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Cystathionine metabolic enzymes play a role in the inflammation resolution of human keratinocytes in response to sub-cytotoxic formaldehyde exposure

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ABSTRACT

Low-level formaldehyde exposure is inevitable in industrialized countries. Although daily-life formaldehyde exposure level is practically impossible to induce cell death, most of mechanistic studies related to formaldehyde toxicity have been performed in cytotoxic concentrations enough to trigger cell death mechanism. Currently, toxicological mechanisms underlying the sub-cytotoxic exposure to formaldehyde are not clearly elucidated in skin cells. In this study, the genome-scale transcriptional analysis in normal human keratinocytes (NHKs) was performed to investigate cutaneous biological pathways associated with daily life formaldehyde exposure. We selected the 175 upregulated differentially expressed genes (DEGs) and 116 downregulated DEGs in NHKs treated with 200 μM formaldehyde. In the Gene Ontology (GO) enrichment analysis of the 175 upregulated DEGs, the endoplasmic reticulum (ER) unfolded protein response (UPR) was identified as the most significant GO biological process in the formaldeyde-treated NHKs. Interestingly, the sub-cytotoxic formaldehyde affected NHKs to upregulate two enzymes important in the cellular transsulfuration pathway, cystathionine γ-lyase (CTH) and cystathionine-β-synthase (CBS). In the temporal expression analysis, the upregulation of the pro-inflammatory DEGs such as *MMP1* and *PTGS2* was detected earlier than that of *CTH*, *CBS* and other ER UPR genes. The metabolites of CTH and CBS, L-cystathionine and L-cysteine, attenuated the formaldehyde-induced upregulation of pro-inflammatory DEGs, *MMP1*, *PTGS2*, and *CXCL8*, suggesting that CTH and CBS play a role in the negative feedback regulation of formaldehyde-induced pro-inflammatory responses in NHKs. In this regard, the sub-cytotoxic formaldehyde-induced CBS and CTH may regulate inflammation fate decision to resolution by suppressing the early pro-inflammatory response.

1. Introduction

Formaldehyde exposure is one of the most common environmental hazards that threaten human health. It has been the focus of many toxicological and epidemiological investigations. Since the early 1900s, formaldehyde has been widely used in a variety of industrial products such as press-wood products, permanent-press fabrics, paper product coatings, insulation supports and general consumer products (Flyvholm and Andersen, 1993; Bostrom et al., 1994). Formaldehyde is also released from vehicle emissions, particle boards, carpets, paints and varnishes. Epidemiological studies have shown that formaldehyde is associated with an increased risk of sino-nasal cancer (Roush et al., 1987), nasopharyngeal cancer (Vaughan et al., 2000), childhood asthma (Garrett et al., 1999), and leukemia (Zhang et al., 2010). Toxicological studies have shown that formaldehyde causes squamous cell carcinoma in rodents (Swenberg et al., 1980; Brown, 1985). Formaldehyde is currently classified as a human carcinogen (Cogliano et al., 2005).

Formaldehyde exposure can result in various respiratory and dermatologic problems in humans, including irritation or allergic reactions in the eyes, skin and the respiratory system (De Jong et al., 2009; Hauksson et al., 2016). Therefore, formaldehyde-induced transcription profile studies have been performed in various kinds of mammalian cells including human nasal epithelial cells (Neuss et al., 2010) and human tracheal fibroblasts (Li et al., 2007). However, few studies have investigating their effects on human skin cells. Irritant and allergic contact dermatitis are common inflammatory human skin diseases induced by repeated exposure to chemicals (Nosbaum et al., 2009). The

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formaldehyde-induced contact allergy in cosmetics has been reported in industrialized countries since the 1960s (Thyssen et al., 2007). According to the FDA's Voluntary Cosmetic Registration Program database, 19.5% of cosmetic products in the USA contained formaldehyde or formaldehyde-releasing preservatives (de Groot and Veenstra, 2010) and formaldehyde is still categorized as a major contact allergen (Warshaw et al., 2015). Despite the causative association of formaldehyde exposure with both irritant and allergic contact dermatitis (Garrett et al., 1999), the molecular mechanism of formaldehyde-induced allergy in human skin is not comprehensively understood. In human skin, epidermal keratinocytes are the primary cells that are in direct contact with environmental chemicals and have an essential role in skin permeability barrier functions (Vandebriel et al., 2010). Human keratinocytes can sense toxic chemicals or organic materials and produce various pro-inflammatory autacoids, cytokines, and anti-microbial peptides (Jin et al., 2014; Bae et al., 2015a; Bae et al., 2015b). Recently, we reported that formaldehyde significantly increased the expression of interleukin-8 (IL-8), interleukin-24 (IL-24), and vascular endothelial growth factor (VEGF) in human keratinocytes (Bae et al., 2015a). However, it is still unclear how human epidermal keratinocytes respond to formaldehyde at a whole genome transcription level.

