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Transcriptional signatures of environmentally relevant exposures in normal human mammary epithelial cells: benzo[a]pyrene

Channa Keshava, Diana Whipkey, Ainsley Weston*

Molecular Epidemiology Team, Health Effects Laboratory Division, National Institute for Occupational Safety and Health-CDC, Centers for Disease Control and Prevention, DHHS, 1095 Willowdale Road, M/S L-3014, Morgantown, WV 26505-2888, USA

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Abstract

Changes in gene expression in a panel of primary normal human mammary epithelial cell strains, developed from healthy breast tissue obtained at reduction mammoplasty from different donors, in response to benzo[a]pyrene exposure have been investigated. It was expected that both gene expression changes common to cell strains derived from different donors as well as inter-individual variation would be observed. Therefore, the strategy that has been adopted is to identify potentially important changes, or useful changes from a biomonitoring perspective, using gene-array technology and a small number of donors; then investigate selected transcription responses using a large number of tissue donors and a cheaper method of transcript detection (real-time polymerase chain reaction). Here we report results from four primary normal human mammary epithelial cell strains that were treated with benzo[a]pyrene in vitro for either 6 or 24 h. Transcription was monitored using high-density oligonucleotide arrays (Affymetrix HuGeneFL). Total RNA was used for the preparation of labeled targets that were hybridized to microarrays containing probes representing more than 6800 human genes and expressed sequence tags. Gene expression data were analyzed using the GeneChip® software (MAS 5.0). Altered gene expression patterns were observed in response to benzo[a]pyrene in human mammary epithelial cell strains from different donors. Specifically, the dioxin inducible cytochrome P450 *CYP1B1* was consistently induced in response to 6 and 24 h exposure to benzo[a]pyrene in cell strains from all four donors. Two other genes that were relatively consistently induced were *IL1β* and *MMP1*. Less consistent changes in other metabolism genes (*CYP1A1*, *CYP11B2*, and *NQO1*) and certain cell cycle control genes *GOS2* and *AF1Q* were also induced, while *EGR1* was suppressed. Although no change in *p53* transcription was observed, an accumulation of *p53* protein was detected using antibodies. A similar accumulation of *Waf1* (*p21*) was also observed using immunohistochemistry, this was expected since *p53* is *p21*'s transcription factor. Significant inter-individual variations in both the levels and patterns of gene expression were observed, in response to benzo[a]pyrene exposure. These studies provide a complementary approach to molecular epidemiology for the investigation of differential susceptibility to chemical carcinogens, and specifically polycyclic aromatic hydrocarbons.
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Keywords: Oligonucleotide arrays; Polycyclic aromatic hydrocarbons; Mammary cells; Carcinogenesis

Abbreviations: BP, benzo[a]pyrene; CHTN, Cooperative Human Tissue Network; FC, Fold Change; NCI, National Cancer Institute; NHMEC, normal human mammary epithelial cell; PAH, polycyclic aromatic hydrocarbon; SLR, signal log ratio.

* Corresponding author. Tel.: +1 304 285 6221; fax: +1 304 285 5708.
E-mail address: agw8@cdc.gov (A. Weston).

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1. Introduction

Previous studies have demonstrated the ability of normal human mammary epithelial cells (NHMECs) to metabolize and activate benzo[a]pyrene [1]. Metabolism and activation of polycyclic aromatic hydrocarbons has also been shown to have wide inter-individual variation among humans [2]. Inter-individual variation in carcinogen metabolism and inter-individual variation in response to carcinogen exposures may impact cancer susceptibility [3].

Gene expression profiling is an emerging field that monitors transcription of thousands of genes simultaneously. Gene expression profiling for toxic chemicals may allow the identification of toxicity mechanisms and potential biomarkers of chemical exposure [4–6]. Despite their potentially profound biological importance, ubiquitous polycyclic aromatic hydrocarbons have received little toxicogenomic attention. Consequently, there have been only few reports concerning expression profiling in mammalian tissues and cells following exposure to these chemicals [7,8].

This investigation seeks to examine the toxicogenomic effects of benzo[a]pyrene in NHMECs. In addition to defining the common changes in gene transcription across several different human genetic backgrounds, this report also demonstrates inter-individual variation in the transcriptional response. This is an important facet because many other studies use a single genotype, often associated with an abnormal phenotype (e.g. MCF-7 cells) [9–13].

