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Gene Expression Patterns of Environmental Chemicals in Human Cell Lines using HazChem Human Array

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Abstract

The human HazChem array includes 16 control genes and 300 environmental toxicity-related genes. In past experiments, the expression levels of these genes were altered in whole genome microarray experiments using VOCs and PAH-treated human cells. In this study, we employed the human HazChem array to determine the gene expression pattern of chemical groups. The chemical groups used in this study were PAHs, POPs, and VOCs. PAHs are one of the most widespread organic pollutants. We used chrysene and phenanthrene as examples of PAHs. POPs are chemical substances that remain in the environment, and bioaccumulate through the food chain. We utilized chlordane and toxaphene as our sample POPs. VOCs are important outdoor air pollutants. They have been shown to cause nervous system disorders through respiration and skin contact, and generally are associated with foul odors. We utilized dichloromethane, ethylbenzene, and trichloroethylene as our sample VOCs. Thus, a total of 7 chemicals were assessed. In this study, HepG2 cells were treated with Polycyclic Aromatic Hydrocarbons (PAHs) and Persistent Organic Pollutants (POPs). HL-60 cells were treated with Volatile Organic Compounds (VOCs). Following comparative analysis, we detected some specific expression patterns in each of the chemical groups. We determined that the 7 chemicals utilized herein were divided into 3 chemical groups on the basis of the following 16 genes : HHEX, HLA-G, C1QBP, RHEBL1, PMAIP1, PHIP, HK2, NOTCH1, PRF1, SGK, PLK3, BGLAP, LOC389844, GDF15, NRF1 and ABCC2. Subsequently, the HazChem Human array was used to group the chemicals.

Keywords: Toxicogenomics, PAHs (Polycyclic Aromatic Hydrocarbons), POPs (Persistent Organic Pollutants), VOCs (Volatile Organic Compounds), Microarray, Environmental hazards

Introduction

Health risk valuation is a process by which the qualitative and quantitative effects that might occur when a person is exposed to environmental toxicities are estimated. Toxic chemicals induce diseases such as cancer and leukemia in humans; this effect is generally attributed to expressional alterations in specific genes. Toxic chemicals can also be grouped according to their characteristics. Chemical groups tend to affect specific genes. By exploiting this tendency, we have attempted to verify the grouping of certain chemicals by their characteristic. In this study, we assessed the PAH, POP, and VOC chemical groups.

Polycyclic Aromatic Hydrocarbons (PAHs) are lipophilic chemical compounds which consist of fused aromatic rings and harbor no heteroatoms or substituents[1]. PAHs are detected in oil, coal, and tar deposits, and are generated as byproducts of fuel combustion. As a pollutant, the PAHs are of concern because certain PAH compounds have been identified as carcinogens, mutagens, and teratogens. Long-term exposure to PAHs via respiration or skin contact has been shown to result in cancers. Some PAHs have been demonstrated to cause cancer in laboratory animals when they breathed air containing them, ingested them in food, or had them applied to their skin[2].

Persistent Organic Pollutants (POPs) are organic compounds, and are resistant to environmental degradation via chemical, biological, and photolytic processes. POPs have been observed to persist in the environment, to be capable of long-range transport, to bioaccumulate in human and animal tissues, to biomagnify in food chains[3], and to exert potentially significant impacts on both human health and the environment.

Some studies have linked POP exposure to declines, diseases, or abnormalities in a variety of wildlife species, including certain fish, birds, and mammals. Wildlife can also function as sentinels for human health : abnormalities ordeclines detected in wildlife populations can be considered an early warning for humans.

Volatile Organic Compounds (VOCs) are organic compounds that evaporate into the atmosphere at room temperature. Artificial VOCs include paint thinners, furnishings, dry cleaning solvents, carpets, and tobacco smoke-all of which are possible sources of indoor VOC exposure. VOCs influence the eyes, nose, liver, kidney, and central nervous system, and can also cause headaches. High VOC concentrations principally induce toxic effects[4]. Additionally, low concentrations of VOCs can still directly exert harmful effects on the environment or on the human body. Persons with respiratory problems such as asthma, as well as young children, the elderly, and persons with heightened sensitivity to chemicals may be more susceptible to illnesses caused by VOC exposure[5,6].