Genome-wide transcription profile studies to elucidate the molecular mechanisms underlying formaldehyde-induced toxicological outcomes such as allergy and carcinogenesis have mainly been performed in respiratory epithelial cells (Li et al., 2007; Neuss et al., 2010). Formaldehyde is normally present in both indoor and outdoor air at low levels (Gustafson et al., 2005; Rovira et al., 2016). Formaldehyde is usually present as a contaminant in foods, albeit at very low concentrations (Jeong et al., 2015). Many consumer products may contain small amounts of formaldehyde due to their use as fumigants, fertilizers, or preservatives. However, most transcriptional profile studies have been performed with high concentrations of formaldehyde that are cytotoxic to target cells as formaldehyde was determined to be a carcinogen in animal models using high-dose exposure. At cytotoxic concentrations, the formaldehyde-induced gene expression signature of target cells is associated with cell death-related biological pathways such as apoptosis (Szende and Tyihak, 2010). Currently, information on the toxicological response to sub-toxic concentrations of formaldehyde is limited in human cells. In addition, a direct cell death in human skin may not be a primary cellular mechanism to explain the pathophysiology of formaldehyde-induced cutaneous allergy in normal life conditions. However, the effects of sub-cytotoxic formaldehyde concentrations on human skin are not fully understood. To investigate the toxicological effect of low-dose formaldehyde exposure in the general environment, we performed a genome-wide transcription profile analysis on cultured human primary keratinocytes treated with sub-cytotoxic formaldehyde concentrations, which did not cause cell death.

2. Materials and methods

2.1. Cell culture and cell viability test

Normal human keratinocytes (NHKs) from neonatal foreskins were purchased from Lonza (first passage, Basel, Switzerland) and cultured in a KBM medium with KGM2 growth supplements containing insulin, human epidermal growth factor, bovine pituitary extract, hydrocortisone, epinephrine, transferrin, and gentamicin/amphotericin B, purchased from Lonza (KBM/KGM-2). We purchased three NHK batches prepared form different donors and performed all experiments in triplicates or quadruplicates. Cells were serially passaged at 80–90% confluence and the third passage of primary NHKs was used in the cytotoxicity and other experiments in this study. Cell viability was evaluated using a Cell Counting Kit-8 assay (CCK-8, Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. 5×10^4 NHKs were seeded to each well in 48-well plates and cultured up to 100% confluence. The 200 μM solution of formaldehyde (#F8775, 37% solution, Sigma-Aldrich, St. Louis, MO, USA) was freshly prepared in KBM/KGM-2 media just before performing experiments. Formaldehyde in KBM/KGM-2 was treated at 24 h after KBM/KGM-2 media were exchanged in confluent NHKs. NHKs were exposed to formaldehyde for 24 h in a KBM/KGM-2 medium and washed with phosphate-buffered saline (PBS) three times. The CCK-8 solution, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H–tetrazolium, monosodium salt (WST-8) diluted in PBS, was used to treat the NHKs cells prior to further incubation for 2 h. The absorbance at 450 nm was measured using a microplate reader (BioTek, Winooski, VT, USA). Absolute optical density was expressed as a percentage of the control value.

2.2. Microarray experiments

Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA), per the manufacturer's instructions. RNA was further purified using the RNeasy Mini Kit (Qiagen, Germantown, MD, USA). The quantity of the total RNA was measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Montchanin, DE, USA). RNA integrity was verified with the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Affymetrix Human Genome U133 2.0 GeneChip arrays (Affymetrix, Santa Clara, CA, USA) were prepared, hybridized and scanned by the local authorized Affymetrix service provider (DNA Link, Inc., Seoul, South Korea). RNA reverse transcribed to cDNA and transcribed into cRNA in the presence of biotinylated ribonucleotides, per standard Affymetrix protocols. The hybridized probe array was stained and washed with a GeneChip hybridization wash and stain kit using the Fluidics Station 450 (Affymetrix). The stained GeneChip probe array was scanned with a GeneChip Scanner 3000 + 7G (Affymetrix). The signal intensity of the gene expression level was calculated using Expression Console Software, Version 1.1 (Affymetrix) based on the MAS 5.0 algorithm. The procedure used to select the differentially expressed genes (DEGs) was as follows: (i) selection of "present" Affymetrix probe sets as control or formaldehyde-treated samples; (ii) selection of Affymetrix probe sets with comparison signal sample/control ratios greater than two as "up-regulated" and less than two as "down-regulated"; and (iii) selection of Affymetrix probe sets with simultaneously significant P values (threshold, 0.05) in the Wilcoxon rank test when compared with vehicle-treated samples.

2.3. Gene Ontology (GO) enrichment analysis

GO enrichment analysis was performed by comparing the frequency of GO biological process (BP) terms assigned for a specific gene included in a group of DEGs with that in all the genes set in Affymetrix Human 133 2.0 GeneChip arrays. The GO annotation files were downloaded from the Gene Ontology Consortium webpage (http://www.geneontology.org) and the August 2015 version of the GO BP terms, which are annotated to a specific gene in the whole genome, was used in the GO enrichment analysis. When redundant probe sets for the same gene in the Affymetrix Human 133 2.0 GeneChip were considered as a single gene unit in the GO enrichment analysis, 23, 624 probe sets were counted as the total number of genes in the analysis. A 2×2 contingency matrix was constructed to determine the frequency of a specific GO BP term in a group of DEGs compared with that in the 23,624 total gene set. The 2×2 contingency matrix data were analyzed by the Fisher's exact test (frequency ≤ 5) or χ^2 test (frequency ≥ 5) using SPSS® for Windows (SPSS Science, Chicago, USA) to calculate the level of significance.

2.4. Validation of microarray results using quantitative real-time PCR

Quantitative real-time PCR (Q-RT-PCR) was performed to validate the microarray data on selected genes from DEGs. Total RNA samples were prepared in independent experiments in the microarray study. cDNA samples were synthesized from the total RNA using SuperScript