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Development of stable *HSPA1A* promoter-driven luciferase reporter HepG2 cells for assessing the toxicity of organic pollutants present in air

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Abstract *HSPA1A* (*HSP70-1*) is a highly inducible heat shock gene up-regulated in response to environmental stresses and pollutants. The aim of our study was to evaluate the sensitivity of the stable metabolically competent HepG2 cells containing a human *HSPA1A* promoter-driven luciferase reporter (HepG2-luciferase cells) for assessing the toxicity of organic pollutants present in air. The HepG2-luciferase cells were validated by heat shock treatment and testing three organic compounds (pyrene, benzo[a]pyrene, and formaldehyde) that are ubiquitous in the air. The maximal level of HSPA1A (HSP70-1) and relative luciferase activity induced by heat shock were over three and nine times the control level, respectively. Pyrene, benzo[a]pyrene, and formaldehyde all induced significantly elevated levels of relative luciferase activity in a dose-dependent manner. Extractable organic matter (EOM) from urban traffic and coke oven emissions in ambient air were tested on the HepG2-luciferase cells. The traffic EOM induced significant increase in relative luciferase activity at concentrations of picogram per liter. The coke oven EOM produced a strong dose-dependent induction of relative luciferase activity up to six times the control value. Significant increases in relative luciferase activity were observed at concentrations that were as low, or lower than the concentrations that the tested organic pollutants decreased cell viability, and increased malondialdehyde concentration, Olive tail moment, and micronuclei frequency. Therefore, we conclude that the HepG2-luciferase cells are a valuable tool for rapid screening of the overall toxicity of organic pollutants present in air.

Keywords *HSPA1A* promoter activity · Luciferase reporter · Organic pollutants · Cell viability · Cellular damage · Toxicity assessment

Abbreviations
EOM   Extractable organic matter
MDA   Malondialdehyde
HSPs   Heat shock proteins
PAHs   Polycyclic aromatic hydrocarbons
DMSO   Dimethylsulfoxide

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Introduction

Increasing concentrations of pollutants present in air are public health problems because of raising rates of cancer and other environmentally related diseases. Everyday, many people are exposed to organic pollutants from urban traffic. Workers in industry are also exposed to high levels of organic pollutants in the air. For example, the coke oven

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emissions represent the coal-burning pollution in the air, and they contain a large variety of toxic compounds including polycyclic aromatic hydrocarbons (PAHs). The toxicity assessment of these pollutants present in air is challenging. To evaluate toxicity, a number of in vitro tests have been developed to examine cell viability, oxidative stress, DNA strand breaks, and micronuclei formation (Ohkawa et al. 1979; Olive and Banáth 2006; Wu et al. 2003; Zheng et al. 2009). These commonly used assays with mammalian cells are time-consuming and often require expertise for staining and microscopic observation and consequently are unsuitable for screening of pollutants in large numbers of environmental samples. In many environmental samples, concentrations of many components are low, and chemical analysis and the commonly used assays are not sensitive enough and are not sufficient to achieve toxicity assessment. The synergistic, antagonistic, or additive toxic effects produced by complex chemical mixtures further complicate toxicity testing. Hence, a rapid and cost-effective toxicity assessment bioassay is needed to determine the toxic potential of complex chemical mixtures. Bioassays using reporter vectors regulated by cellular stress-responsive gene promoters have been suggested as promising tools for toxicity assessment (Simmons et al. 2009).

Among the various cellular stress response pathways, the heat shock response is one of the major pathways characterized by transcriptional up-regulation of a family of genes encoding heat shock proteins (HSPs). HSPs are highly conserved molecular chaperones that play essential roles in nascent protein folding, misfolded protein refolding, or degradation (Hightower 1991; Parsell and Lindquist 1993). The HSPA (HSP70) family, the best characterized HSPs, contains a highly stress-inducible member encoded by the *HSPA1A* gene. The *HSPA1A* gene is up-regulated by a number of pathophysiological stressors, such as ischemia (Truettner et al. 2009), and by environmental stressors, such as air pollutants (Deane and Woo 2006; Luparello et al. 2011). Because of its stress responsiveness, *HSPA1A* expression is a potential marker in toxicological screening (Gupta et al. 2010). In fact, human cells carrying an *HSPA1A* promoter-driven reporter gene have been used to assess the toxicity of heavy metals, organochlorine compounds, and eluates of industrial wastes (Aït-Aïssa et al. 2003; Aït-Aïssa et al. 2000). However, HeLa cells may not be metabolically competent enough to metabolize PAHs to more toxic metabolites, such as benzo[a]pyrene diolepoxide.

The HepG2 cells, derived from a human liver hepatoma, are reported to retain many properties of primary cells such as containing many functional phase I and II enzymes, which are lost in most cultured cell lines (Doostdar et al. 1993; Westerink and Schoonen 2007). The HepG2 cells are metabolically competent and able to metabolize a wide variety of toxicants including PAHs. In the present study,

the HepG2 cells were stably transfected with a human *HSPA1A* promoter-driven luciferase reporter (HepG2-luciferase cells). We assessed the toxicological sensitivity of HepG2-luciferase cells by comparison with a battery of established in vitro toxicity tests, namely, cell viability assay, malondialdehyde (MDA) assay, comet assay, and micronucleus test. Our hypothesis was that transcriptional activation of the *HSPA1A* promoter-driven luciferase reporter is a sensitive indicator of the overall toxicity of complex organic pollutants present in air from urban traffic and coke oven emissions.