The HazChem human array includes a total of 316 genes with 16 control spots and 300 environmental hazard-associated genes. This study applied the HazChem human array, which may prove useful in the discovery of significant genes that evidence toxicity expression. In this work, we processed specific PAHs (chrysene and phenanthrene), POPs (chlordane and toxaphene), and VOCs (ethylbenzene, dichloromethane, and trichloroethylene) in human cells[7]. After our experiments, we detected and recorded the gene expression patterns of each chemical group. The 7 chemicals were then ultimately grouped into 3 chemical groups in accordance with the observed patterns of gene expression.

Results

Cytotoxicity of Chemicals in Human Cell Lines

The relative survival of HepG2 cells after exposure to a range of concentrations of PAHs (chrysene and phenanthrene) and POPs (chlordane and toxaphene) were determined via MTT assays. Additionally, the cell viability of HL-60 cells after exposure to a range of concentrations of VOC (ethylbenzene, dichloromethane, and trichloroethylene) compounds were determined via an MTT assay. The survival percentage relative to the solvent control (DMSO) was determined

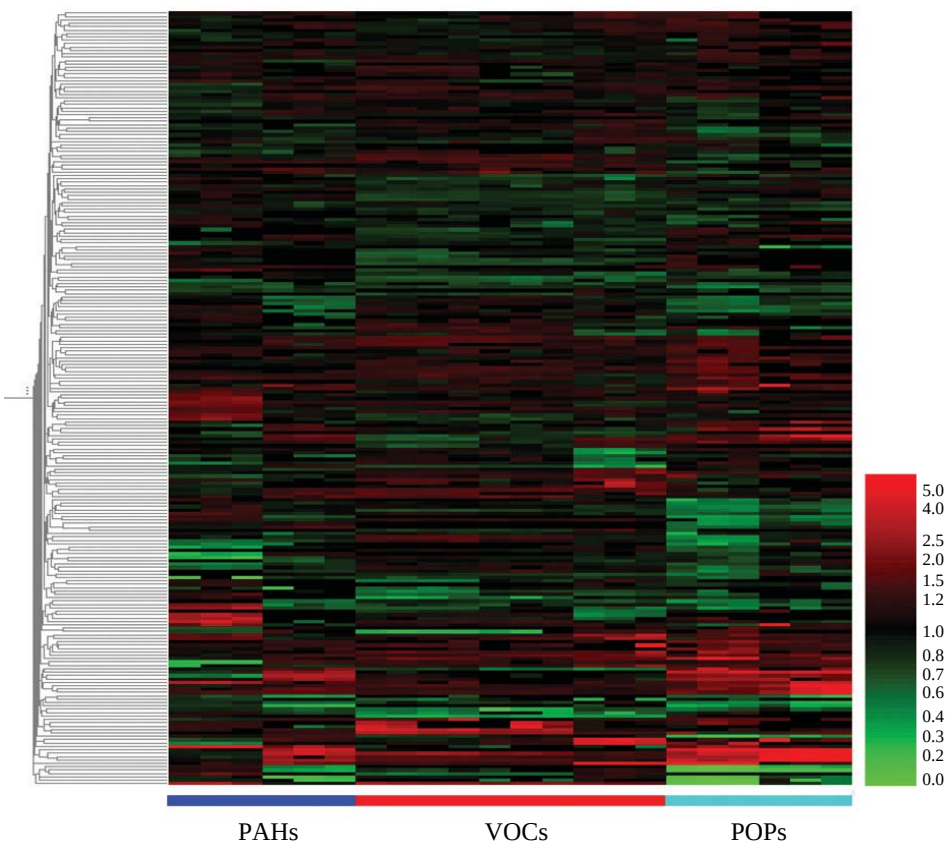

Figure1. Hierarchical clustering analysis of chemical treated cells. We applied chemicals to human cell lines. We used 3 chemical groups (PAHs, VOCs, POPs). The PAHs used were chrysene and phenanthrene. The VOCs used included ethylbenzene, trichloroethylene, and dichloromethane. The sample POPs used were chlordane and toxaphene.

