Genomic and Proteomic Profiling of Responses to Toxic Metals in Human Lung Cells

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Examining global effects of toxic metals on gene expression can be useful for elucidating patterns of biological response, discovering underlying mechanisms of toxicity, and identifying candidate metal-specific genetic markers of exposure and response. Using a 1,200 gene nylon array, we examined changes in gene expression following low-dose, acute exposures of cadmium, chromium, arsenic, nickel, or mitomycin C (MMC) in BEAS-2B human bronchial epithelial cells. Total RNA was isolated from cells exposed to 3 μM Cd(II) (as cadmium chloride), 10 μM Cr(VI) (as sodium dichromate), 3 μg/cm^2 Ni(II) (as nickel subsulfide), 5 μM or 50 μM As(III) (as sodium arsenite), or 1 μM MMC for 4 hr. Expression changes were verified at the protein level for several genes. Only a small subset of genes was differentially expressed in response to each agent: Cd, Cr, Ni, As (5 μM), As (50 μM), and MMC each differentially altered the expression of 25, 44, 31, 110, 65, and 16 individual genes, respectively. Few genes were commonly expressed among the various treatments. Only one gene was altered in response to all four metals (*hsp90*), and no gene overlapped among all five treatments. We also compared low-dose (5 μM, noncytotoxic) and high-dose (50 μM, cytotoxic) arsenic treatments, which surprisingly, affected expression of almost completely nonoverlapping subsets of genes, suggesting a threshold switch from a survival-based biological response at low doses to a death response at high doses. *Key words:* arsenic, cadmium, chromium, DNA microarray, hypoxia inducible factor-1α, kinase, mitomycin C, nickel, toxicogenomics, toxicoproteomics. *Environ Health Perspect* 111:825–838 (2003). doi:10.1289/txg.6249 available via *http://dx.doi.org/* [Online 7 May 2003]

Eight of the top 50 substances on the 1997 Agency for Toxic Substances and Disease Registry (ATSDR) priority list (ATSDR 2001) are toxic metals, including arsenic, chromium, cadmium, and nickel. Exposure to these metals is associated with a variety of adverse health effects; however, the mechanisms that lead to the development of these diseases and the subcellular pathways modified in response to metal exposures are not well understood. Metal-specific biomarkers of exposure, effect, or susceptibility are needed for risk assessment and epidemiologic studies exploring the important health effects of exposure to these metals.

Arsenic exposure can occur through ingestion of contaminated drinking water, particularly in regions with geologic sources of arsenic, including Bangladesh, Taiwan, and Chile and parts of the United States such as New Hampshire, Michigan, Nevada, and California (Gebel 2000, 2001). Arsenic can also enter the body via inhalation, which is particularly important for certain occupational exposures (Abernathy et al. 1999; ATSDR 1999a; IARC 1980). Dermal exposure does not appear to lead to significant systemic uptake, although local dermal exposure such as with Fowler's solution or arsenical pesticides has been associated with skin effects at the site of application (Baudouin et al. 2002). Chronic arsenic exposure has been associated with increased incidence of vascular and cardiovascular disease, diabetes, hyperkeratosis, and cancers of the skin, lung, liver, bladder, kidney, and colon (ATSDR 1999a; Byrd et al. 1996; Leonard and Lauwerys 1980).

The primary route of toxicologic concern for exposure to both nickel and chromium is inhalation, principally in occupational settings, although environmental exposures can also occur as a result of anthropogenic sources (IARC 1991; Leikauf 2002; Williams and Sandler 2001). It has been estimated that 1.5 million workers are exposed to nickel occupationally in the United States (IARC 1991). Particulate nickel is emitted into the atmosphere during oil and coal combustion, metal refining, nickel-alloy manufacturing and grinding, battery manufacturing, municipal incineration, electroplating, and stainless steel manufacturing, as well as from cigarette smoke and motor vehicle emissions, resulting in environmental inhalation exposure (Barceloux 1999; Laden et al. 2000; NiPERA 1999). Dermal exposure can occur through skin contact with soil, water, or metals, including stainless steel or coins containing nickel (ATSDR 1999b) and can result in allergic reactions. Occupational exposure to nickel via inhalation is associated with respiratory distress and lung and nasal cancer (ATSDR 1999b; Denkhaus and Salnikow 2002; Leikauf 2002).

Chromium(VI) enters the air principally as a result of coal and oil combustion, steel production, stainless steel welding, and chemical manufacturing (ATSDR 1998; Barnhart 1997; IARC 1991). Chromium exposure can also occur from cigarette smoke. Discharge from electroplating, leather tanning, textiles, and dye and pigment manufacturing can contaminate water sources. Occupational exposure to chromium(VI) through inhalation causes respiratory tract problems and lung cancer, whereas dermal contact can lead to allergic contact dermatitis and skin ulceration (Alcedo and Wetterhahn 1990; ATSDR 1998; Dayan and Paine 2001).

Cadmium inhalation can occur occupationally during battery manufacturing, metal soldering, or welding, as well as environmentally from burning fossil fuels, municipal waste, or cigarettes, and is associated with respiratory damage and cancer. Exposure can also occur through consumption of food or water containing cadmium, leading to gastrointestinal problems and kidney and bone disease, as well as increased body burdens of cadmium, which has a half-life of greater than 20 years in humans (ATSDR 2002; Beyersmann and Hechtenberg 1997; Jarup et al. 1998).

Identification of genes whose expression is specifically modified by toxic metal exposure would provide a better understanding of their mechanisms of action and allow development of sensitive and specific biomarkers of both exposure and susceptibility for use in both mechanistic laboratory and epidemiology studies. In the

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We thank the technical staff at Clontech for assistance in analyzing the array data in these experiments. We also thank the Dartmouth Molecular Biology and Proteomics Core Facility and the Dartmouth Bioinformatics Group for their assistance and support.

This work was supported by the National Institute of Environmental Health Sciences Superfund Basic Research program grant P42 ES07373.

The authors state there is no conflict of interest.

Received 31 January 2003; accepted 6 May 2003.

current work we used cDNA microarrays to compare the effects of the toxic metals arsenic, cadmium, chromium, and nickel on expression of 1,200 human genes in human bronchial BEAS-2B cells, as lung is a target for effects of all four of these metals. We also confirmed the expression of certain relevant genes at the protein level in both epithelial and vascular smooth muscle cell models.

Methods

Cell treatment and preparation. Human bronchial epithelial cells (BEAS-2B; ATCC, Rockville, MD) were grown to postconfluence in 75-cm^2 flasks (Corning Costar, Corning, NY) on a matrix of 0.01 mg/mL human fibronectin (Collaborative Biomedical Products, Bedford, MA), 0.03 mg/mL Vitrogen 100 (Collagen Biomaterials, Palo Alto, CA), and 0.01 mg/mL bovine serum albumin (Sigma Chemical Co., St. Louis, MO). The cultures were maintained in LHC-9 medium (Biofluids Inc., Rockville, MD) at 37°C under an atmosphere of 5% CO_2/95% air, and medium was changed 24 hr before treatment.

Primary cultures of porcine smooth muscle cells (pSMC) were grown from medial explants of porcine aortas, using established methods (Ross 1971). Briefly, segments of thoracic aorta were cleaned of outer adventitia, opened longitudinally, and scraped to remove endothelial cells. Segments of the remaining intima and media were cut into 1-mm squares and allowed to adhere to scored plastic dishes. The squares were then cultured with complete Dulbecco's modified Eagle's medium (DMEM; Cellgro MediaTech Inc., Herndon, VA) containing 1 mmol/L glucose, 10% fetal bovine serum, and 1% penicillin/streptomycin under an atmosphere of 10% CO_2/90% air. The explants were removed once cells began to grow out. The cells were harvested in trypsin/EDTA and replated for continued subculturing or characterization by immunohistochemistry. All cultures stained greater than 99% positive for α-actin. The cells used in these experiments were from passage 3 or 4.