The overall research strategy is based on DNA-oligonucleotide microarray monitoring of NHMECs from a small number of donors, in this case four, followed by a more comprehensive evaluation of candidate biomarker genes using real-time polymerase chain reaction (RT-PCR) in NHMECs from a larger number of donors. This study sought to document transcriptional signatures of BP exposure in NHMECs documenting both changes common to all cells from different donors as well as differential responses. Using this approach, it may be possible to identify biomarkers of exposure that may be used in occupational epidemiology studies [5].

2. Materials and methods

2.1. Cell strains and chemical exposure

Primary cultures of NHMECs were derived from healthy breast tissues from different donors using standard methods [14]. Tissues that would otherwise be discarded were obtained at reduction mammoplasty through the National Cancer Institute (NCI) supported Cooperative Human Tissue Network (CHTN) [15]. The cell cultures were maintained in mammary epithelial basal medium (Clonetics/Bio-Whittaker, Inc., Walkersville, MD) supplemented with mammary epithelial growth medium (Singlequots™) according to the manufacturer's recommendation. Cell strains (all at passage 6) grown to 70% confluence (37 °C with 5% CO_2 in a humidified chamber) were exposed to BP (4 μM; NCI Chemical Carcinogen Reference Standard Repository, Kansas City, MO [16]) for 6 and 24 h. Acetone:ethanol (1:22.5) was used as the vehicle, at a final concentration of 0.1% in media. Four cell strains were used, these are referenced here as 1, 2, 3 and 4, but they correspond to the strains M98013, M98014, M98016 and M98018, respectively. All experiments were performed in duplicate starting with BP treatment, the 24 h vehicle exposure served as the control for both the 6 and 24 h BP exposures. Cell viability was determined by Trypan Blue dye exclusion.

2.2. Indirect immunofluorescence for p53 and p21 (Waf1) protein detection

Immunofluorescence assays for *p53* and *p21* detection were performed in parallel using eight-well chamber slides (Nunc, Naperville, IL). At the end of a treatment period, the media was aspirated, washed with saline, and the cells fixed with methanol. The fixed cells were pre-incubated with bovine serum albumen for *p53* detection or goat serum for *p21* detection (10%, 20 min), cells were washed (saline) and incubated (1 h, 37 °C) with anti-*p53* or anti-*p21* antibodies (pab 187) (1:1000, pab 1801, Santa Cruz Biotech, Santa Cruz, CA). Unbound antibodies were washed away with saline and the secondary FITC-antibody conjugate was added (goat, anti-mouse,1:1500, 45 min, room temperature). Cell preparations were examined using a laser scanning

confocal microscope (BX50, Olympus, Warrendale, PA).

2.3. High-density oligonucleotide array expression analysis

Following treatment, total RNA was isolated from the cells (TRIzol reagent; Gibco BRL Life Technologies, Rockville, MD), and purified using RNeasy (Qiagen, Valencia, CA). cRNA was prepared exactly as described by the microarray manufacturer (Affymetrix, Santa Clara, CA [17]). Total RNA (10 μg) was used for the preparation of double stranded cDNA using an oligo $(dT)_{24}$ primer with a T7 RNA polymerase promoter sequence at its 5′ end. Following second strand synthesis, a labeled cRNA transcript was generated from the cDNA in an in vitro transcription reaction using Enzo BioArray high yield RNA transcript labeling (Enzo Diagnostics, Inc., Farmingdale, NY). The labeled anti-sense RNA was purified using RNeasy and each cRNA sample (20 μg) was fragmented (94 °C for 35 min) in the presence of Tris–acetate (40 mM, pH 8.1), potassium acetate (100 mM), and magnesium acetate (30 mM).