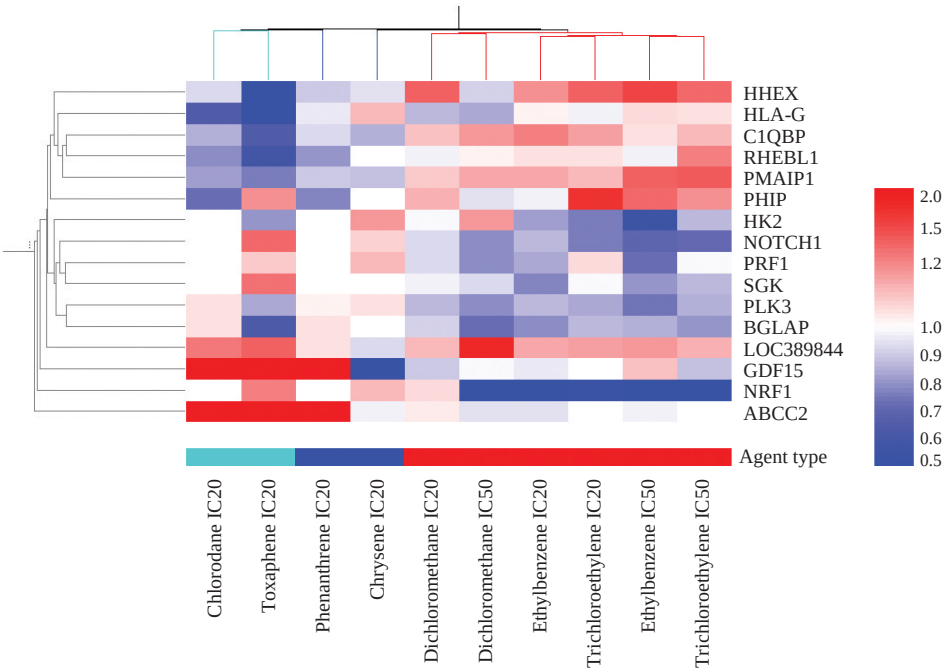

Figure 2. Chemical grouping according to differences in gene expression patterns with 16 genes. We used 16 genes to group the chemicals into 3 chemical groups. In the grouping process, we validated the differences in gene expression patterns among the 3 relevant chemical groups.

Table 1. Gene lists related to gene expression pattern of chemical groups.

GeneBank ID	GeneSymbol	GeneName	PAHs	POPs	VOCs
NM_002729	HHEX	hematopoietically expressed homeobox	↓	↓	↑
NM_002127	HLA-G	HLA-G histocompatibility antigen, class I, G	–	↓	↑
NM_001212	C1QBP	complement component 1, q subcomponent binding protein	↓	↓	↑
NM_144593	RHEBL1	Ras homolog enriched in brain like 1	–	↓	↑
NM_021127	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	↓	↓	↑
NM_017934	PHIP	pleckstrin homology domain interacting protein	–	–	↑
NM_000189	HK2	hexokinase 2	–	–	↓
NM_017617	NOTCH1	Notch homolog 1, translocation-associated (Drosophila)	–	–	↓
NM_005041	PRF1	perforin 1 (pore forming protein)	–	–	↓
NM_005627	SGK	serum/glucocorticoid regulated kinase	–	–	↓
NM_004073	PLK3	polo-like kinase 3 (Drosophila)	–	–	↓
NM_199173	BGLAP	bone gamma-carboxyglutamate (gla) protein	–	–	↓
XM_372202	LOC389844	similar to ferritin, heavy polypeptide-like 17	–	↑	↑
NM_004864	GDF15	growth differentiation factor 15	–	↑	↑
NM_005011	NRF1	nuclear respiratory factor 1	–	–	↓
NM_000392	ABCC2	ATP-binding cassette, sub-family C member 2	–	↑	↑

as the percentage of OD value measured after treatment.