For both cell types, treatments were chosen that did not cause overt signs of toxicity or changes in cell survival or replication as measured by long-term colony-forming assays. The exception was the 50-μM arsenic treatment used for dose–response comparisons. In all other cases, the doses of metal are relevant to those to which humans could be exposed. For example, levels of nickel found in the lungs of autopsied U.S. subjects with no known occupational exposure to nickel range between 1.8 and 2.1 mg Ni/cm^2 of lung surface area (Edelman and Roggli 1989). Nickel refinery workers had much higher levels of nickel in the lung (mean 15 mg Ni/cm^2) (IARC 1991).

cDNA array analysis. One confluent flask of > 10^7 cells BEAS-2B cells per treatment group of control cells or cells exposed to 5 or 50 μM sodium arsenite, 3 μM cadmium chloride, 10 μM sodium dichromate (Aldrich, St. Louis, MO), 3 μg/cm^2 of cell culture flask nickel subsulfide (Sigma), or 1 μM mitomycin C (MMC) for 4 hr was washed and scraped in ice-cold phosphate-buffered saline. Cells were then centrifuged, and the cell pellet was snap frozen in liquid nitrogen. The expression of 1,200 genes was assessed by cDNA microarray analysis using Clontech nylon membrane-based Human Broad Coverage 1.2 I arrays (Clontech Laboratories Inc., Palo Alto, CA). Densitometry was performed on the hybridized membranes using a phosphoimager and the data were analyzed using AtlasImage software (Clontech Laboratories). The presence of nine housekeeping genes per array allowed us to discard housekeeping genes that were induced or repressed by a particular treatment (typically one to two of nine included on the array). The expression of each gene was normalized to the average of the remaining housekeeping genes. The housekeeping genes included on the array were ubiquitin, phospholipase A2, hypoxanthine–guanine, phosphoribosyltransferase (HPRT), liver glyceraldehyde 3-phosphate dehydrogenase (GAPDH), brain-specific tubulin alpha 1 subunit (TUBA1), HLA class I histocompatibility antigen C-4 alpha subunit (HLAC), cytoplasmic beta-actin (ACTB), 23-kDa highly basic protein, 60S ribosomal protein L13A (RPL13A), and 40S ribosomal protein S9.

The normalized ratios (treated divided by control) and differences (treated minus control) in gene expression between treated and control samples were calculated for all genes. Microarray analyses were repeated using $n = 7$ independent cultures for the housekeeping genes as well as two untreated independent cultures for all 1,200 genes, and inter-array variability was estimated to be < 22%. Within a single array, the variability of housekeeping gene expression was estimated to be between 8.4 and 20.9%. The housekeeping genes were used to calculate thresholds for each treatment. Thresholds were determined using fold changes 2 standard deviations outside of the average housekeeping gene value. The following threshold values were assigned on the basis of the underlying distribution of the data: 5 μM arsenic: ratio 1.69, difference 4; 50 μM arsenic: ratio 2.0, difference 13; chromium, cadmium, nickel, and MMC: ratio 1.49, difference 4. In addition to setting a threshold for the ratios, the difference between the treated samples and the controls was used to examine genes with low expression levels in which a fold change would be less reliable (e.g., 400/200 units compared with 4/2 units).

Immunoblot. The effects of arsenic exposure on hypoxia inducible factor-1α (HIF-1α) or β-actin protein levels (used as a loading control) were determined by Western blotting using a polyclonal antibody to HIF-1α (Transduction Laboratories, Lexington, KY) or a monoclonal antibody to β-actin (Sigma). Immunoblotting was performed as described previously (Andrew et al. 2001; Barchowsky et al. 1997).

Kinase expression assay. For the kinase expression assay, the medium of 1-day postconfluent BEAS-2B cells was changed 12–18 hr prior to addition of 5 μM potassium dichromate (Aldrich) and the cells were treated for a time course of 1, 4, and 24 hr. pSMC were grown to 80–90% confluence in 75-cm^2 flasks (Corning Costar), and the medium was changed to a serum-free DMEM containing 1 mg/mL bovine serum albumin 20 hr prior to the addition of sodium arsenite. Cells were treated with 2.5 μM As for 1, 4, and 12 hr. A dose–response experiment using 1, 2.5, and 10 μM As was performed at 24 hr. Following treatment, cells were rinsed with Tris-buffered saline containing protease inhibitors, as described previously. The cells were then prepared as described (Kinexus Bioinformatics Corp. 2001). Briefly, the cells were lysed in 20 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.0; 2 mM EGTA; 5 mM EDTA; 30 mM sodium fluoride; 40 mM β-glycerophosphate, pH 7.2; 10 mM sodium pyrophosphate; 2 mM sodium orthovanadate; and 0.5 % Nonidet P-40, supplemented with protease inhibitors. The cell lysates were sonicated twice for 15 sec and centrifuged for 2 hr at 19,000 rpm at 4°C. A protein assay was performed on the supernatant, and a cell lysate mixture was adjusted to a concentration of 1 μg/μL using a 4 × sample buffer (50% glycerol; 125 mM Tris–HCl, pH 6.8; 4% sodium dodecyl sulfate; 0.08% bromophenol blue; 5% β-mercaptoethanol). Samples were heated at 100°C for 4 min. The samples were analyzed via the Kinetworks' Protein Kinase Screen 1.0, a multiplexed western blot service provided by Kinexus Bioinformatics Corp. (Vancouver, British Columbia, Canada). Kinexus loaded equal amounts of protein, quantified the blots by densitometry, and analyzed them using their proprietary software.

Results

BEAS-2B human lung bronchial epithelial cells were treated for 4 hr either with arsenic (as sodium arsenite, 5 or 50 μM), cadmium (as cadmium chloride, 3 μM), chromium (as sodium dichromate, 10 μM), nickel (as nickel subsulfide, 3 μg/cm^2), or the genotoxic cancer chemotherapy drug MMC (1 μM). cDNA array analysis with 1,200 human genes (Clontech 1.2; Clontech) was performed with each treatment. The selected threshold fold change for each treatment was outside the 1.49- to 2.0-fold range in expression seen in the housekeeping genes (average plus 2 standard deviations).

The genes listed in Figure 1 showed increased or decreased expression following each treatment (see "Materials and Methods" for details). This Boolean schematic representation lists the genes uniquely changed by each exposure within the appropriate shape, with overlapping regions (or underlining for arsenic) indicating genes that were modified by more than one treatment. To summarize the data, low-dose cadmium altered the expression of 25 genes; chromium, 44; nickel, 31; 5 μM arsenic, 110 (Figure 2); 50 μM arsenic, 65; and MMC, 16 genes.

As shown in Figure 1, although there was some overlap, overall each treatment modified expression of a largely unique set of genes. Only heat-shock protein 90A (*HSP 90A*) expression was modified by treatment with all four of the metals tested, and no gene's expression was modified by all five treatments. Several genes were differentially regulated in response to three metals: cadmium, chromium, and nickel. Specifically, these three metals induced expression of erythrocyte glucose transporter 1 (*GLUT1*) and decreased transcriptional activator (*DB1*), collagen type 4 (*COL4A2*), glutathione peroxidase (*GSHPX1*), hepatoma-derived growth factor (*HDGF*), and cytochrome P450 1B1 (*CYP1B1*) (Figure 1, overlapping region). Interestingly, when two or more exposures affected expression of the same gene, the expression was usually altered in the same direction, that is, increased or decreased, with each exposure. The only exception was that treatment with the organic DNA-damaging agent MMC induced expression of early growth response protein 1 (*hEGR1*), whereas chromium, arsenic, and nickel all suppressed expression of this gene (Figure 1, black font).

To explore the effects of dose on the gene expression profile, we exposed cells for 4 hr to two different doses of arsenic: 5 μM, which caused little or no cytotoxicity, or 50 μM, which was highly cytotoxic as determined by a colony-forming assay.