Materials and methods

Chemicals and materials

Pyrene (99% purity) and benzo[a]pyrene (98% purity) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Formaldehyde was purchased from Sinopharm Chemical Reagent Co. Ltd in China. Dulbecco's modified Eagle's minimal essential medium, trypsin, newborn calf serum, and geneticin selective antibiotic (G418) were obtained from GIBCO (Grand Island, NY, USA). The luciferase assay system, pGL4.17 [*luc2*/Neo] vector, and the CellTiter-Glo luminescent cell viability assay kit were purchased from Promega (Madison, WI, USA).

Air sampling and preparation of EOMs

Urban vehicle emissions were collected at a heavily traveled road in Wuhan, China, during the rush hours in May 2010, while the coke oven emissions in the ambient air were collected on the topside of a coke oven battery in a state-run coke oven plant located in Central China in November 2010. At each locality, samplings were performed using GilAir-5 air samplers (Sensidyne, USA). The airflow rate was set at 2 l/min, lasting for 6 h. The sampler was equipped with a glass tube filled with purified XAD-2 (ORBO 43, from Supelco) to collect the organic pollutants in the gas phase and a Teflon filter (Gelman Zefluor 37 mm, 2 μm) to collect the particulate matter. The collected samples were extracted with organic solvents, and then the organic extracts from the gas phase and particulate matter collected at one locality were combined. Quantitative chemical analysis of PAHs was performed by high-performance liquid chromatography with fluorescence detection according to Method 5506 of the US National Institute for Occupational Safety and Health (NIOSH 1998), and the airborne concentrations of the total PAHs were 0.6 and 1.1×10^3 μg/m^3 for the traffic and the coke oven samples, respectively. For the in vitro experiments, EOM samples were evaporated to dryness and dissolved in dimethylsulfoxide (DMSO), and

the stock solution of the traffic and coke oven EOM samples contained 0.063 and 688 μg PAHs/ml DMSO, respectively.

Cell culture

The HepG2 cell line was kindly provided by Dr. Darroudi (Leiden University, The Netherlands). Cells were cultured in Dulbecco's modified Eagle's minimal essential medium supplemented with 10% newborn calf serum, 2 mM glutamine, and antibiotics. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37°C, and medium was refreshed every 2 or 3 days with sub-culturing.

Construction of HSPA1A promoter-driven luciferase plasmid

The human HSPA1A promoter was isolated with primer sequences (He et al. 2009) as follows: forward primer 5′-TAGGGGTACCGACGGCTCCAACTCAGTAATC-3′; reverse primer 5′-ATGCAAGCTTGCCGGTTCCCTGCTCTCTGTCG-3′. The sequence of human HSPA1A promoter was inserted into the KpnI–HindШ site of pGL4.17 [luc2/Neo] (Promega, Madison, WI, USA) luciferase plasmid. The direction and sequence authenticity of the luciferase reporter plasmid were validated by restriction analysis and direct sequencing.

Generation of stable HSPA1A promoter-driven luciferase reporter cells

The luciferase reporter plasmid was transfected into HepG2 cells using the Lipofectamine ™ 2000 (Invitrogen, USA) according to the manufacturer's instructions. HepG2 cells were trypsinized and resuspended in culture medium to a final concentration of 1.5×10^5 cells/ml. To each well of a white six-well culture plate, 2 ml suspension was added. The plate was incubated for 24 h at 37°C, and then a mixture containing 1 μg luciferase reporter plasmid and 2.5 μl Lipofectamine was added to each well for the transfection reaction. Transfected cells were incubated with culture medium containing 0.1 mg/ml G418 for 14 days. Twenty G418-resistant clones were isolated and screened by measuring their inducible luciferase activities in response to heat shock. One positive clone, which showed low background and high inducible luciferase activities, was chosen as the luciferase reporter cell line in the subsequent experiments. This cell line is now called the HepG2-luciferase cells.

Treatment of HepG2-luciferase cells with heat shock

The HepG2-luciferase cells were heated at 42°C for 1 h and then recovered at 37°C for 24 h. Cells were harvested at 0, 1, 2, 4, 8, 12, and 24 h of recovery for analysis of levels of HSPA1A and relative luciferase activity.

Treatment of HepG2-luciferase cells with environmental pollutants

Prior to each experiment, HepG2-luciferase cells were seeded in triplicate at a density of 2.5×10^4 per well in 48-well culture plates or 2.5×10^5 per well in six-well culture plates. After 24 h, the culture medium was replaced with fresh medium supplemented with vehicle alone (DMSO) or increasing concentrations of pyrene (1, 5, 10, 50, and 100 μM), benzo[a]pyrene (2.5, 5, 10, 20, and 40 μM), formaldehyde (1, 5, 10, 20, and 40 μM), traffic EOM (0.006, 0.06, 0.6, 6.2, and 63 ng/l), and coke oven EOM (0.2, 1.1, 5.5, 28, 138, and 688 μg/l) for 24 h. Pyrene, benzo[a]pyrene, the traffic and coke oven EOMs were dissolved in DMSO, and the concentrations of stock solutions were 100 mM, 40 mM, 63 μg/l, and 688 mg/l, respectively. The stock solution of formaldehyde was 40 mM. The final concentrations of DMSO used in all experiments did not exceed 0.1%.

