an energy dispersive X-ray spectrometer (EDS) system (ZEISS Gemini SEM300, Carl Zeiss, Germany) was applied to analyze the particle distribution. SEM images from chosen microareas and EDS maps showing the elemental composition and surface distribution were obtained for analysis.

2.3. Cell culture and treatment of $PM_{2.5}$ or ADSP

Normal human epidermal keratinocytes (NHEKs derived from neonatal, 38-year, 68-year old) were cultured in KBM-GOLD medium with supplements (bovine pituitary extract, insulin, human epidermal growth factor, gentamicin/amphotericin B, epinephrine, transferrin, and hydrocortisone), which were purchased from Lonza (Basel, Switzerland). The experiments were performed when cells were used within two passages. NHEKs were starved for 24 h in KBM-Gold medium without supplements, followed by stimulation with $PM_{2.5}$ (0, 12.5, 25, 50, 100, 200, 400 μg/mL) or ADSP samples (25 μg/mL), for 24 h.

2.4. Cell viability tests

After treatment of airborne particles to NHEKs, cell viability was evaluated using the CCK-8 assay system according to the manufacturer's instructions (Dojindo, MD, USA). The CCK-8 reagent was added to the culture medium of NHEKs, and then the cells were incubated in a CO_2 incubator at 37 °C for 2 h. The absorbance was measured at 450 nm using a spectrophotometer, Spectrostar nano (BMG Labtech, Ortenberg, Germany). Cell viability was calculated as the percentage of the optical density relative to that of each control sample.