The fragmented cRNA (15 μg) was mixed with control oligonucleotides, eukaryotic hybridization controls (20×; *BioB*, *BioC*, *BioD*, *cre* at 1.5, 5, 25 and 100 pM, respectively), control oligonucleotide B2, herring sperm DNA (10 mg/ml), acetylated BSA (50 mg/ml) and hybridization buffer (2×) in the hybridization cocktail. This cocktail was heated (99 °C for 5 min; and 45 °C for 5 min) prior to introduction to the HuGeneFL GeneChip probe cassette. Hybridization was allowed to proceed (45 °C in a rotisserie oven set at 60 rpm) for 16 h. Following hybridization, the arrays were washed with low stringency buffer (6× SSPE [NaCl 3 M, NaH_2PO_4 0.2 M, EDTA 0.02 M], Tween 20 [0.01%], and anti-foam [0.005%] at 25 °C) followed by high stringency buffer (MES [100 mM], Na^+ [0.1 M], and Tween 20 [0.01%] at 50 °C, see Affymetrix protocol EukGE-WS2). The arrays were stained with streptavidin–phycoerythrin (Molecular Probes, Eugene, OR) and biotinylated anti-streptavidin antibodies (Vector Laboratories, Burlingame, CA) using the GeneChip™ fluidics station protocol EukGE-WS2. Following washing and staining, probe arrays were read twice (multiple image scan) at 3 μm resolution using the GeneChip® System confocal scanning laser (Hewlett-Packard). Changes in gene expression were computed from the differences between perfect match and mismatch probes. The differential gene expression data analysis was performed using GeneChip® MAS 5.0 software. The fluorescence intensity was measured for each microarray and normalized by global scaling to1500 and to the average fluorescence intensity for the entire microarray. Absolute analysis for gene expression was performed and then comparison analysis was performed between vehicle control (24 h) and treatment groups, between cell strains and results are presented as fold change from the control baseline expressed as signal log ratio (SLR).

2.4. Quantitative real-time PCR

Relative quantitation with real-time, reverse transcriptase-polymerase chain reaction (RT-PCR) was performed with SYBR Green PCR Core Reagents and an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Reverse transcription was performed using a total of 10 μg of RNA (SuperScript Choice System for cDNA Synthesis, Invitrogen Life Technologies, Carlsbad, CA). Following reverse transcription, the cDNA was diluted 1:50 and 2 μl was used as template to perform PCR in a 50 μl reaction containing: SYBR Green PCR buffer (1×), $MgCl_2$ (3 mM), dNTPs (1 mM), primers (100 pmol each), Ampli Taq Gold (1.25 U) and Amp Erase UNG (0.25 U). PCR consisted of initial denaturation (95 °C, 10 min), then 40 cycles of denaturation (95 °C, 15 s) and annealing/extension (60 °C, 1 min). The PCR primers for determination of *CYP1B1* induction levels were: 5′-AGGGACCGTCTGCCTTGTATGG-3′ and 5′-GTGTTGGCAGTGGTGGCATGAG-3′ (with GAPDH primers 5′-GGCTGAGAACGGGAAGCTTGT-3′ and 5′-TGAAGACG CCAGTGGACTCCAC-3′). Amplicon size and reaction specificity was confirmed by agarose gel eletrophoresis. Each sample was assayed in quadruplicate and the median threshold cycle (C_T) values were used to calculate the fold change (FC) between treated and control samples. Standard deviation and standard error were also calculated [18].

3. Results

Trypan blue dye exclusion was used to determine dose. Greater than 95% of cells from each donor remained viable over the entire treatment period (24 h) when exposed to BP (4 μM, the maximum concentration achievable in serum-free aqueous media) (data not shown). During this period, *p53* protein accumulation was detected by the increasingly fluorescent properties of all of the cell strains when probed with anti-*p53* antibodies and an FITC conjugated secondary antibody (Fig. 1A–C). A concomitant increase in *p21* was also observed (Fig. 1D–F).

To demonstrate consistency of cRNA synthesis and transcript labeling between different samples over the duration of exposure in four NHMEC strains, the cRNA $3'/5'$ transcript ratios were compared between treatment groups for *GAPDH* and *β-actin*. The variability in the cRNA synthesis and microarray hybridization efficiency between the samples were assessed by comparing the signal intensities for the four control bacterial and phage gene cRNAs, *BioB, BioC, BioD*, and *cre* (1.5, 5, 25, and 100 pM, respectively). All four control cRNA were detected in an increasing order of intensities as expected in all the 24 arrays used in this study (data not shown). The $3'/5'$ cRNA transcript ratios for *GAPDH* and *β-actin* were found to be consistent between samples over the duration of exposure (data not shown).

