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Proteomic analysis of MCF-7 cells treated with benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene, coal tar extract, and diesel exhaust extract

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ABSTRACT

Polycyclic aromatic hydrocarbon (PAH) DNA adducts have been associated with carcinogenesis, which is accompanied by multiple alterations in gene expression. We used two-dimensional electrophoresis to distinguish protein expression changes induced in MCF-7 cells by individual PAH (B[*a*]P and DB[*a*,*l*]P) and PAH mixtures (coal tar extract [SRM 1597] and diesel exhaust extract [SRM 1975]). Spots of interest were identified by MALDI-TOF-TOF. Our results have shown alterations in the expression of heat-shock proteins, cytoskeletal proteins, DNA associated proteins, and glycolytic and mitochondrial proteins. The proteins that were universally altered in expression were actin cytoplasmic 1, tubulin alpha and myosin light chain alkali, cyclophilin B, and heterogeneous ribonucleoprotein B1 (a protein involved in access to telomerase and mRNA maturation). Additional proteins with altered expression include histone H2A.1, heat-shock protein 70-2, galectin-3, nucleoside diphosphate kinase, ATP synthase, and electron transfer flavoprotein. While sharing similarities, each PAH treatment exhibited a unique proteomic fingerprint.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are a large group of ubiquitous environmental contaminants formed by the incomplete combustion of carbon compounds. Humans are routinely exposed to these compounds, which are found in sources such as automobile exhaust, tobacco smoke and coal tar. The correlation between human cancer and exposure to PAH has been noted for centuries, and animal studies have confirmed and detailed this association (Harvey, 1991). Contemporary studies observe the relationship between cancer rates and polymorphisms in carcinogen metabolizing enzymes, as well as specific mutational fingerprints believed to be the result of the interaction of PAH with DNA (Hainaut and Pfeifer, 2001; Smith et al., 2000).

The carcinogenic PAH studied have been found to be initially metabolized to diol and subsequently diol epoxide species by cytochrome P450 enzymes (CYPs). CYP1A1 has been implicated in the activation of many carcinogens and has been well characterized. CYP1B1, a more recent discovery, appears to be important in the metabolism of dibenzo[*a*,*l*]pyrene, a very potent carcinogen (Buters et al., 2002; Ralston et al., 1997). CYP1A1 and CYP1B1 are induced when ligands such as B[*a*]P interact with the aryl hydrocarbon receptor (AHR), which regulates the expression of many genes (Carlson and Perdew, 2002; Whitlock, 1999). The charged and water soluble PAH metabolites formed by these enzymes may be excreted, or CYP1 enzymes may further oxidize the PAH. The fate of PAH is thus dependent on the expression and activities of CYPs, epoxide hydrolase (EH), glutathione S-transferase (GSTM1) and other conjugating enzymes (Salama et al., 2001). Resulting diol epoxides have been demonstrated to react with the exocyclic amino group of purine bases to form covalent adducts and result in mutational miscoding when the DNA is replicated (Dipple et al., 1990, 1999).

Some PAH have been found to bind readily to DNA, but are not carcinogenic (Goshman and Heidelberger, 1967; Hughes and Phillips, 1993). Additionally, when the ability of B[*a*]P metabolites to cause tumors in mice is compared to the mutagenicity in V79 cells or the Ames test, a discrepancy is observed. The anti isomer of B[*a*]P 7,8-dihydrodiol-9,10 epoxide is mutagenic as measured in the Ames test, and is tumorigenic. However, the syn isomer of the same compound is also highly mutagenic, but not tumorigenic (Rubin, 2001). In general, bacterial mutagenicity data are poorly coordinated with cancer data (Sjogren et al., 1996). These examples are representative of a body of evidence indicating that the ability of PAH to form DNA adducts is only one contributing factor to their carcinogenicity (Dipple et al., 1984).

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An additional difficulty in studying PAH is their occurrence in complex mixtures. These mixtures may contain hundreds or even thousands of compounds. The carcinogenic potential of the most abundant individual species in these mixtures is often well studied in animal models. Summing these potentials is a method of calculating the risk of exposure to PAH mixtures (Borgert et al., 2004). However, the actual carcinogenicity, which is dependent on the route of exposure, often does not equal, and sometimes far exceeds calculated carcinogenicity (Fitzgerald et al., 2004; Schneider et al., 2002). When individual PAH are used as co-treatments, cocarcinogenic or anticarcinogenic effects have been observed (Baird et al., 1984; Cherng et al., 2001; Courter et al., 2007; Hughes and Phillips, 1990; Marston, 1999; Marston et al., 2001). These effects are thought to be the result of differential induction or competition for metabolizing enzymes.