Gene Expression Analysis

HepG2 cells were treated with PAHs and POPs, and HL-60 cells were treated with VOCs, after which the RNA was subjected to human HazChem array analysis. For each treatment, genes with statistically significant expressional changes were identified via microarray. Hierarchical clustering was applied across the seven agents, using a combined list of genes (Figure 1). The results obtained using the human HazChem array and the comparison of the gene expression clusterings for chrysene IC20, phenanthrene IC20, chlordane IC20, toxaphene IC20, ethylbenzene IC50, dichloromethane IC50, and trichloroethylene IC50 compounds showed differentially expressed gene patterns according to the chemical groups to which the compounds belonged (Figure 1).

Classification

We utilized a statistical method for the classification of chemicals using gene expression profiles.[8,9] We employed ANOVA as a statistical method, with Wel-

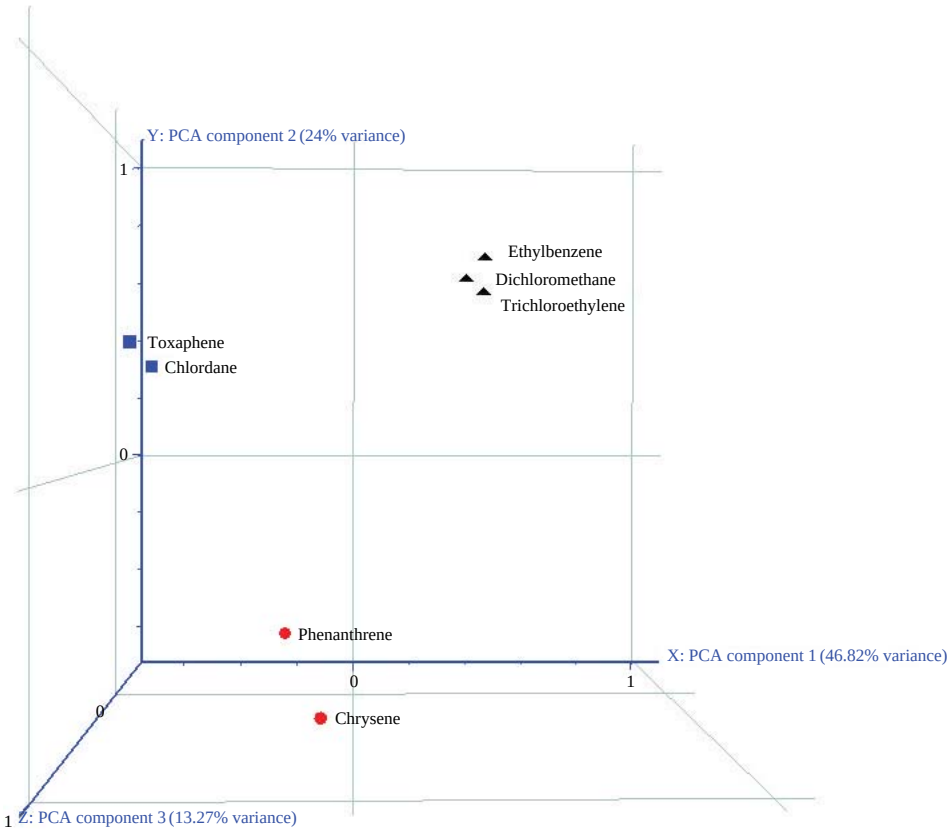

Figure 3. The expression profiles of chemical were divided into 3 groups-PAHs (red), POPs (blue), and VOCs (black) groups. The selected genes were subjected to the PCA algorithm in order to verify their chemical groupings.