Comprehensive analysis of changes in gene expression in response to BP exposure between duplicate samples and over time revealed that sampling error between duplicates was small (data not shown). As a convenient way to make global comparisons in transcriptional changes due to BP exposure between the different cell strains, increases or decreases in transcription of greater than 1.5-fold (SLR ~ 0.7) were arbitrarily selected, as others have done [4,19–21]. Overall changes in gene expression in response to BP exposure at 6 h for cell strain 1 indicated at least 1.5-fold transcriptional induction for 56 genes (1.2%) after 6 h BP exposure and at least 1.5-fold reduction in transcription for 23 genes (0.4%). For all cell strains, at both 6 and 24 h, as few as 51 (cell strain 4) and as many as 201 (cell strain 3) (0.4–3.0%) were either induced by 1.5-fold or reduced by 1.5-fold, depending on cell strain. These data illustrate inter-individual variations in transcriptional response to BP between donors of different cell strains. It should also be noted that in the case of each cell strain, the expression of only a small percentage of the 7000 genes represented on the array were altered by 1.5-fold or more (increased or decreased). More stringent criteria were used to identify robust signals as follows: (1) software call of 'present', (2) >1.5-fold change in all four cell strains, and (3) P-value <0.05 (t-test, MAS 5.0 software).

Only one metabolism gene was found to be consistently induced by more than five-fold in all four cell strains at both 6 and 24 h ($P<0.05$). Altered expression of the dioxin inducible cytochrome *CYP1B1* was observed in all cell strains (Fig. 2, Table 1). Low basal levels of *CYP1B1* transcripts were observed in each case, this is apparent by comparison of the upper panel of features of the untreated samples (indicated in Fig. 2A by arrows) with those directly below (mismatch probes). This observation was confirmed in duplicate samples, which contributed to relatively small standard errors (Fig. 2B). Both the levels and patterns of expression were observed to vary between cell strains from different human donors. An increase in level of RNA transcripts (FC $= 8.5 \pm 0.9$) was observed after 6 h exposure of cell strain 1 to BP, the same level was observed for this cell strain at 24 h. An increase in level of RNA transcripts (FC $= 2.7 \pm 0.5$) was observed after 6 h exposure of cell strain 2 to BP, however, after 24 h exposure, the level was more than twice as high (FC $= 6.0 \pm 0.6$). A similar pattern was observed for cell strain 3 although the absolute levels of increase were higher, FC $= 4.0$ (measured by one microarray) at 6 h and 9.2 (± 1.3) at 24 h. In the case of cell strain 4 after 6 h exposure to BP, the *CYP1B1* RNA transcript had been induced by FC $= 9.4 \pm 0.7$ and similarly at 24 h (FC $= 7.7 \pm 0.3$) (Fig. 2B). These data demonstrate inter-individual variation in both the levels and patterns of *CYP1B1* induction in NHMECs in response to BP exposure in vitro. Levels and patterns of *CYP1B1* induction observed for BP-treated NHMECs using DNA-microarrays were reproduced almost exactly using quantitative real-time PCR (Fig. 2C).

We expected to observe a consistent increase in *CYP1A1* expression in response to BP exposure.

