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Proteome-wide changes in primary skin keratinocytes exposed to diesel particulate extract—A role for antioxidants in skin health

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ABSTRACT

Background: Skin acts as a protective barrier against direct contact with pollutants but inhalation and systemic exposure have indirect effect on keratinocytes. Exposure to diesel exhaust has been linked to increased oxidative stress.
Objective: To investigate global proteomic alterations in diesel particulate extract (DPE)/its vapor exposed skin keratinocytes.
Methods: We employed Tandem Mass Tag (TMT)-based proteomics to study effect of DPE/DPE vapor on primary skin keratinocytes.
Results: We observed an increased expression of oxidative stress response protein NRF2, upon chronic exposure of primary keratinocytes to DPE/its vapor which includes volatile components such as polycyclic aromatic hydrocarbons (PAHs). Mass spectrometry-based quantitative proteomics led to identification 4490 proteins of which 201 and 374 proteins were significantly dysregulated ($\geq$1.5 fold, $p \leq 0.05$) in each condition, respectively.
Proteins involved in cellular processes such as cornification (cornifin A), wound healing (antileukoproteinase) and differentiation (suprabasin) were significantly downregulated in primary keratinocytes exposed to DPE/DPE vapor. These results were corroborated in 3D skin models chronically exposed to DPE/DPE vapor. Bioinformatics analyses indicate that DPE and its vapor affect distinct molecular processes in skin keratinocytes. Components of mitochondrial oxidative phosphorylation machinery were seen to be exclusively overexpressed upon chronic DPE vapor exposure. In addition, treatment with an antioxidant like vitamin E partially restores expression of proteins altered upon exposure to DPE/DPE vapor.

Abbreviations: DPE, Diesel particulate extract; PAHs, Polycyclic aromatic hydrocarbons; MMPs, Matrix metalloproteinases; ROS, Reactive oxygen species; ARE, Antioxidant Response Element; NHEK-Ad, Adult normal human epidermal keratinocytes; ECM, extracellular matrix membrane; HPRD, Human Protein Reference Database; TEABC, Triethyl ammonium bicarbonate; BCA, Bicinchoninic acid; TMT, Tandem Mass Tag; NADPH, Nicotinamide adenine dinucleotide phosphate; HCD, High energy Collision induced Dissociation; AGC, Automatic Gain Control.

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Conclusions: Our study highlights distinct adverse effects of chronic exposure to DPE/DPE vapor on skin keratinocytes and the potential role of vitamin E in alleviating adverse effects of environmental pollution.

1. Introduction

Air pollution is one of the major environmental risk factors known to adversely affect human health. Outdoor air pollution has been classified as carcinogenic to humans in a 2013 assessment report by the International Agency for Research on Cancer (IARC) [1]. The most common sources of outdoor air pollution include industrial and automobile emissions. Emissions from fuel combustion that include carbon monoxide, nitrogen oxides, ozone, sulphur dioxide and particulate matter (PM), are known to cause various lung ailments and cardiomyopathies [2,3]. Outdoor air pollution has also been linked to increasing incidence of cancers such as lung and breast cancer [4,5]. In addition, a growing body of literature highlights the adverse effects of air pollution on skin [6,7].

Diesel emissions are comprised of a heterogeneous mixture of solid and vapor phase components, that includes carbonaceous particulate matter of varying sizes, aliphatic hydrocarbons, polyaromatic hydrocarbons (PAHs) and their derivatives and gases such as carbon monoxide, sulphur and nitrous oxides [8]. Particulate matter in ambient air is one of the major by-products of diesel exhaust emissions and contributes to a number of ill-effects on human health [9]. Diesel exhaust consists of fine particles that range from 2 to 10 μm in size and particulate matter with an average diameter <10 μm (PM_{10}) has a greater likelihood of entering circulation and cause adverse oxidative and inflammatory effects [10]. A major mechanism by which diesel exhaust particles exert their detrimental effects is through the generation of oxidative stress [11] which is an important contributor to extrinsic skin aging [12,13]. Diesel exhaust is known to induce overexpression of genes for phase I [cytochrome P-450 1A1 (CYP1A1)] and phase II [NADPH quinone oxidoreductase-1 (NQO-1)] xenobiotics metabolizing enzymes in human airway epithelial cells [14]. One study also implicates the role of environmental pollutants such as diesel particulate extract in inducing the expression of inflammatory cytokines such as IL-8 and IL-1ß in normal human keratinocytes [15].