ch's T-test as an algorithm. We detected some specific expression patterns of 16 genes in each of the chemical groups. We determined that 10 chemicals (including additional ethylbenzene IC50, dichloromethane IC50, and trichloroethylene IC50) could be divided into 3 chemical groups on the basis of the 16 genes. The genes assessed herein were : HHEX, HLA-G, C1QBP, RHEBL1, PMAIP1, PHIP, HK2, NOTCH1, PRF1, SGK, PLK3, BGLAP, LOC389844, GDF15, NRF1, and ABCC2 (Figure 2) (Table 1). These genes regulate protein binding (GO : 5515), nucleotide binding (GO : 116) and enzyme activities (GO : 4674, GO : 16301, GO : 16491, GO : 16740, GO : 16887).

Using the PCA program[10], we tested 16 genes with regard to their chemical groupings (Figure 3). We were principally interested in marker genes for group prediction. We generated 16 genes as predictive markers for chemical groups.

Discussion

DNA microarrays are a powerful, high-throughput tool for the screening of the expression of genes. In environmental monitoring, this might provide a method by which chemicals can be quickly categorized and assigned a mode of toxic action. This approach also allows for more sensitive end points to be addressed. Recently, a great deal of interest has been focused on the use of microarrays in toxicology for the rapid classification of toxicants on the basis of characteristic expression profiles, as well as the use of these profiles as a means for identifying the putative mechanism of action[11-13].

The HazChem human array chip was designed for the purpose of studying toxicant action in humans[14]. In this study, we utilized a HazChem human array to identify the significantly differentially expressed genes induced by several chemicals in human cell lines. The results of microarray data analysis demonstrated differentially expressed gene patterns as the result of exposure to PAHs (chrysene, phenanthrene), POPs (chlordane, toxaphene), and VOCs (ethylbenzene, dichloromethane, trichloroethylene) in a human cell line. We found 16 discriminatory genes associated with different patterns between PAHs, POPs and VOCs, on the basis of the gene expression profiles and genetic distances (Figure 2).

The genes utilized herein were : HHEX, HLA-G, C1QBP, RHEBL1, PMAIP1, PHIP, HK2, NOTCH1,

PRF1, SGK, PLK3, BGLAP, LOC389844, GDF15, NRF1, and ABCC2. We generated 16 genes as predictive markers for the chemical groups.

In conclusion, our study focused on the chemical grouping of several chemicals using a HazChem human array. We verified in this study that the HazChem human array may prove useful in grouping the chemicals.

Materials and Methods

Chemicals and Reagents

Chrysene, Phenanthrene, Chlordane, and Toxaphene were purchased from Riedel dehaën (Germany) and TCI-EP (Japan), respectively. Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (USA). Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's Phosphate Buffered Saline (PBS), 0.5% trypsin-EDTA, and Fetal Bovine Serum (FBS) were acquired from GIBCO^{TM} (USA).

Ethylbenzene (CAS No, 100-41-4), trichloroethylene (CAS No. 79-01-6), dimethylsulphoxide (DMSO), and 3-(4,5-dimethylthizol-2-yl)-2,5-diphenyltetrazolium bromide were purchased from the Sigma-Aldrich Chemical Company (USA). RPMI-1640 Culture Medium, Dulbecco's Phosphate Buffered Saline (PBS) and Fetal Bovine Serum (FBS) were purchased from GIBCO^{TM} (USA). All other chemicals used in this study were of analytical grade or the highest available grade.