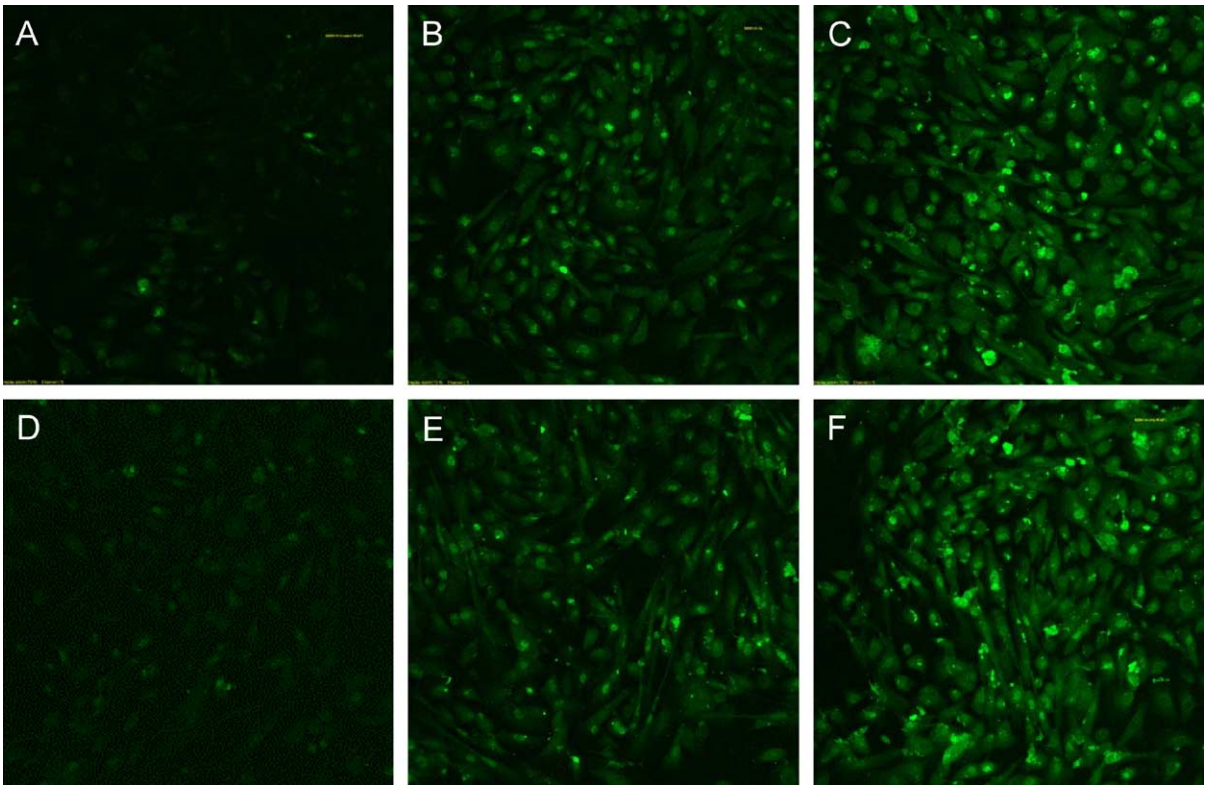

Fig. 1. Immunocytochemical staining of normal human mammary epithelial cells using antibodies directed against either *p53* (A–C) or *p21-Waf-1* (D–F). Cells were treated with vehicle control (A and D) or benzo[a]pyrene for 6 h (B and E) and 24 h (C and F).

However, even though there was a strong correlation between *CYP1B1* and *CYP1A1* expression ($r=0.943$, $P<0.001$), levels of *CYP1A1* were not detected for cell strain 2 (data not shown). These correlations withstood log base 2 transformation of the data. In addition, the steroid hydroxylase (*CYP11B2*) was also highly induced, but levels were inversely correlated with *CYP1B1* ($r=-0.788$, $P<0.02$) and *CYP1A1* ($r=-0.741$, $P<0.03$).

Using the stringent selection criteria (present call, SLR >0.7 in all cell strains, $P<0.05$), only two other genes were identified as potential biomarkers of BP exposure, *1L1β* and *MMP1* (Table 1). In addition to induction of cytochrome p450s *CYP1A1*, *CYP1B1* and *CYP11B2*, *NQO1* was induced by an SLR of >0.7 in at least three cell strains (Table 2), however, *P*-values did not reach significance.

It was noted previously that levels of both *p53* and *p21* protein were increased at 6 and 24 h of BP exposure, indicating favorable conditions for G_1-blockade. However, no change in *p53* transcription was observed for any cell strain using oligonucleotide arrays. This result was confirmed by RT-PCR. Since *p21* probes were not present on the oligonucleotide microarrays, no similar conclusion could be made, however, it is assumed that the accumulation of *p53* caused increased *p21* transcription and *p21* protein synthesis.

A number of other genes, among them transcription factors, growth controlling genes and cell cycle regulatory genes, were consistently increased by SLR >0.7 in at least three cell strains (Table 2). These genes may represent candidate genes that will be useful adjuncts to exposure assessment in future epidemiological studies. With regard to inter-individual variation, the data in Table 2 show that both the levels and patterns of transcription vary in response to BP exposure between cell strains of these four donors.