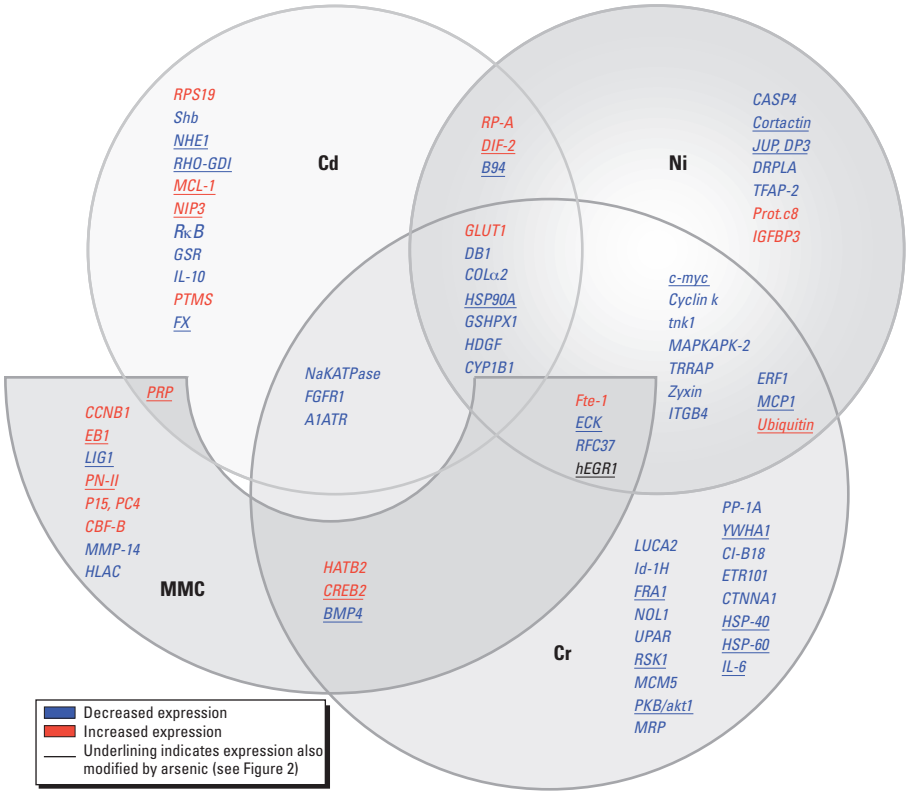

Figure 1. Gene expression profiles for cells treated with cadmium, chromium, nickel, or MMC. Relative expression of 1,200 genes was assessed in human bronchial BEAS-2B cells exposed for 4 hr to cadmium (10 μM Cd^{2+} as cadmium chloride), chromium(VI) (10 μM Cr(VI) as sodium dichromate), nickel (3 μg/cm^2 Ni^{2+} as nickel subsulfide), and mitomycin C (1.0 μM MMC) using cDNA microarray analysis. Genes with expression changes above a statistically derived threshold for an exposure (minimum 1.5-fold) are listed within each designated shape using the abbreviations defined in Table 1. Font color indicates an increase (red) or decrease (blue) in expression relative to control. Black font indicates a gene whose expression was induced by one treatment but suppressed by another. Genes with modified expression following exposure to more than one metal are found in the relevant overlapping areas. Genes modified by arsenic in addition to chromium, cadmium, nickel, or MMC are underlined.

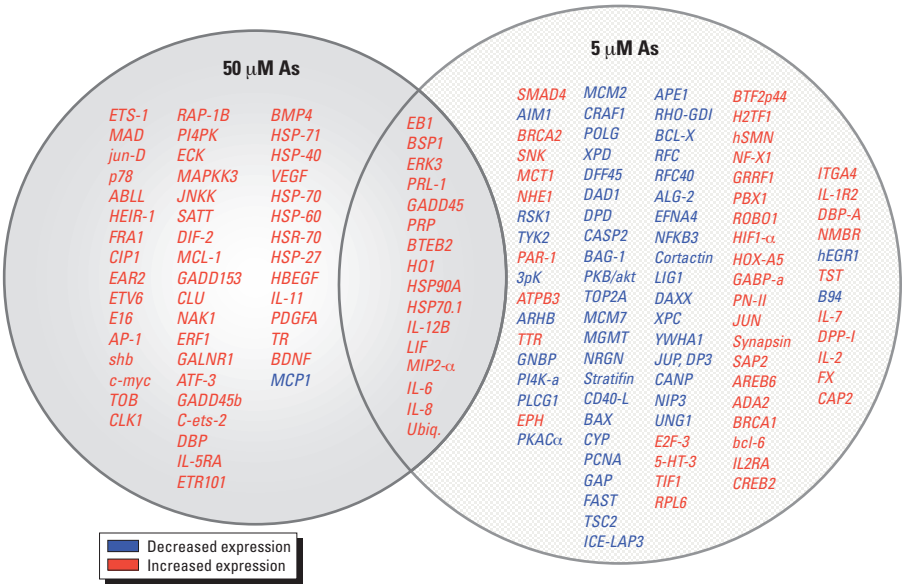

Figure 2. Gene expression profiles for cells treated with 5 or 50 μM arsenic. Ubiq., ubiquitin. Relative expression of 1,200 genes was assessed in human bronchial BEAS-2B cells exposed for 4 hr to arsenic [5 or 50 μM As(III) as sodium arsenite] as described in Figure 1. Font color indicates an increase (red) or decrease (blue) in expression relative to control. The overlapping region contains a list of genes affected by both 5 and 50 μM arsenic.

Of the 1,200 genes examined at both doses, only 16 of 158 affected genes were altered at both doses (Figure 2, overlapping region). All genes altered by 50 μM arsenic were increased in expression (red font) with the exception of monocyte chemotactic protein 1 precursor (*MCP1*) (blue font). In contrast, 5 μM arsenic increased (red font) or decreased (blue font) expression of genes in similar numbers. Interestingly, more total genes were affected by the lower dose than by the higher cytotoxic dose. As might be expected, at the higher dose, stress response and apoptotic genes predominated. Interestingly, most of these genes were unaffected at the lower dose.

Western immunoblot and kinase expression assays were performed for certain genes to determine whether the altered gene expression seen in the microarray assays was paralleled by a change in protein expression. Immunoblots (Figure 3) demonstrated increases in protein levels of the transcription factor HIF-1α, following exposure to arsenic for 4, 8, or 24 hr, which are consistent with the increased *HIF-1α* mRNA levels observed after 5-μM arsenic exposure (Figure 2). This level of arsenic exposure did not affect β-actin expression, which was used as a loading control.

Assays for kinase protein expression changes were performed on cells exposed to arsenic or chromium over a range of doses and time points in two separate cell types. The ratio of mRNA levels in exposed versus control cells (Figure 4) was compared with the ratio of protein levels observed in the kinase expression assay for each kinase (Figure 4). ERK3 gene and protein levels were increased at most arsenic doses and time points tested (Figure 4A), whereas RSK1, PKACα, and PKBα/akt1 all showed consistent decreases in expression following arsenic exposure in both assays (Figure 4B–D). Chromium exposure also decreased mRNA and protein levels of PKBα/akt1 (Figure 4E). Thus, for the genes and gene products examined, changes in mRNA expression were similar to changes in protein expression.

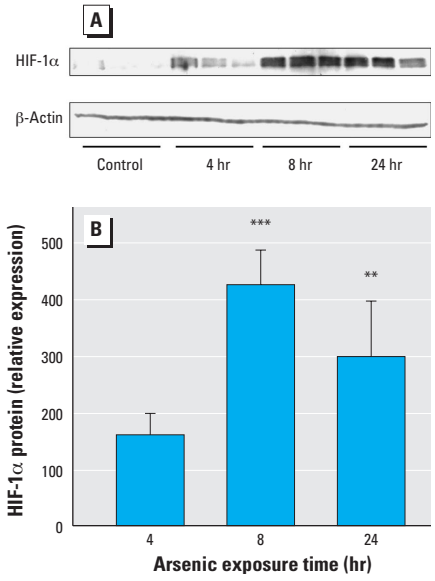

Figure 3. Effects of arsenic on HIF-1α protein expression. (*A*) Confluent BEAS-2B cells were exposed to 2.5 μM arsenic for 4, 8, or 24 hr. Cells were harvested for total protein and Western blots were performed as described in "Materials and Methods," using antibodies to HIF-1α or the loading control β-actin. (*B*) The band density ratio of HIF-1α to β-actin density of bands shown in *A*. Data represent protein collected from independent experiments. Values are means SD; $n = 3$. 8-hr arsenic, $^{**}p < 0.001$; 24-hr arsenic, $^{***}p < 0.05$ vs. control.