Luciferase activity in HepG2-luciferase cells

After the treatments, cells were washed with 0.5 M phosphate-buffered saline (pH 7.4). Subsequently, 100 μl lysis buffer was added to each well. Plates were shaken at room temperature for 15 min, and luciferase activities were measured using the luciferase assay system with a luminometer (LUMAT LB9507; Berthold, Germany). The ratio of the luciferase activities (test sample/control group) is the relative luciferase activity (versus control). In each experiment, the relative luciferase activities for three replicates were averaged. Each assay was repeated at least twice to corroborate the results.

Determination of cell viability

The number of viable HepG2-luciferase cells in culture was determined using the CellTiter-Glo Luminescent Cell Viability Assay kit. This assay is a homogeneous method to determine the number of viable metabolically active cells based on the quantification of ATP present. The untreated cells were the 100% viable control.

Measurement of oxidative damage

Levels of oxidative damage in HepG2-luciferase cells were measured using the MDA assay kit (Jiancheng Bio Company, Nanjing, China). This assay is based on the reaction of MDA with thiobarbituric acid, at moderate temperature, which yields a stable chromophore with maximal absorbance at

532 nm wavelength. The results are reported as MDA concentration and calculated as nanomoles per milliliter.

Determination of genotoxic damage

We measured genotoxic damage in HepG2-luciferase cells by comet assay and micronucleus test. The comet assay was performed under alkaline conditions using the methods described previously (Olive and Banáth 2006). The level of DNA damage was recorded under the fluorescence microscope, and images of 100 random selected cells were analyzed for each sample by Comet Assay Software Project (CASP, http://casplab.com/). The means of Olive tail moment were used to assess the DNA damage. The micronuclei frequency was determined by micronucleus test as previously described (Wu et al. 2003) with some minor modifications. A total of 1,000 binucleated cells for each sample were examined microscopically for micronuclei as previously described (Fenech 2007). The micronuclei frequency is reported as the number of micronuclei per 1,000 binucleated cells.

Detection of HSPA1A protein levels

Western analysis was carried out as described previously (Towbin et al. 1979) with some minor modifications. About 10 μg total proteins were loaded in each well and separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. After running, the proteins were transferred onto nitrocellulose membranes. The membranes were saturated and blocked with 5% fat-free milk at 37°C for 1 h, before being incubated with mouse anti-HSPA1A (SPA 810, StressGen) or mouse anti-β-actin antibodies (A5441, Sigma). After extensive washing, the second antibody (goat anti-mouse HRP, SPH-101, 4A biotech) was added. Specific antibody–antigen complexes were detected using the supersignal west pico trail kit (Thermo Scientific, USA). Immunoreactive bands on membranes were quantified for the level of specific induction using GeneTools software from SynGene.

Statistical analysis

Three individual experiments were performed for each time point or dose group, and the results represented means±SD. Statistical analysis was performed by one-way analysis of variance followed by the least significant difference test or Dunnett's test. The bivariate correlations between the relative luciferase activities and levels of cellular damage were evaluated using the Spearman rank test. In all tests, differences were considered significant at $p < 0.05$. All the data analysis was carried out using the statistical analysis software SPSS 12.0 for windows (SPSS Inc., Chicago, IL, USA).

Results

Validation of the HepG2-luciferase cells

The HepG2-luciferase cells were validated by the classic inducer of heat shock response, heat shock treatment. Heat shock induced a progressive increase in the abundance of HSPA1A and relative luciferase activity peaking at 4 h of recovery (Fig. 1a–c). The relative level of HSPA1A at 4 h of recovery was over three times the control level. Surprisingly, at this recovery time, the relative luciferase activity was greater than nine times the control level. The Spearman rank test
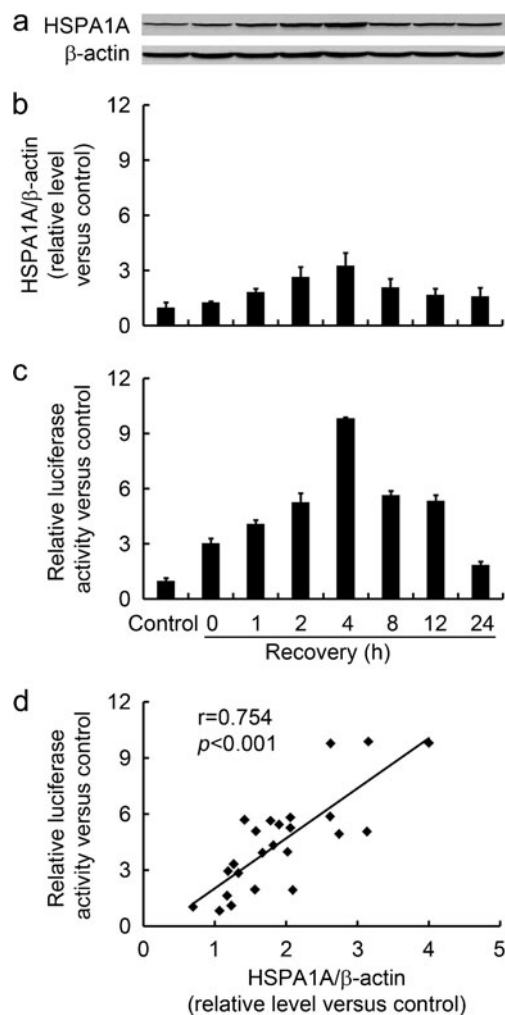

