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/cm2 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 (hp90), 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, nickel, toxicogenomics, toxicoproteomics. Environ Health Perspect 111:825–838 (2003). doi:10.1289/tex.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 be associated 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 Painé 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 Hechteng 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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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-cm² 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% CO2/95% 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 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% CO2/95% air, and medium was changed 24 hr before treatment.

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 metals 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/cm² of lung surface area (Edelman and Roggli 1989). Nickel refinery workers had much higher levels of nickel in the lung (mean 15 mg Ni/cm²) (IARC 1991).

cDNA array analysis. One confluent flask of >10⁶ 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/cm² of cell culture flask nickel subsulfide (Sigma), or 1 µM miromycin 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 glycoldehyde 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 (RP13L3A), 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 control 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-cm² 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 x 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/cm²), 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; and no gene’s expression was modified by treatment with all four of the metals tested, nickel (as cadmium chloride, 3 µM), chromium (as sodium dichromate, 10 µM), nickel (as nickel subsulfide, 3 µg/cm²), 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.

As shown in Figure 1, although there was some overlap, 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), heptoma-derived growth factor (HDPF), and cytomegalo 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 Cd²⁺ as cadmium chloride), chromium(VI) (10 µM Cr(VI) as sodium dichromate), nickel (3 µg/cm² Ni²⁺ 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. Ubiquitin (Ubiqu) was used as a housekeeping gene. 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 increased 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, PKCα, 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 blotts 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.

**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.

**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...
### Table 1. Relative expression of genes for cells treated with arsenic, chromium, cadmium, nickel, or MMC.

| Gene abbreviation* | Gene name# | Arsenic (5 μM) ratio | Arsenic (50 μM) ratio | Chromium ratio | Cadmium ratio | Nickel ratio | MMC ratio | GenBank* |
|--------------------|------------|----------------------|----------------------|----------------|--------------|--------------|-----------|----------|
| 3pK                | MAPKAP kinase (3pK) | 2.00 |  |  |  |  |  | | U09578 |
| S-HT-3             | 5-Hydroxytryptamine 3 receptor precursor (S-HT-3); serotonin-gated ion channel receptor | 2.00 |  |  |  |  |  | | D49394 |
| A1ATR              | Alpha-1-antitrypsin precursor; alpha-1 protease inhibitor; alpha-1-antitrypsinase | 1.54 | 1.65 |  |  |  |  | | X02920 |
| ABL2               | 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              | ALG2 calcium-binding protein | 2.40 |  |  |  |  |  | | AFO35606 |
| 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; | X86133 |
| AREB6              | Transcription factor AREB6 | 5.00 |  |  |  |  |  | | D15050 |
| ARH6               | Transforming protein rhoB; ARH6 | 1.83 |  |  |  |  |  | | X06820 |
| ATF-3              | Cyclic-AMP-dependent transcription factor ATP-3 (activating factor 3); sodium/potassium-transporting ATPase | 6.33 |  |  |  |  |  | | L18871 |
| ATPB3              | Beta 3 subunit (ATPB3); sodium/potassium-dependent ATPase | 1.83 |  |  |  |  |  | | U51478 |
| B94                | B94 protein | 2.00 | 3.50 | 2.33 |  |  |  | | M02357 |
| BAG-1              | BCL-2 binding anagene-1 (BAG-1); glucocorticoid receptor-associated protein RAP46 | 1.70 |  |  |  |  |  | | S83171; | Z35491 |
| BAX                | Apoptosis regulator bax | 2.11 |  |  |  |  |  | | L24744 |
| 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 |  |  |  |  | | 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; MSMD1 | 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 B | 2.00 |  |  |  |  |  | | L40377 |
| CASP2              | Caspase-2 precursor (CASP2); ICH-1L protease + ICH-1S protease | 4.00 |  |  |  |  |  | | U13021; | U13022 |