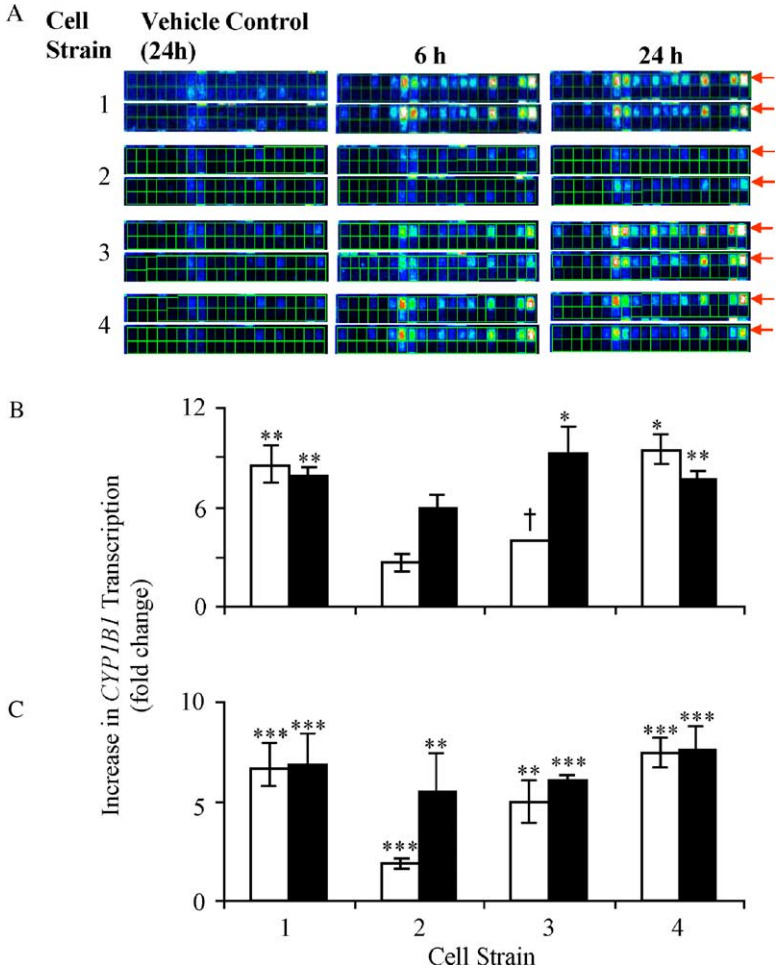

Fig. 2. Induction of *CYP1B1* due to 6 h (□) and 24 h (■) exposure to BP in four NHMEC strains. (A) Probe sets from hybridized oligonucleotide arrays corresponding to the *CYP1B1* gene in four different cell strains are represented. Perfect-match oligonucleotides are tiled across the upper rows and indicated by arrows, each of their mismatch counter-parts reside immediately below. The column on the left side indicates low levels of expression in all cell strains prior to exposure. The column in the center shows variable induction of *CYP1B1* transcription in all cell strains after 6 h exposure to BP. The column on the right shows variable induction of *CYP1B1* transcription in all cell strains after 24 h exposure to BP. (B) Quantitation of DNA-microarray data presented in A. (C) Confirmation of DNA-microarray data by quantitative real-time PCR. Asterisks indicate significant increase in level of transcripts over untreated cellls: $*P<0.05$, $**P<0.01$, $***P<0.001$.† Results of single determination.

Both increases and decreases in BP-driven transcription indicate qualitative and quantitative human inter-individual variation (Table 2). Modulation of transcription for three genes (*CYP1B1, IL1β*, and *MMP1*) suggest themselves as promising biomarkers of carcinogenic polycyclic aromatic hydrocarbon (PAH) exposure. All data from these experiments can be found at http://www.cdc.gov/niosh/ext-supp-mat/GeneChipPaper-1/index.htm.

4. Discussion

Modulation of gene transcription in a panel of primary NHMEC strains in response to BP exposure has been studied using high-density oligonucleotide arrays. The response to BP exposure involved the induction as well as repression of genes of various biochemical and regulatory pathways. Thus, BP metabolism genes, transcription factors and cell

Table 1
Genes which are induced by SLR≧0.9 (1.8-fold) in at least three cell strains

Category Accession number	Gene symbol	1		2		3		4		Gene description
		6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	
U03688	CYP1B1	3.09*	3.09*	1.43	2.58	1.89	3.20	3.23*	2.94*	Human dioxin-inducible cytochrome p450 (CYP1B1)
X04500	IL1β	0.85	2.98*	0.93*	1.14	1.89	1.85*	0.93	1.20*	Human gene for prointerleukin 1 beta
X54925	MMP1	1.85*	0.93	1.96	NC	1.93	0.77*	NC	0.14	*H.sapiens* mRNA for type 1 interstitial collagenase

NC, no change; *statistically significance indicated by 5.0 software MAS.
[a] Down-regulated.

cycle regulation genes were all involved. These data contrast with an earlier report of BP exposure and microarray monitoring of gene expression in mouse liver, where only *CYP1A1* and *CYP1A2* were found to be elevated [7].