Apart from particulate matter, diesel exhaust also comprises of a mixture of volatile components such as aldehydes (formaldehyde, acetaldehyde and acrolein), benzene, 1,3-butadiene, PAHs and nitro-PAHs which can act as irritants in humans [16]. More than 300 putative and 40 established PAHs are known to be adsorbed on PM surface and account for majority of the volatile components in diesel exhaust emissions [17,18]. A number of studies have linked cellular metabolism of PAHs and ROS production to cytotoxicity in *in vitro* and *in vivo* systems. ROS-mediated oxidative DNA damage, DNA adduct formation and their associated cellular toxicity has been attributed not only to exposure to PM but also to surface adsorbed PAHs and nitro-PAHs [19,20]. Studies have shown that PAHs from diesel exhaust and cigarette smoke can trigger inflammatory responses in the respiratory tract [21,22]. In addition, the role of PAHs in contact hypersensitivity or dermatitis in skin has also been well-studied [23,24]. PAHs such as benzopyrene (BP) are known to induce oxidative stress related damage in skin through aryl hydrocarbon (AHR)-related pathway [25]. A recent study also highlights the toxic effect of PAHs in synergy with UVA1 by impairment of cellular homeostasis of skin keratinocytes [26]. Although there are some studies investigating the effects of vapor phase of diesel exhaust, they focus mainly on PAH components, and an unbiased study of total vapor phase is lacking. It is intuitive to expect that PM and volatile PAHs may exert distinct effects on cellular systems. To the best of our knowledge, there are no high throughput studies investigating the adverse effects of chronic exposure to DPE and its vapor in skin.

We therefore aimed to elucidate the effect of chronic diesel exhaust exposure on primary skin keratinocytes. We employed a quantitative proteomics approach to understand the molecular alterations brought about in primary human skin keratinocytes upon exposure to diesel particulate extract (DPE) or its vapor. Mass spectrometry-based proteomic analysis of primary skin cells exposed to DPE or its vapor led to quantitation of 4490 proteins. Of these, 201 and 374 proteins were found to be dysregulated by $\geq$1.5 fold (p-value ≤ 0.05) in DPE and DPE vapor phase exposed skin keratinocytes, respectively. Our study indicates that DPE and its vapor results in significantly altered expression of several proteins reported to be involved in maintenance of skin epithelial integrity, regulation of skin hydration and oxidative stress. Vitamin E is a well-studied antioxidant with known beneficial roles in skin [27]. We observed that treatment with vitamin E alleviated the adverse effects of chronic exposure to environmental pollution.

2. Materials and methods

2.1. Keratinocyte culture

Adult normal human epidermal keratinocytes (NHEK-Ad) from a single Hispanic non-smoker donor were purchased from Lonza (Walkersville, MD, USA (Catalog #00192627)). NHEK-Ad cells were cultured in KGM-Gold™ BulletKit™ (Lonza, Basel, Switzerland) supplemented with bovine pituitary extract, human epidermal growth factor, bovine insulin, hydrocortisone, gentamicin, amphotericin-B, epinephrine and transferrin. The cells were cultured in a 37 °C humidified air incubator with 5% CO_2.

2.2. Adapting skin keratinocytes to diesel particulate extract

Diesel particulate extract (DPE) was purchased from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). NHEK-Ad cells were chronically treated with 0.05% (v/v) DPE in medium in a DPE exposure dedicated incubator. DPE concentration of 0.05% was selected based on cell cytotoxicity assays with varying concentrations of DPE (data not shown). Cells maintained in the DPE dedicated incubator and not directly treated with DPE were considered as DPE vapor exposed cells. Cells were chronically exposed to DPE or its vapor for 20 days. NHEK-Ad cells cultured in a regular incubator without DPE exposure were considered as control. Hereafter, unexposed parental cells will be referred to as NHEK-Ad cells, cells exposed directly to 0.05% DPE will be referred to as NHEK-Ad-DPE and cells exposed to the vapor effect of DPE will be referred to as NHEK-Ad-DPE-V. Parental cells and DPE and DPE vapor exposed keratinocytes treated with vitamin E (Sigma Aldrich, St. Louis, MO) (α-tocopherol ≥95.5%, 9 IU/ml for 72 h) will be referred to as NHEK-Ad-Vit-E and NHEK-Ad-DPE-Vit-E and NHEK-Ad-DPE-V-Vit-E, respectively.

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