Fig. 1 HepG2-luciferase cells respond to heat shock with increased levels of HSPA1A and relative luciferase activity. The HepG2-luciferase cells contain a human *HSPA1A* promoter-driven luciferase reporter. Cells were heated at 42°C for 1 h and then recovered at 37°C for the indicated times. The untreated cells were used as control. **a** HSPA1A levels detected by Western analysis after heat shock. β-Actin was used as the loading control. **b** Levels of HSPA1A and β-actin were determined by densitometric analysis. **c** Relative luciferase activity was measured using the luciferase assay system. **d** Correlation between relative luciferase activity and relative level of HSPA1A was evaluated using the Spearman rank test. Data represent mean±SD of three individual experiments

showed that the relative luciferase activity was positively correlated with the level of HSPA1A (Fig. 1d).

Pyrene induced a dose-dependent increase in relative luciferase activity up to 2.4 times the control level ($p<0.01$) at 100 μM (Fig. 2a), where the cell viability declined to 60% of the control at this dose (Fig. 2b). Pyrene treatment

resulted in significant increases in MDA concentration (Fig. 2c). The increases in Olive tail moment and micronuclei frequency induced by pyrene were not statistically significant (Fig. 2d, e).

Benzo[a]pyrene led to a 2.7 times induction of relative luciferase activity at 20 μM and was effective at a
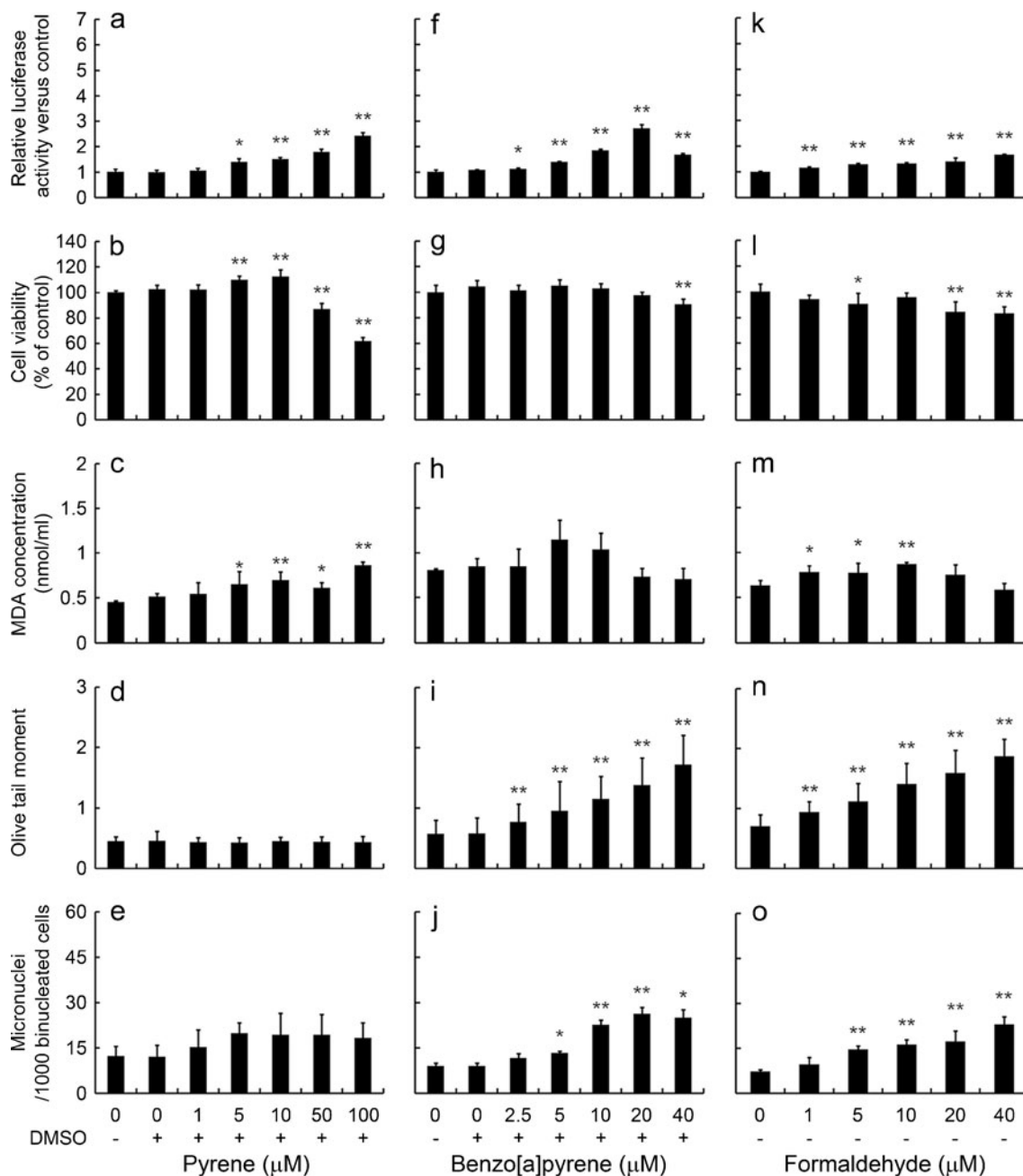