Modulation of gene expression was different both in the levels and apparently pattern depending on the donor origin of the cell strain. The suggestion that the temporal patterns of expression are different between cell strains has prompted us to embark on a more

Table 2
Genes which are altered by SLR≧0.6 (1.5-fold) in at least three cell strains

Category Accession number	Gene symbol	1		2		3		4		Gene description (GO)[a]
		6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	
J03934_s_at	NQO1	0.4	0.7	NC	0.4	0.8	0.5	0.8	0.9	NAD(P)H dehydrogenase (quinone) activity
M31551_s_at	SERPINB2	1.5	0.8	0.6	0.9	0.8	1.7	NC	2.3	Serpin; serine-type endo-peptidase inhibitor activity; 6.5e–156
M32011_at	NCF2	NC	0.2[b]	0.9	NC	0.6	NC	0.2[b]	0.6[b]	Superoxide metabolism cellular defense response
M32879_s_at	CYP11B2	NC	0.9[b]	1.3	NC	0.5[b]	0.7[b]	0.6[b]	NC	Electron transport
M34057_at	LTBP1	0.4[b]	0.0	0.9[b]	0.7[b]	1.2[b]	0.2	NC	0.5[b]	Latent transforming growth factor beta binding protein 1
M72885_rna1_s_at	GOS2	1.0	0.6	0.4	0.6	0.0	NC	NC	0.7	Regulation of cell cycle
U01828_at	MAP2	1.6	2.3[b]	0.8[b]	0.3	0.3[b]	0.8	NC	1.1[b]	Microtubule stabilization
U05861_at	AKR1C2	NC	0.7	1.2*	1.1	1.5	1.2	0.6	1.1	Lipid metabolism
U16954_at	AF1Q	0.5[b]	0.6	NC	1.1*	0.6[b]	0.8	NC	0.5	Cell growth and/or maintenance
U90551_at	HIST1H2AC	0.8	0.7[b]	1.0*	0.3	1.3*	0.4	0.9	0.5	Histone; DNA binding; 1.4×10^{-53}
X02612_at	CYP1A1	2.5*	2.1	0.2	0.9	0.6	1.7*	2.3	1.7	p450; electron transport; 1.8×10^{-171}
X52541_at	EGR1	1.0[b]	0.7[b]	0.9[b]	0.2	1.2[b]	0.5	0.2	0.4*	Regulation of transcription, DNA-dependent
X57985_rna2_at	HIST2H2BE	1.6*	NC	0.8	0.2[b]	1.3	NC	0.3	0.9[b]	Nucleosome assembly

NC=no change; *statistically significance indicated by 5.0 software MAS.
[a] GO biological process description.
[b] Down-regulated.

extensive time-course. Here, 51–201 RNA transcripts were altered. Among them, 33–96 were up-regulated and 3–104 were observed to be down-regulated with at least 1.5-fold change although most did not reach statistical significance ($P < 0.05$). Some of the commonly induced genes included *CYP1B1, IL1β, MMP1, NQO1, NCF2* and *AKR1C2*. Activation of some of these genes may represent changes in the metabolic enzyme activity, deregulation in cell cycling or inflammatory response when exposed to BP. These data are consistent with earlier reports that documented large inter-individual variation in carcinogen metabolism among humans [2]. However, relatively consistent induction of these genes in response to BP exposure suggests their possible use as biomarkers of occupational exposures. To validate potential biomarkers of exposure, the next step is to extend these findings to larger numbers of cell strains using efficient and cheaper technology (RT-PCR).

The gene encoding cytochrome P4501B1, *CYP1B1*, has important endocrine metabolism function in the breast, thus it catalyzes the formation of genotoxic 4-hydroxyestradiol from estradiol [22]. In addition, it has also been shown to be involved in the metabolic activation of certain environmental procarcinogens, including polycyclic aromatic hydrocarbons (e.g. BP) and heterocyclic aromatic amines [23,24]. In this study, using four cell strains obtained from different individuals, we observed the induction of *CYP1B1* in every case. However, the levels of expression were different for different cell strains indicating inter-individual variation in *CYP1B1* transcript levels. So far, six single nucleotide polymorphisms have been described for *CYP1B1* [22–25]. It is known that the codon 432 polymorphism (V/L) has a significant impact on *CYP1B1* enzyme kinetics, where the Michaelis constant (K_m) of the leucine variant is three-fold higher for the 2- and 4-hydroxylation of estadiol than the valine variant [24]. In contrast, this polymorphism has no impact on the oxidation kinetics of BP-7,8-dihydroxy-7,8-dihydrodiol. It is not known if these polymorphisms are related to the inter-individual variation in inducibility that was observed here, but this question is being addressed. However, our data provide a basis for the idea that inter-individual variation in carcinogen metabolism could contribute to differential susceptibility to chemical carcinogenesis, and this theory is extended to human female breast cancer. Consistent with these ideas are reports that have implicated inheritance of polymorphisms in codons 119 (A/S) and 432 (V/L) in breast cancer susceptibility [25–27]. Also consistent with enzyme kinetics studies, no statistically significant association between the *CYP1B1* polymorphisms in exon 3 and breast cancer risk has been observed [26].