(Continued on next page)
| Gene abbreviation | Gene name | Arsenic (5 µM) ratio | Arsenic (50 µM) ratio | Chromium ratio | Cadmium ratio | Nickel ratio | MMC ratio | GenBank* |
|-------------------|-----------|----------------------|----------------------|----------------|--------------|--------------|-----------|----------|
| 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 SUM1 | 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; L28222 |
| CLK1              | CDC-like kinase 1 (CLK1) | 2.78 | | | | | | M74816 |
| CLU                | Clusterin precursor (CLU); complement-associated protein SP-40,40; complement cytolsis inhibitor (CLU); 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 | | | | | | M98343 |
| C/EBF1            | CD40 receptor-associated factor 1 (CRAF1) | 5.00 | | | | | | |
| CREB2             | cAMP-dependent transcription factor ATF-4; DNA-binding protein TAXREB67; cAMP-response element binding protein (CREB2) | 2.62 | 1.72 | 1.58 | | | | D90209 |
| CTXN4A1           | Alpha1 catenin (CTXNA1); cadherin-associated protein; alpha E-catenin | 1.76 | | | | | | |
| Cycin k           | Cyclin K | 2.13 | 2.13 | | | | | AF060515 |
| CYP1              | 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 |
| DFF4S             | 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 | | | | | | |
| DPP-1             | Dipeptidyl-peptidase I precursor (DPP-1); cathepsin C; cathepsin J; dipeptidyl transferase | 1.83 | | | | | | X87212 |
| DRPLA             | Atrophin-1; dentatorubral-pallidolysian atrophy protein (DRPLA) | 3.00 | | | | | | D31840 |
| E16               | E16 amino acid transporter | 2.63 | | | | | | AF077286 |
| E2F-3             | E2F-3 | 2.17 | | | | | | Y10479 |