Fig. 2 HepG2-luciferase cells respond in a dose-dependent manner to three organic compounds ubiquitous in the air. Chemicals tested were pyrene, benzo[a]pyrene, and formaldehyde. The luciferase activity in HepG2-luciferase cells was measured and expressed as relative luciferase activity versus control (a, f, k). Cell viability was evaluated using the CellTiter-Glo Assay kit (b, g, l). Oxidative damage was measured by MDA concentration (c, h, m). DNA damage was assessed with the alkaline comet assay and was expressed as Olive tail moment (d, i, n). The micronuclei frequency was determined by CBMN cytome assay and was expressed as the number of micronuclei per 1,000 binucleated cells (e, j, o). Chemical concentrations are at the *bottom*. DMSO (0.1%) was the vehicle for pyrene and benzo[a]pyrene. Data represent mean±SD of three individual experiments. *$p<0.05$ compared with untreated controls. **$p<0.01$ compared with untreated controls

concentration as low as 2.5 µM (Fig. 2f). The decrease of relative luciferase activity at 40 µM compared with the maximal level at 20 µM may be attributable to the saturation of PAH activating enzymes or decrease in cell viability (Fig. 2g). Even at the highest concentration tested, benzo [a]pyrene had no effect on MDA concentration (Fig. 2h). The significant increases in Olive tail moment and micronuclei frequency showed dose-dependent relationships with benzo[a]pyrene concentration (Fig. 2i, j).

The low dose of formaldehyde (1 µM) led to a significant increase in relative luciferase activity that progressively increased to 1.6 times the control level at 40 µM (Fig. 2k). This induction rate was low, possibly due to the decrease in cell viability (Fig. 2l). Formaldehyde treatment resulted in significant increases in MDA concentration (Fig. 2m). The increases in Olive tail moment and micronuclei frequency were significant and showed dose-dependent relationships with formaldehyde concentration (Fig. 2n, o).

EOM treatment activated *HSPA1A* promoter-driven luciferase reporter

The traffic EOM induced low, but detectable relative luciferase activity at concentrations of picogram per liter (Fig. 3a). However, traffic EOM at much higher concentrations had no effect on cell viability (Fig. 3b). The traffic EOM resulted in significant increases in MDA concentration (Fig. 3c). The significant increases in Olive tail moment and micronuclei frequency showed dose-dependent relationships with traffic EOM concentration (Fig. 3d, e).

The coke oven EOM produced a strong dose-dependent induction of relative luciferase activity up to six times the control level at the highest concentration tested (Fig. 3f). Only at the highest concentration of coke oven EOM tested, cell viability was decreased to 86% of the control (Fig. 3g). The coke oven EOM resulted in significant increases in MDA concentration (Fig. 3h). The significant increases in Olive tail moment and micronuclei frequency showed dose-dependent relationships with coke oven EOM concentration (Fig. 3i, j).

Correlations between relative luciferase activity and cellular damage

Relative luciferase activity was correlated with MDA concentration, Olive tail moment, and micronuclei frequency (Table 1). In HepG2-luciferase cells treated with pyrene, relative luciferase activity only positively correlated with MDA concentration ($r=0.786$, $p<0.001$). In cells exposed to benzo[a]pyrene, relative luciferase activity positively correlated with Olive tail moment ($r=0.869$, $p<0.001$) and micronuclei frequency ($r=0.904$, $p<0.001$). For formaldehyde, positive correlations were observed between relative luciferase activity and Olive tail moment ($r=0.967$, $p<0.001$) and micronuclei frequency ($r=0.813$, $p<0.001$). In HepG2-luciferase cells treated with traffic EOM, relative luciferase activity was positively correlated with both Olive tail moment and micronuclei frequency ($p=0.001$ and $p=0.007$, respectively). In cells treated with coke oven EOM, relative luciferase activity was positively correlated with MDA concentration ($r=0.634$, $p=0.001$), Olive tail moment ($r=0.964$, $p<0.001$), and micronuclei frequency ($r=0.533$, $p=0.007$).