Discussion

Chronic exposure to the toxic metals arsenic, chromium, cadmium, and nickel has been associated with a wide variety of adverse health effects (ATSDR 1998, 1999a,1999b, 2002). Previous studies of individual genes have demonstrated that these metals can each substantially alter gene expression in various cell and whole animal systems (Andrew and Barchowsky 2000; Hamilton and Wetterhahn 1989; Hamilton et al. 1998; Ihnat et al. 1997; McCaffrey et al. 1994). The development of gene array technology has provided a means for examination of alterations in gene expression on a more global level. The current study describes profiles of early changes in gene and protein expression that

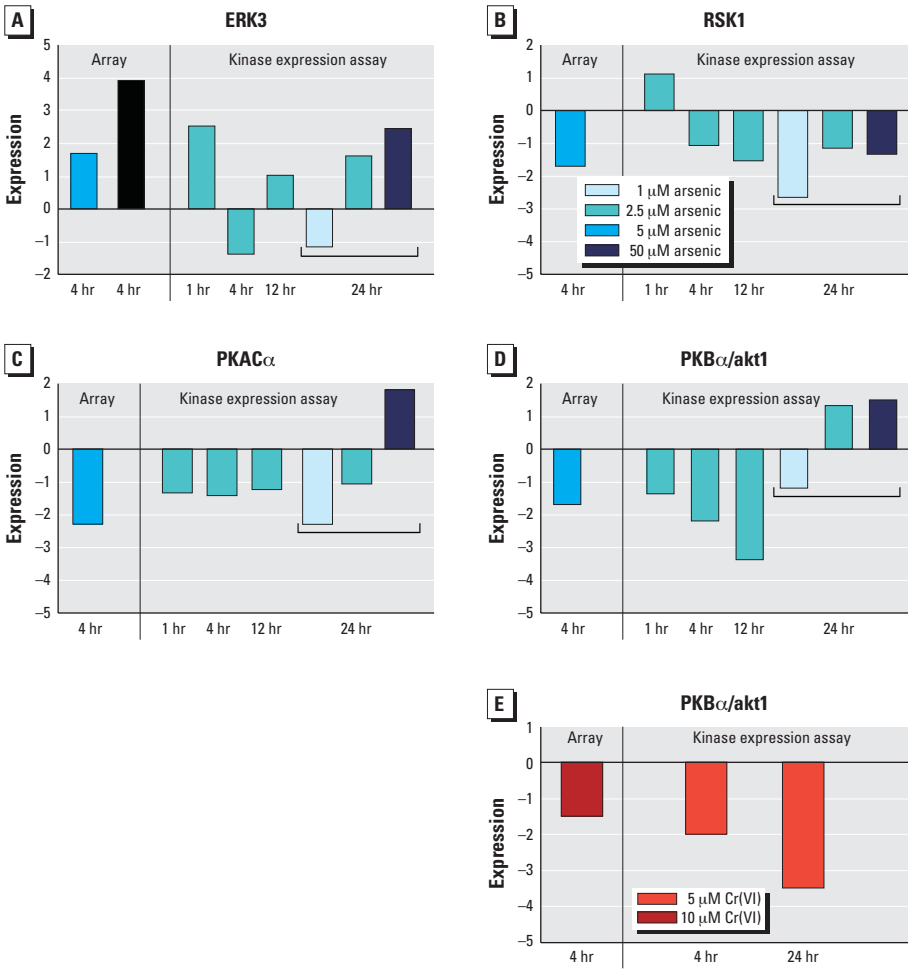

Figure 4. Comparison of gene expression and protein kinase expression profiles for cells treated with chromium and arsenic. Changes in the kinases (*A*) ERK3, (*B*) RSK1, (*C*) PKCα, and (*D*) PKBα in response to arsenic exposure were measured at both the gene level by cDNA array analysis and at the protein level by a kinase assay as described in "Materials and Methods." Data under the heading "Array" represent the normalized ratio of gene expression for arsenic-exposed cells compared with that of control, whereas data under the heading "Kinase expression assay" represent the ratio of protein levels for arsenic-exposed smooth muscle cells compared with that of control. (*E*) This graph shows the ratio of changes in PKBα gene and protein expression after chromium treatment, as described above for arsenic. Treatment time is indicated on the graph below each bar, and bar shading designates the dose of arsenic or chromium, as shown in the legend.

Table 1. Relative expression of genes for cells treated with arsenic, chromium, cadmium, nickel, or MMC.[a]

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio		Arsenic (50 μM) ratio		Chromium ratio		Cadmium ratio		Nickel ratio		MMC ratio		GenBank[a]
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
3pK	MAPKAP kinase (3pK)		2.00											U09578
5-HT-3	5-Hydroxytryptamine 3 receptor precursor (5-HT-3); serotonin-gated ion channel receptor	2.00												D49394
A1ATR	Alpha-1-antitrypsin precursor; alpha-1 protease inhibitor; alpha-1-antiproteinase						1.54		1.65					X02920
ABLL	Tyrosine-protein kinase ABL2; tyrosine kinase ARG (ABLL)			2.44										M35296
ADA2	ADA2-like protein	2.33												AF069732
AIM1	Aurora- & IPL1-like midbody-associated protein kinase 1 (AIM1); ARK2		2.00											AF008552
ALG-2	ALG-2 calcium-binding protein		2.40											AF035606
AP-1	Proto-oncogene c-jun; transcription factor AP-1			15.42										J04111
APE1	DNA-(apurinic or apyrimidinic site) lyase; AP endonuclease 1; APEX nuclease (APEN; APE1); REF-1 protein		2.07											X59764; X66133
AREB6	Transcription factor AREB6	5.00												D15050
ARHB	Transforming protein rhoB; ARHB; ARH6		1.83											X06820
ATF-3	Cyclic-AMP-dependent transcription factor ATF-3 (activating factor 3) sodium/potassium-transporting ATPase			6.33										L19871
ATPB3	Beta 3 subunit (ATPB3); sodium/ potassium-dependent ATPase	1.83												U51478
B94	B94 protein		2.00						3.50		2.33			M92357
BAG-1	BCL-2 binding athanogene-1 (BAG-1); glucocorticoid receptor-associated protein RAP46		1.70											S83171; Z35491
BAX	Apoptosis regulator bax		2.11											L22474
bcl-6	B-cell lymphoma 6 protein (bcl-6); zinc finger protein 51 (ZNF51); LAZ-3 protein	2.14												U00115
BCL-X	Apoptosis regulator bcl-x		1.89											Z23115; L20121; L20122
BDNF	Brain-derived neurotrophic factor (BDNF)			6.33										M61176
BMP4	Bone morphogenetic protein 4 (BMP4) + bone morphogenetic protein 2B (BMP2B)			2.00			3.00						3.00	D30751 + M22490
BRCA1	BRCA1-associated ring domain protein	2.17												X82200
BRCA2	Breast cancer type 2 susceptibility protein (BRCA2)	2.17												U43746
BSP1	Transforming growth factor-beta signaling protein 1 (BSP1); mothers against dpp homolog (MAD); MADR1; MSMAD1	3.50		2.44										U57456
BTEB2	Basic transcription element-binding protein 2 (BTEB2); GC-box binding protein 2	2.26		2.59										D14520
BTF2p44	Basic transcription factor 2 44-kDa subunit (BTF2p44)	1.90												Z30094
CANP	Calpain 2 large (catalytic) subunit; M-type calcium-activated neutral proteinase (CANP)		1.82											M23254
CAP2	Cytoplasmic antiproteinase 2 (CAP2); protease inhibitor 8	2.00												L40377
CASP2	Caspase-2 precursor (CASP2); ICH-1L protease + ICH-1S protease		4.00											U13021 + U13022