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| Gene abbreviation | Gene name                       | Arsenic (5 µM) ratio | Arsenic (50 µM) ratio | Chromium ratio | Cadmium ratio | Nickel ratio | MMC ratio | GenBank* |
|------------------|---------------------------------|----------------------|-----------------------|----------------|--------------|--------------|-----------|----------|
|                  |                                 | Up       | Down     | Up       | Down     | Up       | Down     | Up       | Down     |
| EAR2             | v-erbA-related protein (EAR2)   |           |          | 2.08     |          |          |          |          |          | X17294   |
| 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 | M53971 | 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 (ERF-1) | 3.37 | 2.00 | 2.00 | | | | X79067 |
| ERK3             | Extracellular signal-regulated kinase 3 (ERK3); MAP kinase 3 (MAPK3; p97-MAPK); PRKMK5 | 1.70 | 3.92 | | | | | X80692 |
| ETR101           | Transcription factor ETR101     | 4.03     | 3.00     |          |          |          |          | M62631   |
| ETS-1            | Erythroleukemia virus oncogene homolog 1 (ETS-1); p54 | 3.21 | | | | | | J04101 |
| ETv6             | eTS-related protein tel; eTS translation 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 | | | | | | | |
| FRA1             | fos-related antigen (FRA1)      | 8.59     | 1.80     |          |          |          |          |          | X16707   |
| Fsa-1            | fse-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-α) | 2.33 | | | | | | D13316 |
| GADD45           | Growth arrest and DNA-damage-inducible protein 153 (GADD45); DNA-damage-inducible transcript 3 (DDIT3); C/EBP homologous protein (CHOP) | 10.35 | | | | | | S40706 |
| GADD45β          | Growth arrest and DNA-damage-inducible protein 45 beta (GADD45 beta) | 2.60 | 13.88 | | | | | M60974 |
| GALNR1           | Galanin receptor type 1 (GALNR1; GALR1) | | | | | | | |
| GAP              | GAP-associated protein          | 2.00     | | | | | | L34339 |
| GLUT1            | Erythrocyte glucose transporter 1 (GLUT1) | | 1.86 | 1.64 | 1.93 | | | K03195 |
| GNB1             | Guanine nucleotide-binding protein G-i/G-s/G-t beta subunit 2, transducin beta 2 subunit 2 | | 2.50 | | | | | M06429 |
| GRRF1            | Glucocorticoid receptor repression factor 1 | | 2.00 | | | | | M73077 |
| GSHPX1           | Glutathione peroxidase (GSHPX1; GPX1) | | 2.00 | 1.68 | 1.62 | | | Y00483 |
| GSR              | Glutathione reductase (GRase; GSR, GR) | | | | | | | M21304 |
| 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 | Gene name | Arsenic (5 µM) | Arsenic (50 µM) | Chromium | Cadmium | Nickel | MMC |
|-------------------|-----------|----------------|----------------|----------|---------|--------|-----|
|                   |           | ratio | ratio | ratio | ratio | ratio | ratio |
| HATB2             | Histone acetyltransferase B subunit Z; retinoblastoma-binding protein p46; retinoblastoma-binding protein 7 | 1.80 | 1.80 | | | | | | U93143 |
| HBEGF             | Heparin-binding EGF-like growth factor (HBEGF); diphtheria toxin receptor (DTR) | 16.29 | | | | | | | M66278 |
| HDGF              | Hepatoma-derived growth factor (HDGF) | | | 1.67 | 1.55 | 1.55 | | | D16431 |
| HEGR1             | Early growth response protein 1 (HGR1); transcription factor ETR103; KROX24; zinc finger protein Z25; AT2Z25 | 2.36 | 13.00 | | | 2.17 | 1.62 | | X52541; M662629 |
| HEIR-1            | Helix-loop-helix protein HLH 1R2; 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) | | | | | | | | 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-AS            | Homeobox protein HOX-AS; HOX-IC | 1.91 | | | | | | | M36679 |
| 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 | | | | | | | D49547 |
| HSP-60            | Mitochondrial matrix protein P1 precursor; p50 lymphocyte protein; chaperonin homolog, HUCHA20; heat-shock protein 60 (HSP-60); HSP01 | 3.13 | | | | | | | 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); HSPB6 | 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; IGBP3) | | | | | | 2.00 | | | M311159; M39579 |
| 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 | | | | | | | | X58770 |
| 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 | Gene name | Arsenic (5 µM) ratio | Arsenic (50 µM) ratio | Chromium ratio | Cadmium ratio | Nickel ratio | MMC ratio | GenBanka |
|-------------------|-----------|----------------------|-----------------------|----------------|--------------|--------------|-----------|-----------|
| **IL-6**          | Interleukin-6 precursor (IL-6); B-cell stimulatory factor 2 (BSF2); interferon beta-2 (IFNβ2); hybridoma growth factor | 3.11 | 2.78 | 2.45 | | | | X04602; M14564 |
| **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 | | | | | | |
| **ITGA4**         | Integrin alpha 4 precursor (ITGA4); VLA4; CD49D antigen | 2.54 | | | | | | L12002; X16983; X15396 |
| **ITGB4**         | Integrin beta 4 (ITGB4); CD104 antigen | 1.76 | 1.61 | | | | | X53567; J52188; X51841 |
| **JNK**           | c-jun N-terminal kinase 1 (JNKK); JNK activating kinase 1 (JNKK1); MAP kinase kinase 4 (MKK4) | 3.00 | | | | | | L38870 |
| **JUN**           | jun activation domain binding protein | 2.43 | | | | | | U65928 |
| **JUN-D**         | jun-D | 5.67 | | | | | | X86681 |
| **JUP,DP3**       | Junction plakoglobin (JUP); desmoplakin III (DF3) | 2.33 | 1.60 | | | | | M23410; Z00228 |
| **JUN**           | Leukemia inhibitory factor precursor (LIF); differentiation-stimulating factor (D factor); melanoma-derived LIF inhibitor (MLPLI); HILDA | 2.15 | 7.63 | | | | | X13967; M53420 |