Discussion

In the present study, we examined the suitability of the HepG2-luciferase cells for assessing toxicity of organic pollutants present in air. The HepG2-luciferase cells had a dose-dependent increase in relative luciferase activity induced by a group of organic compounds and complex mixtures, suggesting their suitability as a toxicological screening tool. The results obtained from the two EOMs suggest that the HepG2-luciferase cells can be used to assess the overall toxicity of unknown environmental samples. The HepG2-luciferase cells have several advantages over some of the well tested models, such as transgenic fly, worms, or other cell lines. The advantages of using the metabolically competent HepG2-luciferase cells to assess the toxicity of organic pollutants present in air are the following: (1) it does not require the expertise for staining or microscopic observation, (2) it is easier to conducted, and (3) it is less time-consuming. It only costs about 30 s to measure the luciferase activity of one sample, (4) allowing for qualitative and quantitative assessment of reporter gene induction in a high through setup (48-well culture plates); (5) compared with other cultured cell lines, the HepG2 cells are metabolically competent enough to metabolize a wide variety of toxicants to more toxic metabolites. The HepG2-luciferase cells can be used to screen larger numbers of organic pollutants present in air. (6) It is highly sensitive; the HepG2-luciferase cells can be used to assess the toxicity of low concentrations of organic complex mixtures such as extractable organic matter (EOM) from urban traffic (picogram per liter). Compared with other in vitro toxicity tests examined in the present study, the HepG2-luciferase cells have higher sensitivity, reproducibility, and lower costs that facilitate rapid screening of organic pollutants in large numbers of environmental samples.

Validation of the HepG2-luciferase cells

Expression of HSPA1A is regulated mainly at the transcriptional level (Morimoto 1993). In HepG2-luciferase cells, the endogenous *HSPA1A* promoter is driving the expression of

Fig. 3 HepG2-luciferase cells respond in a dose-dependent manner to two complex mixtures of EOM from urban traffic and coke oven emissions. The complex mixtures were traffic EOM and coke oven EOM. The luciferase activity in HepG2-luciferase cells was measured and expressed as relative luciferase activity versus control (**a**, **f**). Cell viability was evaluated using the CellTiter-Glo Assay kit (**b**, **g**). Oxidative damage was measured by MDA concentration (**c**, **h**). DNA damage was assessed with the alkaline comet assay and was expressed as Olive tail moment (**d**, **i**). The micronuclei frequency was determined by CBMN cytome assay and was expressed as the number of micronuclei per 1,000 binucleated cells (**e**, **j**). EOM concentrations are at the *bottom*. DMSO (0.1%) was the vehicle for the EOMs. Data represent mean±SD of three individual experiments. *$p < 0.05$ compared with untreated controls. **$p < 0.01$ compared with untreated controls
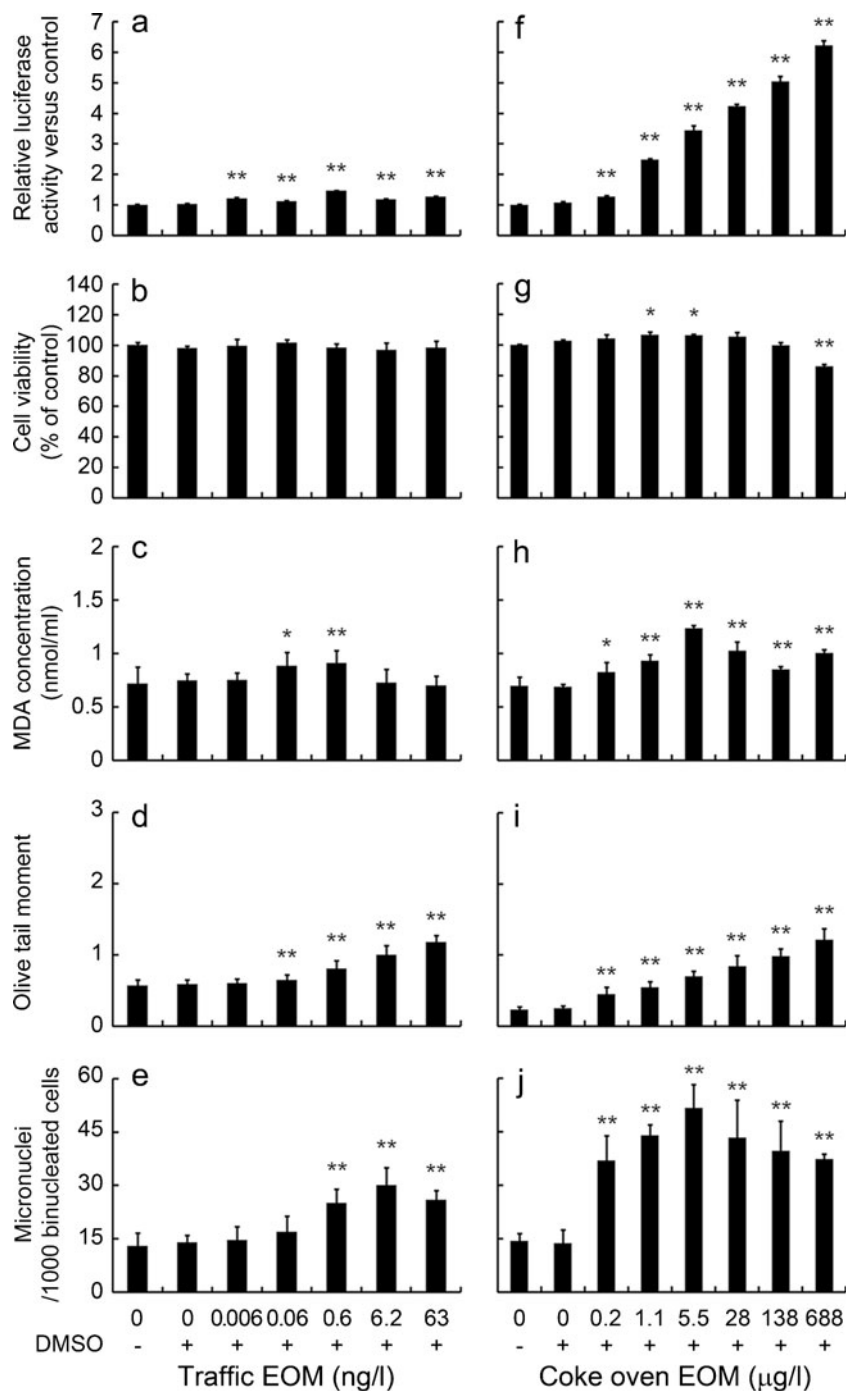
the (endogenous) *HSPA1A* gene and the transfected plasmid containing the *HSPA1A* promoter is driving the luciferase reporter. Heat shock treatment activates the heat shock factor that binds to the heat shock element in the *HSPA1A* promoter to initiate transcription (Morimoto 1993). Interestingly, both the endogenous *HSPA1A* gene and the transfected *HSPA1A* promoter-driven luciferase reporter responded to heat shock with maximal levels of HSPA1A (over three times the control level) and luciferase activity (greater than nine times the control level), respectively, at 4 h of recovery. The newly