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Table 1—*Continued.*

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio		Arsenic (50 μM) ratio		Chromium ratio		Cadmium ratio		Nickel ratio		MMC ratio		GenBank[a]
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
CASP4	Caspase-4 precursor (CASP4); ICH-2 protease; TX protease; ICE(REL)-II + caspase-5 precursor (CASP5); ICH-3 protease; TY protease; ICE(REL)-III										1.53			U28014 + U28015
CBF-B	CCAAT-binding transcription factor subunit B (CBF-B); NF-Y protein subunit A (NF-YA); Hap2; CAAT-box DNA-binding protein subunit A											1.50		M59079
CCNB1	G2/mitotic-specific cyclin B1 (CCNB1)											1.51		M25753
CD40-L	CD40 ligand (CD40-L); tumor necrosis factor (TNF)-related activation protein (TRAP); T-cell antigen GP39		2.80											L07414
C-ets-2	C-ets-2			3.33										J04102
CI-B18	NADH-ubiquinone oxidoreductase B18 subunit; complex I-B18 (CI-B18); cell adhesion protein SQM1						1.67							M33374
CIP1	Cyclin-dependent kinase inhibitor 1 (CDKN1A); melanoma differentiation-associated protein 6 (MDA6); CDK-interacting protein 1 (CIP1); WAF1			3.78										U09579; L25610 L29222
CLK1	CDC-like kinase 1 (CLK1)			2.78										M74816
CLU	Clusterin precursor (CLU); complement-associated protein SP-40,40; complement cytolysis inhibitor (CLI); apolipoprotein J (APO-J); TRPM-2; sulfated glycoprotein 2			2.79										
c-myc	c-myc oncogene			3.49			3.42				1.95			V00568
COLα2	Procollagen alpha 2(IV) subunit precursor						2.00		1.75		1.75			X05562
Cortactin	Cortactin; amplaxin; ems-1 oncogene		1.75								1.76			M98343
CRAF1	CD40 receptor-associated factor 1 (CRAF1)		5.00											U21092
CREB2	cAMP-dependent transcription factor ATF-4; DNA-binding protein TAXREB67; cAMP-response element binding protein (CREB2)	2.62				1.72						1.58		D90209
CTNNA1	Alpha1 catenin (CTNNA1); cadherin-associated protein; alpha E-catenin						1.76							D13866; D14705; L23805; L22080
Cycin k	Cyclin K						2.13				2.13			AF060515
CYP	Cytochrome P450 reductase		3.00											S90469
CYP1B1	Dioxin-inducible cytochrome P450 1B1 (CYP1B1)						3.50		3.50		2.33			U03688
DAD1	Defender against cell death 1 (DAD1)		2.07											D15057
DAXX	DAXX		2.50											AF015956
DB1	Putative transcription activator DB1						2.33		1.75		1.75			D28118
DBP	DNA-binding protein TAXREB302; albumin D box-binding protein (DBP)			2.27										D28468
DBP-A	DNA-binding protein A	1.76												M24069
DFF45	DNA fragmentation factor 45 (DFF45)		3.00											U91985
DIF-2	IEX-1L anti-death protein; PRG-1; DIF-2			3.09			2.60		1.80					AF039067; AF071596
DPD	DNA polymerase delta catalytic subunit		2.18											M80397
DPP-1	Dipeptidyl-peptidase I precursor (DPP-I); cathepsin C; cathepsin J; dipeptidyl transferase	1.83												X87212
DRPLA	Atrophin-1; dentatorubral-pallidoluysian atrophy protein (DRPLA)										3.00			D31840
E16	E16 amino acid transporter			2.63										AF077866
E2F-3	E2F-3	2.17												Y10479

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Table 1—*Continued.*

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio		Arsenic (50 μM) ratio		Chromium ratio		Cadmium ratio		Nickel ratio		MMC ratio		GenBank[a]
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
EAR2	v-erbA-related protein (EAR2)			2.08										X12794
EB1	EB1 protein	2.20		2.04								1.53		U24166
ECK	Ephrin type-A receptor 2 precursor; epithelial cell kinase (ECK); tyrosine-protein kinase receptor ECK			2.54			2.00				1.80		1.50	M59371; M36395
EFNA4	Ephrin A4 precursor (EFNA4); EPH-related receptor tyrosine kinase ligand 4 (EPLG4); LERK4		7.00											U14188
EPH	Ephrin type-A receptor 1 precursor; tyrosine-protein kinase receptor eph	2.88												M18391
ERF1	TIS11B protein; EGF response factor 1 (ERF1)			3.37			2.00				2.00			X79067
ERK3	Extracellular signal-regulated kinase 3 (ERK3); MAP kinase 3 (MAPK3); p97-MAPK); PRKM5	1.70		3.92										X80692
ETR101	Transcription factor ETR101			4.03			3.00							M62831
ETS-1	Erythroblastosis virus oncogene homolog 1 (ETS-1); p54			3.21										J04101
ETV6	ets-related protein tel; ets translocation variant 6 (ETV6)			2.27										U11732
FAST	fas-activated serine/threonine (FAST) kinase		2.75											X86779
FGFR1	N-sam; fibroblast growth factor receptor1 precursor (FGFR1); basic fibroblast growth factor receptor precursor (bFGFR); fms-like tyrosine kinase-2 (FLT2) + heparin-binding growth factor receptor (HBGF-R-alpha-A1) + HBGF-R-alpha-A2 + HBGF-R-alpha-A3						3.00		3.00					X66945; M34641; M34186; M37722 + M63887 + M63888 + M63889
FRA1	fos-related antigen (FRA1)			8.59			1.80							X16707
Fte-1	fte-1; yeast mitochondrial protein import homolog; 40S ribosomal protein S3A (RPS3A)					1.50				1.52		1.55		M77234
FX	Thymosin beta 4; FX	2.21							1.74					M17733
GABP-α	GA-binding protein alpha subunit (GABP-alpha); transcription factor E4TF1-47; nuclear respiratory factor-2 alpha subunit	2.33												D13316
GADD153	Growth arrest and DNA-damage-inducible protein 153 (GADD153); DNA-damage-inducible transcript 3 (DDIT3); C/EBP homologous protein (CHOP)			10.35										S40706; S62138
GADD45	Growth arrest and DNA-damage-inducible protein (GADD45); DNA-damage-inducible transcript 1 (DDIT1)	2.60		13.88										M60974
GADD45β	Growth arrest and DNA-damage-inducible protein 45 beta (GADD45 beta)			3.23										AF078077
GALNR1	Galanin receptor type 1 (GALNR1; GALR1)			2.78										L34339
GAP	GAP-associated protein		2.00											U17032
GLUT1	Erythrocyte glucose transporter 1 (GLUT1)					1.86		1.64		1.93				K03195
GNBP	Guanine nucleotide-binding protein G-i/G-s/G-t beta subunit 2; transducin beta 2 subunit 2		2.50											M36429
GRRF1	Glucocorticoid receptor repression factor 1	2.00												M73077
GSHPX1	Glutathione peroxidase (GSHPX1; GPX1)						2.00		1.68		1.62			Y00483; M21304
GSR	Glutathione reductase (GRase; GSR; GR)								1.60					X15722
H2TF1	Nuclear factor NF-kappa-B p100 subunit; nuclear factor NF-kappa-B p52 subunit; H2TF1; oncogene lyt-10	1.71												X61498