| **LIG1**          | DNA ligase I; polydeoxyribonucleotide synthase (ATP) (DNL1) (LIG1) | 2.94 | | | | | | M36067 |
| **LUCA2**         | LUCA2; lysosomal hyaluronidase 2 (HYAL2); PH-20 homolog | 2.11 | | | | | | U69577 |
| **MAO**           | MAD protein, MAX dimerizer | 14.00 | | | | | | L08895 |
| **MAPKAPK2**      | MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2) | 1.50 | 1.50 | | | | | U12779 |
| **MAPKK3**        | Dual specificity mitogen-activated protein kinase 3 (MAP kinase kinase 3; MAPKK3; MMK3); 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 JC; 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 |
| **MGT1**          | 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 | Gene name | Arsenic (5 µM) ratio | Arsenic (50 µM) ratio | Chromium ratio | Cadmium ratio | Nickel ratio | MMC ratio | GenBank |
|------------------|-----------|----------------------|-----------------------|----------------|---------------|--------------|-----------|---------|
|                  |           | Up       | Down    | Up       | Down    | Up       | Down    | Up       | Down    | GenBank  |
| MMP-14           | Matrix metalloproteinase 14 precursor (MMP14); membrane-type matrix metalloproteinase 1 (MT-MMP1); MMP-X1 | 1.67 | D26512; X83535 | 1.80 | X70326 | 7.83 | L13740 | 1.55 | 1.55 | D00099 |
| MRP              | macMARCKS; MARCKS-related protein (MRP); MLP | 1.80 | X70326 | 2.00 | L19067 | 3.00 | U15306 | 1.70 | 1.80 | M81768 |
| NAK1             | Early response protein NAK1; TR3 orphan receptor | 7.83 | L13740 | 1.55 | 1.55 | 2.00 | L19067 | 3.00 | 3.00 | U15306 |
| NaKATPase        | Sodium/potassium-transporting ATPase alpha 1 subunit (Na+/K+-ATPase) | 1.55 | 1.55 | D00099 | 1.55 | 1.55 | 2.00 | L19067 | 3.00 | U15306 |
| NFKB3            | NF-kappaB transcription factor p65 subunit; RELA; NFKB3 | 2.00 | L19067 | 1.70 | 1.80 | 2.00 | L19067 | 1.70 | 1.80 | M81768 |
| NF-X1            | Transcriptional repressor NF-X1 | 3.00 | U15306 | 1.70 | 1.80 | 2.00 | L19067 | 1.70 | 1.80 | M81768 |
| NHE1             | Sodium/hydrogen exchanger 1 (Na+/H+ exchanger 1; NHE1); amiloride-sensitive Na+/H+ antiporter | 1.70 | 1.80 | M81768 | 1.70 | 1.80 | 2.00 | L19067 | 1.70 | 1.80 | M81768 |
| NIP3             | NIP3 (NIP3) | 2.40 | U15174 | 2.40 | U15174 | 2.00 | U15174 | 2.00 | U15174 | M73482 |
| NMBR             | Neuromedin B receptor (NMBR); neuromedin-B-prefering bombesin receptor | 1.76 | U15174 | 1.76 | U15174 | 1.83 | X55504 | 1.83 | X55504 | U15174 |
| NOL1             | Proliferating cell nuclear antigen P120; NOL1 | 1.83 | X55504 | 1.83 | X55504 | 1.75 | Y09689 | 1.75 | Y09689 | U12979 |
| NRGN             | Neurogranin (NRGN); RC3 | 1.75 | Y09689 | 1.75 | Y09689 | 1.74 | U12979 | 1.74 | U12979 | M80359 |
| p15; PC4         | Activated RNA polymerase II transcriptional coactivator p15; PC4 | 1.74 | U12979 | 1.74 | U12979 | 2.36 | M80359 | 2.36 | M80359 | M85456 |
| p78              | p78 putative serine/threonine-protein kinase | 2.36 | M80359 | 2.36 | M80359 | 2.00 | M85456 | 2.00 | M85456 | M85456 |
| PAR-1            | Thrombin receptor (TR); F2R; PAR1 | 2.40 | M62424 | 2.40 | M62424 | 2.00 | M62424 | 2.00 | M62424 | M62424 |
| PBX1             | Pre-B-cell leukemia transcription factor-1; homeobox protein pbx1; Homeobox protein prl | 2.00 | M85456 | 2.00 | M85456 | 1.75 | M62424 | 1.75 | M62424 | M62424 |
| PCNA             | Proliferating cyclic nuclear antigen (PCNA); cyclin | 1.75 | M62424 | 1.75 | M62424 | 2.52 | M85456 | 2.52 | M85456 | M15796; J04718 |
| PDGFA            | Platelet-derived growth factor A subunit precursor (PDGFA; PDGF-1) | 2.52 | M85456 | 2.52 | M85456 | 3.00 | X08374 | 3.00 | X08374 | L36151 |
| PI4K-α           | Phosphatidylinositol 4-kinase alpha (PI4-kinase; PTINKS-4-kinase; PI4K-alpha) | 3.00 | X08374 | 3.00 | X08374 | 2.86 | L36151 | 2.86 | L36151 | U12979 |
| PI4PK            | 88-kDa type I phosphatidylinositol-4-phosphate 5-kinase alpha (PTINKS(4P)-5-kinase); 1-phosphatidylinositol-4-phosphate kinase; diphosphoinositide kinase | 2.86 | L36151 | 2.86 | L36151 | 2.33 | X07767 | 2.33 | X07767 | X07767 |
| PKAαx            | cAMP-dependent protein kinase alpha-catalytic subunit (PKA C-alpha) | 2.33 | X07767 | 2.33 | X07767 | 1.74 | M63167 | 1.74 | M63167 | M63167 |
| PKB/akt          | rac-alpha serine/threonine kinase (rac-PK-alpha); protein kinase B (PKB); c-akt; akt | 1.74 | M63167 | 1.74 | M63167 | 1.53 | M63167 | 1.53 | M63167 | M63167 |
| PLCG1            | Phospholipase C gamma 1 (PLC-gamma 1; PLCG1); 1-phosphatidylinositol 4, 5-bisphosphate phosphodiesterase gamma 1; PLC-II; PLC-148 | 2.11 | M63167 | 2.11 | M63167 | 3.00 | X98093 | 3.00 | X98093 | M34687 |
| PN-II            | Alzheimer’s disease amyloid A4 protein precursor; protease nexin-II (PN-II); APP | 1.74 | Y00264 | 1.74 | Y00264 | 1.86 | Y00264 | 1.86 | Y00264 | X98093 |
| POLG             | DNA polymerase gamma (POLG); mitochondrial DNA polymerase catalytic subunit (MDP1) | 3.00 | X98093 | 3.00 | X98093 | 1.65 | M63960 | 1.65 | M63960 | M63960 |
| PP-1A            | Serine/threonine protein phosphatase PP1-alpha 1 catalytic subunit (PP-1A) | 1.65 | M63960 | 1.65 | M63960 | 3.71 | U48296 | 3.71 | U48296 | U48296 |
| PRL-1            | PTPCAAX1 nuclear tyrosine phosphatase (PRL-1) | 1.82 | U48296 | 1.82 | U48296 | 3.71 | U48296 | 3.71 | U48296 | U48296 |
| Prot.c8          | Proteasome component C8; macropain subunit C8; multicalytic endopeptidase complex subunit C8 | 1.80 | D00762 | 1.80 | D00762 | 1.80 | D00762 | 1.80 | D00762 | D00762 |