developed *HSPA1A* promoter-driven luciferase reporter is several times more sensitive than the expression of HSPA1A. These findings show the sensitivity of the HepG2-luciferase cells.

Pyrene, a non-carcinogen, increased relative luciferase activity at non-cytotoxic concentrations (5–10 μM) in HepG2-luciferase cells. Positive correlation between relative luciferase activity and oxidative damage, detected by MDA concentration, suggests that pyrene is non-carcinogenic but may cause protein damage (Hightower 1991).

Table 1 Correlations between relative luciferase activity and levels of cellular damage in HepG2-luciferase cells treated with organic pollutants

Group	MDA concentration		Olive tail moment		Micronuclei frequency	
	r	p value	r	p value	r	p value
Pyrene-exposed cells						
Relative luciferase activity	0.786	0.000	−0.061	0.793	0.269	0.238
Benzo[a]pyrene-exposed cells						
Relative luciferase activity	−0.089	0.700	0.869	0.000	0.904	0.000
Formaldehyde-exposed cells						
Relative luciferase activity	−0.039	0.879	0.967	0.000	0.813	0.000
The traffic EOM-exposed cells						
Relative luciferase activity	0.125	0.590	0.677	0.001	0.573	0.007
The coke oven EOM-exposed cells						
Relative luciferase activity	0.634	0.001	0.964	0.000	0.533	0.007

Benzo[a]pyrene is metabolized to benzo[a]pyrene diolepoxide that intercalates into and distorts the DNA helix. This makes benzo[a]pyrene carcinogenic and mutagenic. Benzo[a] pyrene increased relative luciferase activity at non-cytotoxic concentrations in HepG2-luciferase cells. The correlations of relative luciferase activity with Olive tail moment and micronuclei frequency suggest that the genotoxic benzo[a]pyrene may increase the relative luciferase activity through an indirect mechanism involving DNA repair processes (Kotoglou et al. 2009; Mendez et al. 2003).

Formaldehyde cross-links primary amino groups in proteins and nitrogen atoms in DNA causing protein misfolding and DNA damage. Formaldehyde is carcinogenic and mutagenic. The low level increase in relative luciferase activity induced by formaldehyde was at concentrations that were also cytotoxic to HepG2-luciferase cells. This small molecular compound may easily pass through the cytoplasmic membrane and cause decrease in cell viability by altering vital cellular functions (Hahn et al. 1985). The positive correlations between relative luciferase activity and Olive tail moment and micronuclei frequency suggest that formaldehyde may stimulate the heat shock response by the mechanism involving DNA repair processes (Kotoglou et al. 2009; Mendez et al. 2003).

Pyrene and formaldehyde induced oxidative damage detected by MDA concentration. Benzo[a]pyrene and formaldehyde induced genotoxic damage indicated by Olive tail moment and micronuclei frequency, consistent with previous studies (NTP 2010; Yang et al. 2007a). The lowest effective concentrations of pyrene (5 μM), benzo[a]pyrene (2.5 μM) and formaldehyde (1 μM) for increasing cellular damage detected by increased MDA concentration, Olive tail moment, and both MDA concentration and Olive tail moment (Table 2), respectively, also increased relative luciferase activity. This suggests that relative luciferase activity is a sensitive indicator of cellular damage induced by these individual organic compounds. These findings validate the relative luciferase activity in HepG2-luciferase cells as a sensitive and responsive indicator of the toxicity of organic compounds.