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Table 1—*Continued.*

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio		Arsenic (50 μM) ratio		Chromium ratio		Cadmium ratio		Nickel ratio		MMC ratio		GenBank[a]
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
HATB2	Histone acetyltransferase B subunit 2; retinoblastoma-binding protein p46; retinoblastoma-binding protein 7					1.80						1.80		U35143
HBEGF	Heparin-binding EGF-like growth factor (HBEGF); diphtheria toxin receptor (DTR)			16.29										M60278
HDGF	Hepatoma-derived growth factor (HDGF)						1.67		1.55		1.55			D16431
hEGR1	Early growth response protein 1 (hEGR1); transcription factor ETR103; KROX24; zinc finger protein 225; AT225		2.36				13.00				2.17	1.62		X52541; M62829
HEIR-1	Helix-loop-helix protein HLH 1R21; DNA-binding protein inhibitor Id-3; HEIR-1			2.41										X69111
HIF1-α	Hypoxia-inducible factor 1 alpha (HIF1 alpha); ARNT-interacting protein; member of PAS protein 1 (MOP1)	3.00												U22431
HLAC	HLA class I histocompatibility antigen C-4 alpha subunit (HLAC)												1.52	M11886
HO1	Heme oxygenase 1 (HO1); HSOXYGR	50.93		54.95										X06985
HOX-A5	Homeobox protein HOX-A5; HOX-1C	1.91												M26679
hSMN	Survival of motor neuron (hSMN)	2.33												U18423
HSP-27	Heat-shock 27-kDa protein (HSP27); stress-responsive protein 27 (SRP27); estrogen-regulated 24-kDa protein; HSPB1			4.28										X54079
HSP-40	Heat-shock protein 40 (HSP40)			13.86			1.50							D49547
HSP-60	Mitochondrial matrix protein P1 precursor; p60 lymphocyte protein; chaperonin homolog; HUCHA60; heat-shock protein 60 (HSP-60); HSPD1			3.13			2.00							M34664
HSP-70	Heat-shock 70-kDa protein 6 (heat-shock 70-kDa protein B)			108.50										X51757; M11236
HSP70.1	70-kDa heat-shock protein 1 (HSP70.1; HSPA1)	5.48		45.83										M11717
HSP-71	Heat-shock cognate 71-kDa protein			4.19										Y00371
HSP-90A	Heat-shock 90-kDa protein A (HSP90A; HSPCA); HSP86	2.33		3.96			3.25		1.86		2.36			X07270
HSR-70	Heat-shock–related 70-kDa protein 2			4.53										L26336
ICE-LAP3	Cysteine protease ICE-LAP3		2.33											U39613
Id-1H	DNA-binding protein inhibitor ID-1; Id-1H						5.00							D13889
IGFBP3	Insulin-like growth factor-binding protein 3 precursor (IGF-binding protein 3; IGFBP3; IBP3)									2.00				M31159; M35878
IL-10	Interleukin-10 precursor (IL-10); cytokine synthesis inhibitory factor (CSIF)								1.54					M57627
IL-11	Interleukin-11 (IL-11); adipogenesis inhibitory factor (AGIF)			13.33										M57765
IL-12B	Interleukin-12 beta subunit precursor (IL-12B); cytotoxic lymphocyte maturation factor 40-kDa subunit (CLMF p40); NK cell stimulatory factor subunit 2 (NKSF2)	2.50		6.00										M65290
IL-1R2	Interleukin-1 receptor type II precursor (IL-1R2); IL-1R-beta	2.40												X59770
IL-2	Interleukin-2 precursor (IL-2); T-cell growth factor (TCGF)	1.90												A14844
IL2RA	Interleukin-2 receptor alpha subunit precursor (IL-2 receptor alpha subunit; IL2RA); p55; TAC antigen; CD25	1.74												X01057; X01058; X01402
IL-5RA	Interleukin-5 receptor alpha subunit precursor (IL-5R-alpha; IL5RA); CD125 antigen			2.66										M75914

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Table 1—*Continued.*

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio		Arsenic (50 μM) ratio		Chromium ratio		Cadmium ratio		Nickel ratio		MMC ratio		GenBank[a]
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
IL-6	Interleukin-6 precursor (IL-6); B-cell stimulatory factor 2 (BSF2); interferon beta-2 (IFNB2); hybridoma growth factor	3.11		2.78			2.45							X04602; M14584
IL-7	Interleukin-7 (IL-7)	1.73												J04156
IL-8	Interleukin-8 precursor (IL-8); monocyte-derived neutrophil chemotactic factor (MDNCF); T-cell chemotactic factor; neutrophil-activating protein 1 (NAP1); lymphocyte-derived neutrophil-activating factor (LYNAP); protein 3-10C	2.95		4.46										Y00787
ITGA4	Integrin alpha 4 precursor (ITGA4); VLA4; CD49D antigen	2.54												L12002; X16983; X15356
ITGB4	Integrin beta 4 (ITGB4); CD104 antigen						1.76				1.61			X53587; ,X52186; X51841
JNKK	c-jun N-terminal kinase kinase 1 (JNKK); JNK activating kinase 1 (JNKK1); MAP kinase kinase 4 (MKK4)			3.00										L36870
JUN	jun activation domain binding protein	2.43												U65928
jun-D	jun-D			5.67										X56681
JUP,DP3	Junction plakoglobin (JUP); desmoplakin III (DP3)		2.33								1.60			M23410; Z68228
LIF	Leukemia inhibitory factor precursor (LIF); differentiation-stimulating factor (D factor); melanoma-derived LPL inhibitor (MLPLI); HILDA	2.15		7.63										X13967; M63420
LIG1	DNA ligase I; polydeoxyribonucleotide synthase (ATP) (DNL1) (LIG1)		2.94										1.57	M36067
LUCA2	LUCA2; lysosomal hyaluronidase 2 (HYAL2) ; PH-20 homolog						2.11							U09577
MAD	MAD protein; MAX dimerizer			14.00										L06895
MAPKAPK-2	MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2)						1.50				1.50			U12779
MAPKK3	Dual specificity mitogen-activated protein kinase kinase 3 (MAP kinase kinase 3; MAPKK 3; MKK3); ERK activator kinase 3; MAPK/ERK kinase 3 (MEK3)			2.78										L36719
MCL-1	Induced myeloid leukemia cell differentiation protein MCL-1			2.23				2.00						L08246
MCM2	MCM2 DNA replication licensing factor; nuclear protein BM28; KIAA0030		2.24											D21063
MCM5	MCM5 DNA replication licensing factor; CDC46 homolog						1.72							X74795
MCM7	MCM7 DNA replication licensing factor; CDC47 homolog; p1.1-MCM3		2.24											D55716
MCP1	Monocyte chemotactic protein 1 precursor (MCP1); monocyte chemotactic and activating factor (MCAF); monocyte secretory protein JE; monocyte chemoattractant protein 1; HC11; small inducible cytokine A2 (SCYA2)				2.21		7.00				2.33			M24545
MCT1	Monocarboxylate transporter 1 (MCT1)	3.50												L31801
MGMT	6-O-methylguanine-DNA methyltransferase (MGMT); methylated-DNA-protein-cysteine methyltransferase		1.83											M29971
MIP2-α	Macrophage inflammatory protein 2 alpha (MIP2-alpha); growth-regulated protein beta (GRO-beta)	2.03		2.11										X53799