The products of PAH metabolism also influence gene expression. Hydroxy metabolites, epoxides, and quinones formed from PAH can be further metabolized to reactive oxidative species (ROS), which can also contribute to DNA damage (Miller and Ramos, 2001; Shen et al., 2006). The resulting perturbation in redox balance can induce genes responsive to the antioxidant response element (ARE), including enzymes associated with glutathione biosynthesis, redox proteins, and drug-metabolizing enzymes (Nguyen et al., 2003).

Some PAH are thought of as complete carcinogens, participating both in initation and promotion of tumors. A large portion of these effects are mediated by the AHR, and the affinity of PAH with the AHR has been investigated as a measure of carcinogenic potency (Sjogren et al., 1996). However, non-AHR mediated effects may also contribute to carcinogenesis (Sharovskaya et al., 2006), and focusing on selected changes in expression does not sufficiently describe the initiation/promotion processes induced by PAH.

To adequately describe the process of PAH induced carcinogenesis, it is necessary to track multiple cellular changes. Several research groups have addressed this challenging problem by observing global expression changes following treatment with PAHs using microarrays and rapid analysis of gene expression (RAGE) (Akerman et al., 2004; Bartosiewicz et al., 2001; Cunningham et al., 2000; Hockley et al., 2006; Lee et al., 2006; Luo et al., 2005; Mahadevan et al., 2005; Papaconstantinou et al., 2006; Sen et al., 2007; Staal et al., 2007). A complimentary approach, proteomics employs a variety of technologies to survey the protein expression profile that determines the functional molecular phenotype of a given sample. The application of two-dimensional gel electrophoresis (2DE) has identified new potential biomarkers in cancer, and further elucidated the roles of existing cancer effectors (Cho, 2007).

In order to investigate the multiple effects of PAH on gene expression, we have undertaken a proteomic analysis of MCF-7 cells treated with B[*a*]P, DB[*a*,*l*]P, and standardized extracts from coal tar and diesel exhaust. Although MCF-7 is a cancer cell line it exhibits many features of normal human breast epithelial cells, including the ability to form domes and to process estradiol via cytoplasmic estrogen receptors. Many aspects of MCF-7 gene expression have been characterized such as the expression of wild-type p53, the Tx-4 oncogene (ATCC, 2008), and the AHR (Loaiza-Perez et al., 2002). This accumulated knowledgebase makes the MCF-7 cell line a useful model system for many types of studies. In contrast, normal breast epithelial cells offer both limited sample size and interindividual variability (Keshava et al., 2005). Here we compare the similarities and differences in the acute response to individual PAH and environmental mixtures. Each PAH treatment yielded unique patterns of alterations in protein expression of heat-shock proteins, cytoskeletal proteins, DNA associated proteins, and glycolytic and mitochondrial proteins.

2. Materials and methods

2.1. Cell culture, treatment, and harvest

MCF-7 cells originated from human mammary carcinoma tissue (Soule et al., 1973) were provided to the Baird lab by the Purdue University Cell Culture Laboratory. MCF-7 cells were grown in 1:1 F12 Nutrient Mixture and Dulbecco's Modified Eagle Medium (Gibco BRL, Grand Island, NY), with 10% fetal bovine serum (Integren, Purchase, NY), 15 mM HEPES buffer, 14.3 mM $NaHCO_3$, 200 units/ml penicillin, 200 μg/ml streptomycin, and 25 μg/ml ampicillin in T75 cm2 flasks and maintained at 37 °C with 5% CO_2.

B[*a*]P and DB[*a*,*l*]P were obtained from Chemsyn Science Laboratories (Lenexa, KS). Toluene was evaporated under nitrogen from standard reference material extracts of coal tar (SRM 1597) and diesel exhaust (SRM 1975 obtained from the National Institute of Standards and Technology (Gaithersberg, MD) and replaced with DMSO. MCF-7 cells were treated for 24 h with 1 μg/ml B[*a*]P, 0.01 μg/ml DB[*a*,*l*]P, 20 μg/ml SRM 1597, or 20 μg/ml SRM 1975 in DMSO, or DMSO alone (vehicle control). The SRM 1597 dose contains 0.2 μg/ml B[*a*]P. The cells were harvested using trypsin-versene (0.05% Trypsin, 0.14 NaCl, 3 mM KCl, 0.1 M Na_2HPO_4, 1.5 mM KH_2PO_4, and 0.5 mM EDTA) and the resulting cell pellet stored at −80 °C.