Cell Line and Culture

A human hepatocellular carcinoma cell line (HepG2) was utilized throughout the study, and the line was purchased from the Korean Cell Line Bank (Korea). HepG2 cells were grown in DMEM medium supplemented with 10% inactivated FBS plus 0.044 M sodium bicarbonate, 10 mM sodium pyruvate, and 1% penicillin at 37°C in a 5% CO_2 atmosphere. For cell growth, the medium was renewed every two or three days. HepG2 at an approximate 80% confluence was achieved by plating 6×10^6 cells/mL in a 100 mm culture dish. In the case of HL-60, we referred to the paper.[7]

Determination of Cell Viability

MTT assays[15] were conducted for the detection of cell viability. A 24-well plate was utilized for the cytotoxicity assay. Cells were seeded at a seeding density of 80×10^4 cells/mL per well in 500 μL of media. The cells were exposed to various concentrations of chrysene, phenanthrene, chlordane, and toxaphene in culture medium at 37°C for 48 hrs. 75 μL of MTT (4 mg/mL in PBS) solution was added to each well and incubated for 3 hrs. DMSO solution was added to each well and transferred to 96-well plates. The optimal density (OD) of the purple formazan product was measured at a wavelength of 540 nm. The 20% inhibitory concentration (IC_{20}) of cell proliferation in a particular chemical was defined as the concentration required to induce a 20% reduction in the cell viability versus the solvent-treated control. The IC_{20} values were directly determined from the linear dose-response curves.

In the case of the MTT assay of HL-60 cells, we referred to the paper.[7]

Preparation of HazChem Array

The HazChem human array[7] (GenoCheck, Ansan, Korea) was utilized in this study. This array includes 300 environmental hazard-related genes and 16 control spots.

RNA Extract & Hybridization

Total RNA was extracted from the cells using Trizol reagent (Invitrogen Life Technologies) and purified with an RNeasy Mini Kit (Qiagen, USA) in accordance with the manufacturer's instructions. Genomic DNA was removed using an RNase-free DNase set (Qiagen, USA) during RNA purification. The quantity of each RNA concentration was quantified using Nanodrop, and the RNA quality was evaluated using an Agilent Bioanalyzer 2100 (Agilent Technologies).

Each extracted total RNA sample (30 μg) was labeled with Cyanine (Cy3)- or Cyanine (Cy5)- conjugated dCTP (Amersharm, Piscataway, NJ) via a reverse transcription reaction using reverse transcriptase, PrimeScrip Reverse Transcriptase (TaKaRa, Japan). The labeled cDNA mixture was then concentrated via ethanol precipitation. The concentrated Cy3- and Cy5-labeled cDNAs were resuspended in 10 μL of hybridization solution (GenoCheck, Korea). The two labeled cDNAs were then mixed, placed on a HazChem array Human 300 (GenoCheck, Korea) and covered with a MAUI M4 chamber (Biomicro Systems, Inc. UT). The slides were then hybridized for 12 hr at 62°C with a MAUI system (Biomicro Systems, Inc. UT). The hybridized slides were washed in 2 X SSC, 0.1% SDS for 2 min, 1 X SSC for 3 min, and then in 0.2 X SSC for 2 min at room temperature. The slides were subsequently centrifuged for 20 seconds at 3,000 rpm to dry.

Microarray Data Analysis

The hybridized slides were scanned with an Axon Instruments GenePix 4000B scanner and the scanned

images were analyzed with GenePix Pro 5.1 (Axon, CA) and GeneSpring GX 7.3.1 (Sillicongenetics, CA) software. Spots that were adjudged as substandard via the visual examination of each slide were flagged and excluded from further analysis. Spots that harbored dust artifacts or spatial defects were manually flagged and excluded. In an attempt to filter out the unreliable data, spots with signal-to-noise (signal-background-background SD) ratios below 10 were not included in the data. Data were normalized via Global, lowess, print-tip, and scaled normalization for data reliability. The data were clustered groups of genes that behaved similarly across the drug treatment experiments using GeneSpring GX 7.3.1 software (Silicongenetics, CA). We utilized an algorithm based on the Pearson's correlation to separate genes exhibiting similar patterns[16].

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