EOM treatment increased relative luciferase activity

Complex mixtures of organic compounds in emissions from urban vehicles and coke oven batteries contain a wide variety of toxic chemicals including carcinogens and mutagens (IARC 1983, 1985). For example, coke oven emissions

Table 2 The lowest effective concentrations of organic pollutants detected by *HSPA1A* promoter-driven luciferase reporter gene assay and other in vitro toxicity tests

NE no effect at highest concentration tested

Tested environmental pollutants	Luciferase reporter gene assay	Other in vitro toxicity tests			
		Cell viability assay	MDA assay	Comet assay	Micronucleus test
Pyrene (μM)	5	50	5	NE	NE
Benzo[a]pyrene (μM)	2.5	40	NE	2.5	5
Formaldehyde (μM)	1	5	1	1	5
Traffic EOM (ng/L)	0.006	NE	0.06	0.06	0.6
Coke oven EOM (μg/L)	0.2	688	0.2	0.2	0.2

collected at a state-run steel company located in northwest China contain as many as 17 PAHs (Yang et al. 2007b).

Traffic EOM produced a low level increase of relative luciferase activity at non-cytotoxic concentrations in HepG2-luciferase cells. Relative luciferase activity was elevated at the low concentration of 0.006 ng/l, where no other indicator of cellular injury was evident. This suggests that HepG2-luciferase cells are sensitive to low concentrations of complex organic pollutants. Relative luciferase activity was increased with obvious oxidative damage across a suitable range of traffic EOM concentration (0.06–0.6 ng/l). In addition to the positive correlations of relative luciferase activity with Olive tail moment and micronuclei frequency, the heat shock response in HepG2-luciferase cells may be induced by several mechanisms involving the proteotoxicity and genotoxicity of the traffic EOM (Kotoglou et al. 2009; Mendez et al. 2003; Voellmy 1996).

Coke oven EOM produced a steady and progressive increase of relative luciferase activity at non-cytotoxic concentrations (0.2–138 μg/l) in HepG2-luciferase cells. The positive correlations of relative luciferase activity with MDA concentration, Olive tail moment, and micronuclei frequency suggest that the coke oven EOM may increase the relative luciferase activity through several mechanisms that involve the generation of abnormal proteins and the DNA repair processes (Kotoglou et al. 2009; Mendez et al. 2003; Voellmy 1996).

Overall, the increases in relative luciferase activity induced by both the traffic and coke oven EOM occurred at concentrations clearly below that causing a decrease in cell viability. The higher relative luciferase activities induced by the coke oven EOM compared with those induced by the traffic EOM may be attributable to the differences in components and concentrations between the two EOMs. The toxic nature of coke oven EOM is suggested by the concentration-dependent progressive and steady increase in relative luciferase activity. In addition to the positive correlations obtained between relative luciferase activities and levels of cellular damage, the response of relative luciferase activity in HepG2-luciferase cells may reflect an integrated stress response summarizing the total cellular damage (de Pomerai 1996) induced by complex mixtures of organic compounds.

Sensitivity of relative luciferase activity

The major aim of this study was to determine if the *HSPA1A* promoter-driven luciferase reporter in HepG2-luciferase cells could be a sensitive indicator of the overall toxicity of organic pollutants present in air. Although a limited number of organic pollutants were screened, linear relationships between relative luciferase activities and levels of cellular damage were detected. The relative luciferase activity was increased by all the tested organic pollutants

including the non-carcinogenic pyrene and low concentrations of EOMs. Significant increases in relative luciferase activity were observed at concentrations that are generally lower than those doses at which the tested organic pollutants decreased cell viability, increased oxidative and genotoxic damage. In a time course study, significant increase in relative luciferase activity was already evident after 1 h of formaldehyde exposure, when no oxidative or genotoxic damage was detected (data not shown). These findings indicate that levels of relative luciferase activity in HepG2-luciferase cells can serve as an early indicator of toxicant exposure. The relative luciferase activity in HepG2-luciferase cells is generally as sensitive, or more sensitive to the organic pollutants tested, compared with the cell viability assay, MDA assay, comet assay, and micronucleus test (Table 2), strengthening the suitability of the HepG2-luciferase cells for assessing the toxicity of noxious organic compounds present in air. Furthermore, compared with those in vitro toxicity tests that are developed to evaluate the specific toxicological endpoints, the HepG2-luciferase cells can be used to detect the overall toxicity of complex organic pollutants. However, it must be kept in mind that our positive results were obtained from a small number of typical chemicals and complex mixtures of organic compounds. More data with other types of toxicants or environmental samples are necessary to confirm our findings and further validate the application of the HepG2-luciferase cells in toxicity assessment.

Conclusions

In summary, our study reports the establishment of the stable metabolic competent HepG2 cells containing a human *HSPA1A* promoter-driven luciferase reporter. The HepG2-luciferase cells have several advantages over other in vitro toxicity tests used in this study. The advantages of HepG2-luciferase cells are the following: (1) highly sensitive and responsive, (2) cost-effective, and (3) highly reproducible since it uses a stable luciferase reporter cell line. In addition, the toxicity assessment with the HepG2-luciferase cells is rapid and easy to perform. Consequently, the stable HepG2-luciferase cells, based on transcriptional activation of the *HSPA1A* promoter, provide a valuable tool for rapid screening of the overall toxicity of complex organic pollutants present in air.

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