2.2. Sample preparation and fractionation

200 μl 40 mM filtered Tris with 0.1 μl endonuclease (Sigma, St. Louis, MO) was added to cell pellet. The sample was sonicated for 5 s at level 4 on a Sonic Dismembrator (Fisher Scientific), and incubated at room temperature for 20 min. The sample was removed to a 1.5 ml centrifuge tube, and centrifuged at 15,000 × *g* for 10 min. The supernatant was reserved as the "Tris fraction" and immediately stored at −80°. The pellet was twice rinsed by vortexing in 500 μl 40 mM Tris and centrifuged at 15,000 × *g* for 10 min. The supernatant was removed, and 200 μl multiple surfactant solution composed of 5 M urea, 2 M thiourea, 2 mM tributylphosphine, 2% CHAPS, 2% SB 3–10, 0.2% 3–10 carrier ampholytes (BioRad, Hercules, CA), and 0.0002% Bromophenol blue was added to the pellet. The pellet was sonicated at level 4 for 5 s, and centrifuged at 15,000 × *g* for 10 min. The supernatant was reserved as "Multiple Surfactant fraction" and stored at −80°. 5 μl of the Tris samples, and 25 μl of the multiple surfactant samples were quantified in duplicate using the RCDC Protein Assay (BioRad, Hercules, CA) using the microfuge tube assay protocol per manufacturer's protocol and pre-diluted bovine serum albumin protein assay standards as standards (Pierce, Rockford, IL).

2.3. IPG strip rehydration and isoelectric focusing

150 μg of protein was brought to a volume of 185 μl in multiple surfactant solution. 11 cm pH 3–10 nonlinear IPG strips (BioRad, Hercules, CA) were rehydrated overnight at room temperature in this solution, covered in mineral oil. The strips were loaded in a Protean IEF isoelectric focusing cell (BioRad, Hercules, CA) and covered in mineral oil. Hydrated electrode wicks were mounted over the electrodes. The strips were conditioned at 250 V for 30 min at 20 °C, then ramped to 8000 V over 2.5 h, and maintained at 8000 V for an additional 3.5 h, and held at 500 V. The strips were removed and stored at −80 °C.

2.4. Equilibration and SDS-PAGE

The strips were equilibrated with SDS-PAGE equilibration base buffer composed of 6 M Urea, 0.375 M Tris pH 8.8, 2% SDS, and 20% glycerol, with 2% (w/v) dithiothreitol was or 2.5% (w/v) of iodoacetamide added per manufacturer's instructions. The strips were dipped several times in SDS-PAGE running buffer, composed of 25 mM Tris, 192 mM glycine, and 0.1% sodium dodecyl sulfate. The strips were mounted over an 8–16% Criterion pre-cast IPG + 1 acrylamide gel and covered in ReadyPrep overlay agarose (BioRad, Hercules, CA). 3 μl of Precision Plus unstained molecular weight standards (BioRad) was loaded in the small well of the gel. 50 V was applied to the gels for 30 min, and then 200 V for 65 min at 4 °C. The gels were shaken gently in 75 mls gel fixative (10% methanol, 7% acetic acid) for 45 min at room temperature, rinsed with ultrapure water (Millipore, Bedford, MA) and placed in 75 ml Sypro Ruby gel stain (BioRad, Hercules, CA). After shaking gently overnight, the stain was replaced with gel fixative and shaken for 45 min. The gels were rinsed and stored in ultrapure water at 4 °C until they were imaged.

2.5. Imaging and analysis

Gels were imaged using a Model 1000 VersaDoc imaging system (BioRad, Hercules, CA). Multiple exposures were recorded directly in PDQuest (BioRad) format. The gels were stored at 4 °C in ZipLoc® bags in a small volume of 0.005% sodium azide. A matchset was created of triplicate gels of each sample. Automated spot detection and matching was initially used, with additional manual refinement. Analysis sets were created of spots that varied quantitatively greater than 1.5-fold or less than −1.5-fold from the DMSO control. Additional sets were formed of spots found to vary in expression according to a Student's *t*-test, with a significance level of