IL1β was also consistently induced by BP exposure. *IL1β* is a proinflammatory cytokine that is normally synthesized by activated mononuclear phagocytes. It has also been found to be differentially expressed in the bronchiolar lavage fluid of smokers and non-smokers [28]. The *IL1β* expression response in this study may be part of an anti-oxidant mechanism since BP exposure stimulates redox cycling as noted by the induction of *CYP1B1*. Interestingly, a gene-array study of tobacco smokers has implicated both CYP1B1 and IL1β as biomarkers of tobacco smoking [29]. Similarly, in a cultured human macrophage cell line (THP-1), both *CYP1B1* and *IL1β* were induced by 3- and 1.7-fold, respectively, when treated with diesel exhaust particles [21].

Matrix metalloproteinaes (MMPs) are a family of proteolytic enzymes involved in tissue remodeling. MMP1 is produced in a variety of cell types and is specifically involved in wound healing. Inappropriate expression of this enzyme has been linked to several diseases like emphysema, melanoma [30], and chondrosarcoma [31]. Polymorphic variants in *MMP1* have been shown to be associated with smoking-related lung injury and heart disease [32,33]. Altered *MMP1* expression is associated with invasive behavior and metastatic potential of tumors. Increased gene expression in *IL1β*, a potent inducer of *MMP1*, was observed in addition to increase in MMP1 in breast cancer cell lines in response to calcium hydroxyapatite [34,35]. We have also observed the BP–DNA adduct levels in the four cell strains studied here are comparable (15–100 adducts per 10^9 nucleotides) at 12 h [36] to those reported by others after 1 h exposure to BPDE [8]. No change in *p53* transcription levels were observed, however, there was an accumulation of *p53* protein as determined by immunofluorescence microscopy. This finding is also consistent with changes that occur in mammary cells that were treated with BPDE [37]. Consistent with the accumulation of *p53* was

the increased transcription of genes whose transcription factor is *p53*, like *p21* [38]. These findings are also consistent with studies using lung cancer cells [39,40].

A gene that might represent a candidate biomarker of PAH exposure is *NQO1*, which has been noted to be induced in abnormal human breast cells (MDA-MB231 and MCF-7) by TCDD exposure [41].

In summary, exposure to environmental carcinogens such as polycyclic aromatic hydrocarbons including BP may pose an increased risk of breast cancer through a complex transcriptional response. There are few if any compelling epidemiological studies that implicate environmental carcinogens, including PAHs, in human breast cancer [42,43]. However, data from human mammary cells treated in vitro suggest that this is not an unlikely possibility [1,8,44]. In this study expression, profiles or transcriptional signatures, in response to BP exposure were generated for four NHMEC strains developed from different donors. The profiles were used to compare overall patterns of gene expression and to identify differentially expressed genes. We have identified a number of genes differentially expressed between cell strains. Some of the genes identified are already known to be over-expressed in breast cancer (e.g. *CYP1B1*), however, several represent novel candidates that could be used as exposure biomarkers [45]. In addition, new studies will be required to determine whether over-expression of these genes are involved in hydrocarbon carcinogenesis. The use of DNA microarrays containing >6800 genes and expressed sequence tags in our analysis has provided a global view of the transcriptional response of NHMECs to BP exposure. Further analysis will provide insights into the mechanisms and functional activities of individual genes. Here, we have shown inter-individual variations in transcriptional response to BP exposure between cell strains from different donors. Our experiments provide a basis for response to carcinogen exposure that may have a bearing on inter-individual variation in cancer susceptibility. These studies are complementary to molecular epidemiologic studies of cancer susceptibility. DNA microarrays provide a useful tool for gene discovery; however, our database for transcriptional modulation of specific genes will be extended to NHMECs of more donors using RT-PCR.

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