Mutation Research 770 (2014) 94–105

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Analysis of gene expression changes in A549 cells induced by organic compounds from respirable air particles

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ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 3 October 2014
Accepted 24 October 2014
Available online 3 November 2014

Keywords:
Ah receptor
A549 cells
Gene expression profile
PAHs
PM2.5

ABSTRACT

A number of toxic effects of respirable ambient air particles (genotoxic effects, inflammation, oxidative damage) have been attributed to organic compounds bound onto the particle surface. In this study, we analyzed global gene expression changes caused by the extractable organic matters (EOMs) from respirable airborne particles <2.5 μm (PM2.5), collected at 3 localities from heavily polluted areas of the Czech Republic and a control locality with low pollution levels, in human lung epithelial A549 cells.

Although the sampled localities differed in both extent and sources of air pollution, EOMs did not induce substantially different gene expression profiles. The number of transcripts deregulated in A549 cells treated with the lowest EOM concentration (10 μg/ml) ranged from 65 to 85 in 4 sampling localities compared to the number of transcripts deregulated after 30 μg/ml and 60 μg/ml of EOMs, which ranged from 90 to 109, and from 149 to 452, respectively. We found numerous commonly deregulated genes and pathways related to activation of the aryl hydrocarbon receptor (AhR) and metabolism of xenobiotics and endogenous compounds. We further identified deregulation of expression of the genes involved in pro-inflammatory processes, oxidative stress response and in cancer and developmental pathways, such as TGF-β and Wnt signaling pathways. No cell cycle arrest, DNA repair or pro-apoptotic responses were identified at the transcriptional level after the treatment of A549 cells with EOMs.

In conclusion, numerous processes and pathways deregulated in response to EOMs suggest a significant role of activated AhR. Interestingly, we did not observe substantial gene expression changes related to DNA damage response, possibly due to the antagonistic effect of non-genotoxic EOM components. Moreover, a comparison of EOM effects with other available data on modulation of global gene expression suggests possible overlap among the effects of PM2.5, EOMs and various types of AhR agonists.

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Abbreviations: A549, human adenocarcinoma alveolar basal epithelial cells type II; AhR, aryl hydrocarbon receptor; B[a]P, benzo[a]pyrene; BMP, bone morphogenetic proteins; cDNA, complementary DNA; c-PAH, carcinogenic polycyclic aromatic hydrocarbon; cRNA, complementary RNA; CYP, cytochrome P450; DEP, diesel exhaust particles; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; EMT, epithelial–mesenchymal transition; EOM, extractable organic matter; FBS, fetal bovine serum; HEL 12469, human embryonic lung fibroblasts; HPLC, high performance liquid chromatography; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDH, lactate dehydrogenase; NFkB, nuclear factor kappa B; NLR, NOD-like receptor; NOD, nucleotide-binding oligomerization domain; PAH, polycyclic aromatic hydrocarbon; PBS, phosphate-buffered saline; PLP, pyridoxal-5′-phosphate; PM2.5, particulate matter < 2.5 μm; RT-qPCR, reverse transcription quantitative real-time PCR; ROS, reactive oxygen species; TCDD, 23,7,8-tetrachlorodibenzo-p-dioxin; TGF-β, transforming growth factor beta; TLR, toll-like receptor; RIN, RNA integrity number; Wnt, Wingless/Int-1.

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http://dx.doi.org/10.1016/j.mrfmmm.2014.10.002
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1. Introduction

Respirable airborne particles < 2.5 μm (PM2.5) are comprised of a complex mixture of numerous chemicals including those which are toxic or carcinogenic. The important toxicological mechanisms of the adverse health effects of PM include: (i) genotoxicity (generation of stable DNA adducts and/or oxidative DNA damage); (ii) the aryl hydrocarbon receptor (AhR)-mediated gene expression; (iii) generation of ROS, oxidative stress; and (iv) induction of inflammation. These effects have been reported to be induced by organic extracts of PM containing PAHs in lung cells [1–3]. Organic compounds adsorbed on the particles are mostly desorbed in the alveolar region of the lung [4]. Some researchers have suggested that the adverse health effects of respirable ambient air particles are related to the toxic effects induced by the particles themselves, including reactive oxygen species (ROS) formation, inflammation and oxidative damage. However, the toxic effects of PM2.5 are mainly attributed to chemicals adsorbed on the particle surface, including organic compounds, such as PAHs and their derivatives.

Although PAHs are responsible for many genotoxic and carcinogenic effects, numerous other organic compounds may further contribute to the disruption of biological processes and pathways. The rapid development of toxicogenomics has provided a tool to gather mechanistic information on the changes occurring simultaneously in the cellular transcriptome in response to xenobiotic exposure, including complex mixtures of environmental pollutants, such as whole aiborne particles or their extracts [5,6].