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Table 1—*Continued.*

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio		Arsenic (50 μM) ratio		Chromium ratio		Cadmium ratio		Nickel ratio		MMC ratio		GenBank[a]
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
MMP-14	Matrix metalloproteinase 14 precursor (MMP14); membrane-type matrix metalloproteinase 1 (MT-MMP1); MMP-X1												1.67	D26512; X83535
MRP	macMARCKS; MARCKS-related protein (MRP); MLP						1.80							X70326
NAK1	Early response protein NAK1; TR3 orphan receptor			7.83										L13740
NaKATPase	Sodium/potassium-transporting ATPase alpha 1 subunit (Na+/K+ ATPase)						1.55		1.55					D00099
NFKB3	NF-kappaB transcription factor p65 subunit; RELA; NFKB3		2.00											L19067
NF-X1	Transcriptional repressor NF-X1	3.00												U15306
NHE1	Sodium/hydrogen exchanger 1 (Na+/H+ exchanger 1; NHE1); amiloride-sensitive Na+/H+ antiporter	1.70							1.80					M81768
NIP3	NIP3 (NIP3)		2.40					2.00						U15174
NMBR	Neuromedin B receptor (NMBR); neuromedin-B-preferring bombesin receptor	1.76												M73482
NOL1	Proliferating cell nucleolar antigen P120; NOL1						1.83							X55504
NRGN	Neurogranin (NRGN); RC3		1.75											Y09689
p15, PC4	Activated RNA polymerase II transcriptional coactivator p15; PC4											1.74		U12979
p78	p78 putative serine/threonine-protein kinase			2.36										M80359
PAR-1	Thrombin receptor (TR); F2R; PAR1	2.40												M62424
PBX1	Pre-B-cell leukemia transcription factor-1; homeobox protein pbx1; Homeobox protein prl	2.00												M86546
PCNA	Proliferating cyclic nuclear antigen (PCNA); cyclin		1.75											M15796; J04718
PDGFA	Platelet-derived growth factor A subunit precursor (PDGFA; PDGF-1)			2.52										X06374
PI4K-α	Phosphatidylinositol 4-kinase alpha (PI4-kinase; PTDINS-4-kinase; PI4K-alpha)		3.00											L36151
PI4PK	68-kDa type I phosphatidylinositol-4-phosphate 5-kinase alpha (PTDINS(4)P-5-kinase); 1-phosphatidylinositol-4-phosphate kinase; diphosphoinositide kinase			2.86										X80907
PKACα	cAMP-dependent protein kinase alpha-catalytic subunit (PKA C-alpha)		2.33											X07767
PKB/akt	rac-alpha serine/threonine kinase (rac-PK-alpha); protein kinase B (PKB); c-akt; akt1		1.74				1.53							M63167
PLCG1	Phospholipase C gamma 1 (PLC-gamma 1; PLCG1); 1-phosphatidylinositol 4, 5-bisphosphate phosphodiesterase gamma 1; PLC-II; PLC-148		2.11											M34667
PN-II	Alzheimer's disease amyloid A4 protein precursor; protease nexin-II (PN-II); APPI	1.74										1.86		Y00264
POLG	DNA polymerase gamma (POLG); mitochondrial DNA polymerase catalytic subunit (MDP1)		3.00											X98093
PP-1A	Serine/threonine protein phosphatase PP1-alpha 1 catalytic subunit (PP-1A)						1.65							M63960
PRL-1	PTPCAAX1 nuclear tyrosine phosphatase (PRL-1)	1.82		3.71										U48296
Prot.c8	Proteasome component C8; macropain subunit C8; multicatalytic endopeptidase complex subunit C8									1.80				D00762

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Table 1—*Continued.*

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio		Arsenic (50 μM) ratio		Chromium ratio		Cadmium ratio		Nickel ratio		MMC ratio		GenBank[a]
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
PRP	Major prion protein precursor (PRP); PRP27-30; PRP33-35C; ASCR	2.05		2.54				1.62				1.90		M13667
PTMS	Parathymosin							2.10						M24398
RAP-1B	ras-Related protein RAP-1B; GTP-binding protein SMG p21B			2.25										X08004
RFC	Activator 1 140-kDa subunit (A1 140-kDa subunit); replication factor C large subunit; DNA-binding protein PO-GA		2.00											L14922
RFC37	Activator 1 37-kDa subunit; replication factor C 37-kDa subunit (RFC37); RFC4						2.59				1.73		1.54	M87339
RFC40	Activator 1 40-kDa subunit; replication factor C 40-kDa subunit (RFC40); RFC2		1.90											M87338
RHO-GDI	rho GDP dissociation inihibitor 1 (RHO-GDI 1); RHO-GDI alpha (GDIA1); ARHGDIA		1.81						1.67					X69550
RκB	R kappa B DNA-binding protein								1.62					U08191
ROBO1	Roundabout 1 (ROBO1)	1.75												AF040990
RP-A	Replication protein A 14-kDa subunit (RP-A) (RF-A); replication factor A protein 3							1.90		1.70				L07493
RPL6	60S ribosomal protein L6 (RPL6); TAX-responsive enhancer element binding protein 107 (TAXREB107); neoplasm-related protein C140	2.10												X69391
RPS19	40S ribosomal protein S19 (RPS19)							1.95						M81757
RSK1	Ribosomal protein S6 kinase II alpha 1 (S6KII-alpha 1); ribosomal S6 kinase 1 (RSK1)		1.72				1.75							L07597
SAP2	ets Domain protein elk-3; NET; SRF accessory protein 2 (SAP2)	1.91												Z36715
SATT	Neutral amino acid transporter A (SATT); alanine/serine/cysteine/threonine transporter (ASCT1)			2.08										L14595
Shb	shb proto-oncogene			3.01					1.62					X75342
SMAD4	Mothers against dpp homolog 4 (SMAD4); MADR4; pancreatic carcinoma gene 4 (DPC4)	3.50												U44378
SNK	Serum-inducible kinase (SNK)	2.75												AF059617
Stratifin	14-3-3 Protein sigma; stratifin; epithelial cell marker protein 1		1.88											AF029082
Synapsin	Synapsin IIIA	1.90												AF046873
TAFAP-2	Transcription factor AP-2 (TFAP2; AP2TF)										1.80			M36711
TIF1	Transcription intermediary factor 1 (TIF1)	1.80												AF009353
tnk1	Tyrosine kinase tnk1						1.78				1.78			U43408
TOB	Transducer of ERBB2 (TOB)			2.31										D38305
TOP2A	DNA topoisomerase II alpha (TOP2A)		3.50											J04088
TR	Thioredoxin reductase			3.29										X91247
TRRAP	TRRAP protein						2.00				1.75			AF076974
TSC2	Tuberin; tuberous sclerosis 2 protein (TSC2)		2.22											X75621
TST	Thiosulfate sulfurtransferase; rhodanese	5.00												D87292
TTR	Transthyretin precursor (TTR); prealbumin; TBPA	1.89											K02091	
TYK2	tyk2 non-receptor protein tyrosine kinase		1.86											X54637
Ubiquitin	Ubiquitin	2.03		7.94		2.16				1.82				M26880
UNG1	Uracil-DNA glycosylase precursor (UNG1)		5.57											X15653
UPAR	Urokinase-type plasminogen activator receptor GPI-anchored form precursor (U-PAR); monocyte activation antigen MO3; CD87 antigen						1.62							U08839; M83246; X51675

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Table 1—*Continued.*

Gene abbreviation[a]	Gene name[a]	Arsenic (5 μM) ratio Up	Arsenic (5 μM) ratio Down	Arsenic (50 μM) ratio Up	Arsenic (50 μM) ratio Down	Chromium ratio Up	Chromium ratio Down	Cadmium ratio Up	Cadmium ratio Down	Nickel ratio Up	Nickel ratio Down	MMC ratio Up	MMC ratio Down	GenBank[a]
VEGF	Vascular endothelial growth factor precursor (VEGF); vascular permeability factor (VPF)			5.86										M32977; M27281
XPC	DNA-repair protein complementing XP-C cells; xeroderma pigmentosum group C complementing protein (p125)		5.00											D21089
XPD	Xeroderma pigmentosum group D complementing protein (XPD); DNA excision repair protein ERCC2		2.33											X52221
YWHA1	14-3-3n protein eta; protein AS1; YWHAH; YWHA1		2.89				1.50							L20422
Zyxin	Zyxin + zyxin-2						2.25				3.00			X94991; X9573

[a]Information from GenBank (*http://www.ncbi.nlm.nih.gov/GenBank/index.html*).

are observed in response to toxic metal exposure. These early changes may provide further insight into mechanisms underlying development of metal-induced diseases. These early gene and protein responses are also candidate biomarkers of metal exposure and/or effect that could potentially be used diagnostically in molecular and epidemiologic studies.