The present study focused on the gene expression responses to complex mixtures of organic compounds bound to the PM2.5 aerosol collected in 3 heavily polluted areas of the Czech Republic and a control locality. We recently demonstrated that extractable organic matter (EOM) from these PM2.5 samples induced significant gene expression changes in normal human lung fibroblasts (HEL 12469) [6]. In this study numerous biological processes and signaling pathways have been found to be deregulated in response to various organic extracts from PM2.5, and the key role of the activated AhR has been identified. In a follow-up study, performed in human lung epithelial adenocarcinoma cells A549, we have further observed that EOM-induced DNA adduct levels are relatively low, as compared to levels induced by similar concentrations of benzo[a]pyrene (B[a]P) [7]. We suggested that this might be due to competitive inhibitory effects of some non-genotoxic EOM components requiring identical enzymatic activities for their metabolism as genotoxic PAHs, which are mostly responsible for DNA adduct formation by EOM [8]. The same set of EOMs was used in the present study to analyze both AhR-dependent and other modulations of global gene expression in the A549 cell line. This study provides a direct comparison of global gene expression changes observed recently in normal human lung fibroblasts with those observed in model of human lung epithelial cells A549, one of the most frequently used cell models in toxicology of the air pollution. Identification of deregulated pathways in both cell models may help to design future studies focusing more deeply on specific deregulated pathways. Such an approach may help to clarify mechanisms of the major toxic events and PM2.5 components responsible for adverse health effects of the air pollution.

2. Methods

2.1. PM2.5 collection, sampling sites, EOM extraction and chemical analysis

PM2.5 were collected at four localities in the Czech Republic with different extent and major sources of air pollution: Ostrava-Bartovice, Ostrava-Poruba, Karvina and Trebon as previously reported [9]. In short, PM2.5 were collected using HiVol 3000 air sampler (model ECO-HVS3000, Ecotech, Australia) and Pallflex filters T60A20 (20 × 25 cm) for 24 h each day for 30–35 days in the winter season of 2008/2009. Organic matter was extracted from filters by dichloromethane and cyclohexane. Dichloromethane extractions of EOMs and the chemical analyses of PAHs were performed uniformly in the laboratories of the certified company ALS Czech Republic, Prague (EN ISO CSN IEC 17025). More detailed chemical analysis is described in [6]. For the in vitro experiments, EOM samples were evaporated to dryness under a stream of nitrogen and the residue re-dissolved in DMSO. The stock solutions of each EOM sample contained 50 mg of EOM/ml DMSO. The samples were kept in at −80 °C until further analyses.

Table 1
Cytotoxicity following 24-h treatment of A549 cells with extractable organic matters (EOMs) obtained by organic extraction of PM2.5 samples collected in various sampling sites. Lactate dehydrogenase (LDH) test of cytotoxicity was used as described in Section 2.

Sampling locality	EOM concentration [μg/ml]	Cytotoxicity [%]
Ostrava-Bartovice	10	6.8
	30	n.d.[a]
	60	n.d.
	100	n.d.
Ostrava-Poruba	10	n.d.
	30	n.d.
	60	n.d.
	100	n.d.
Karvina	10	n.d.
	30	4.8
	60	n.d.
	100	n.d.
Trebon	10	n.d.
	30	n.d.
	60	8.4
	100	n.d.

[a] No cytotoxicity was detectable.

2.2. Cell cultures and cytotoxicity

A549 (human adenocarcinoma alveolar basal epithelial cells, type II) were grown in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 1.0 g/l glucose and pyruvate, 10% FBS, 200 mM glutamine and gentamycin sulfate (10 mg/ml). The cells were cultivated in plastic cell culture dishes (21 cm^2) at 37 °C in 5% CO_2. After reaching 70–80% confluency, the medium was replaced with fresh medium supplemented with 1% FBS. EOM samples were diluted with DMSO and added to the medium at the test concentrations: 10, 30 and 60 μg/ml. The cells were treated for 24 h. Each concentration was tested in triplicate including control cell cultures incubated with DMSO only. The harvested cells were washed three times in PBS and the final concentration of DMSO did not exceed 0.1% of the total incubation volume. The cytotoxicity of A549 cells treated with EOMs was tested using the LDH-Cytotoxicity Assay Kit (Bio Vision) at concentrations of 10, 30, 60 and 100 μg EOM/ml. Significant cytotoxicity was not observed at any of the tested EOM concentrations (Table 1).

2.3. RNA isolation and quality control

Total RNA from lysed A549 cells was obtained using NucleoSpin RNA II (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer's instructions. RNA concentration was quantified with a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The integrity of RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). All samples

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