Results of the cDNA microarray experiments indicate that exposure to these toxic metals modifies expression of only a small subset of the 1,200 total genes examined (Figures 1 and 2), which is consistent with the concept that these were low, relatively nontoxic doses that did not activate large numbers of nonspecific pathways of toxicity response (with the exception of 50 μM arsenic). Although there is some overlap in the genes modified between different metals, these data suggest that each metal modifies expression of a largely unique set of genes that may be characteristic of each treatment. Although this microarray does not contain all known metal-responsive genes, the results show metal-specific patterns of expression among the genes examined. No gene was modified by all five chemical treatments, and only *HSP-90A* was modified by all four metals. Only three to seven genes overlapped among any two treatments, and similarly, only a few genes were common to any three treatments. A similar unique pattern of gene expression has been observed in yeast exposed to equitoxic doses of several different alkylating agents (Jelinsky et al. 2000) as well as in rats treated with different classes of drugs (Hamadeh et al. 2002a, 2002b). Likewise, cadmium chloride, benzo[*a*]pyrene and trichloroethylene produced different patterns of gene expression in the livers of exposed mice (Bartosiewicz et al. 2001).

In this study the genes that were altered commonly by more than one treatment were all changed in the same direction, that is, either increased or decreased expression. This supports the idea that these represent biologically relevant responses to these treatments. Cadmium, chromium, and nickel exposures all increased expression of *GLUT1* and decreased levels of transcription activator DB1 (*DB1*), procollagen alpha 2(IV) subunit precursor (*COL4A2*), glutathione peroxidase (*GSHPX1*), hepatoma-derived growth factor (*HDGF*), and cytochrome P450 1B1 (*CYP1B1*). Despite the known ability of Cr(VI) and MMC to cause both monoadducts and cross-links in DNA, only 7 genes were modified in common by both of these agents. A previous 148-gene microarray experiment showed changes in expression of 12 genes in the liver following cadmium exposure (Bartosiewicz et al. 2001). Organ-specific effects as well as differences in the particular genes included in each microarray may explain the lack of overlap between these two studies. Previous studies indicate that 4-hr nickel exposure stabilizes HIF-1α protein resulting in transcriptional activation of hypoxia-inducible genes (Andrew et al. 2001; Salnikow et al. 2000). Consistent with these findings, HIF-1α–inducible genes, including the insulin-like growth factor binding protein (*IGFBP3*) and *GLUT1*, were up-regulated following nickel exposure (Figure 1) (Minet et al. 2001).

In addition to the metal-specific effects, we examined the effect of arsenic dose on gene expression. The lower-dose arsenic exposure (5 μM) modified expression of a wide variety of genes representing a diverse range of protein classes such as transcription factors, inflammatory cytokines, kinases, and DNA repair proteins, as shown previously in human fibroblasts (Yih et al. 2002) and keratinocytes (Bae et al. 2002). The literature supports the observed induction of heme oxygenase (*HO1*) (Menzel et al. 1998; Taketani et al. 1989; Yih et al. 2002) and the transcription factor junD (*junD*) (Liu et al. 2001). In addition, the immunoblot in Figure 3 confirmed that the *HIF-1*α gene expression changes were correlated with higher protein levels. We have also demonstrated dose-dependent increases in HIF-1α protein and mRNA levels in vascular smooth muscle cells (data not shown), suggesting that these effects of low-level arsenic are not confined to a single cell type. Further investigation will be needed to determine the downstream consequences of increases in levels HIF-1α and the other 11 transcription factors induced in response to 5-μM arsenic exposure. Arsenic exposure has also been associated with increased expression of the inflammatory cytokines, interleukin (IL)-6 and IL-8 via a mechanism that may also involve MAP kinase signaling pathways, as well as induction of other cytokines such as IL-12B, IL-7, and IL-2 (Wu et al. 1999).

The decreased expression of genes involved in DNA damage recognition and repair support the hypothesis that arsenic exposure may decrease the ability of exposed cells to recognize and repair DNA damage, potentially contributing to its carcinogenic and co-carcinogenic activity (Abernathy et al. 1999; Hartwig et al. 1997; Hartwig 1998; Rossman et al. 2001; Vogt and Rossman 2001). For example, the following DNA repair genes were altered after arsenic treatment: Xeroderma pigmentosum group D–complementing protein (*XPD*, DNA excision repair protein *ERCC2*), Xeroderma pigmentosum group C–complementing protein (*XPC*), AP endonuclease 1 (*APE1*), DNA ligase-1 (*DNL1*), DNA polymerase delta catalytic subunit (*DPD*), DNA topoisomerase II alpha (*TOP2A*), DNA damage–inducible protein *GADD45* (Chen et al. 2000; Liu et al. 2001), MCM DNA

replication licensing factors 2 and 7 (*MCM2, MCM7*), proliferating cyclic nuclear antigen (*PCNA*), O^6-methylguanine–DNA methyltransferase (*MGMT*), replication factor C large and 40-kDa subunits (*RFC, RFC40*), and uracil–DNA glycosylase precursor (*UNG1*). Other studies in our laboratory using human lymphocytes from an epidemiologic study have demonstrated a dose-dependent correlation between decreased expression of nucleotide excision repair genes and chronic exposure to arsenic in the drinking water (Andrew et al. 2003).

Surprisingly, increasing the dose of arsenic to 50 μM did not simply increase the magnitude of the change in the same set of genes or add additional genes. Rather, we observed a striking shift in the gene response profile between the lower and the higher dose. Exposure to 50 μM arsenic for 4 hr resulted in increased rather than decreased expression of nearly all genes that were modified, including many genes that prepare cells to deal with adverse conditions. Consistent with the concept of high-dose arsenic acting as a heat-shock mimetic, 50 μM arsenic induced a variety of heat-shock proteins [*HSP-40, HSP-71, HSP-70* (Liu et al. 2001), *HSP-60* (Liu et al. 2001), *HSP-27, HSP90A, HSP-70.1*]. Many of the other genes induced in response to higher doses of arsenic are involved in stress response pathways. Higher doses of arsenic increase levels of jun kinases (*JNK3*), possibly via mitogen-activated protein kinase kinases, such as *MAPKK3*, and also activate MAP kinases such as extracellular signal regulated kinase (*ERK3*) (Cavigelli et al. 1996; Liu et al. 1996; Porter et al. 1999; Samet et al. 1998; Wu et al. 1999). Comparison between the microarray and kinase assay results shown in Figure 4 indicates a correlation between protein and gene-level changes in response to arsenic exposure for all genes that were examined in both studies: ERK3, ribosomal S6 kinase 1 (RSK1), cAMP-dependent protein kinase alpha-catalytic subunit (PKACα), and protein kinase B (PKB/akt1). This correlation between gene- and protein-level changes was also seen for PKB/akt following chromium treatment. Further investigation is necessary to determine how the observed changes in kinase expression levels affect signal transduction pathways. These experiments were performed in the BEAS-2B human bronchial epithelial cell line as well as primary pSMC, indicating that the observed changes are universal rather than cell-type or cell-line specific.

This study demonstrates the feasibility of using gene expression profiling to understand toxin-induced biological responses. Overall, the number of genes modified in response to metal exposures was relatively small. Although a few genes were modified in response to more than one metal, each metal largely altered expression of a unique set of genes. The profile of genes induced by high-dose arsenic exposure clearly indicated a stress response, whereas the other nonovertly toxic doses of metals led to more subtle modification of cell signaling pathways. Future work will focus on using these data to explore basic mechanisms of metal toxicity and to generate new hypotheses. We invite other researchers to consider our data (Table 1) from the perspective of their own specialized areas of expertise. These metal response patterns may shed new light on the mechanisms of toxic metal–induced human diseases and may also be useful for development of molecular biomarkers of exposure and/or effect in mechanistic, epidemiologic, and risk assessment studies.

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