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Table 1—Continued.

| Gene abbreviation | Gene name | Arsenic (5 µM) | Arsenic (50 µM) | Chromium | Cadmium | Nickel | MMC |
|-------------------|-----------|----------------|----------------|----------|----------|--------|------|
|                   |           | ratio Up       | ratio Down     | ratio Up | ratio Down | Up     | Down |
| PRP               | Major prion protein precursor (PRP); PRP27-30, PRP33-35C, ASCR | 2.05 | 2.54 | 1.62 | 1.90 | M13667 |
| PTMS              | Parathyminosin | 2.10 | | | | M24398 |
| RAP-1B            | ras-Related protein RAP-1B; GTP-binding protein SMG p21B | 2.25 | X0B004 |
| RFC               | Activator 1 140-kDa subunit (A1 140-kDa subunit); replication factor C large subunit; DNA-binding protein PO-QA | 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 | M87339 |
| Rho-GDI           | rho GDP dissociation inhibitor 1 (RHO-GDI 1); RHO-GDI alpha (GDIA1); ARHGDIA | 1.81 | 1.67 | X69950 |
| 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) (RP-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); neoplasms-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 |
| Stratfin          | 14-3-3 Protein sigma; stratfin; epithelial cell marker protein 1 | 1.88 | AF029082 |
| Synapsin          | Synapsin IIIA | 1.90 | AF046873 |
| TAFAP-2           | Transcription factor AP-2 (TAFAP-2; AP2Tf) | 1.80 | M36711 |
| TIF1              | Transcription intermediary factor 1 (TIF1) | 1.80 | AF009353 |
| tsk1              | Tyrosine kinase tsk1 | 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 |
| TPRAP             | TPRAP protein | 2.00 | 1.75 | AF076974 |
| TSC2              | Tuberin; tuberous sclerosis 2 protein (TSC2) | 2.22 | X76521 |
| TST               | Thiosulfate sulfurtransferase; rhodanese | 5.00 | D87292 |
| TTR               | Transthyretin precursor (TTR); prealbumin; TBPA | 1.89 | K02091 |
| TUK2              | tyk2 non-receptor protein tyrosine kinase | 1.86 | X56367 |
| Ubiquitin         | Ubiquitin | 2.03 | 7.94 | 2.16 | 1.82 | M28880 |
| UNG1              | Uracil-DNA glycosylase precursor (UNG1) | 5.57 | X15653 |
| UPAR              | Urokinase-type plasminogen activator receptor GPI-anchored form precursor (UPAR); monocye activation antigen MO3; CD87 antigen | 1.62 | U08839; M83246; X51675 |
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 a few genes were common to any two 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), procoagulation alpha 2(IV) subunit precursor (COLA4A2), 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 monoaadducts 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-1α 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 (DNLI), DNA polymerase delta catalytic subunit (DPD), DNA topoisoaerase II alpha (TOP2A), DNA damage–inducible protein GADD45 (Chen et al. 2000; Liu et al. 2001), MCM DNA

| Gene abbreviation | Gene name | Arsenic (5 µM) | Arsenic (50 µM) | Chromium | Cadmium | Nickel | MMC |
|-------------------|-----------|---------------|---------------|----------|----------|--------|------|
| VEGF              | Vascular endothelial growth factor precursor (VEGF), vascular permeability factor (VPF) | 5.86 | | | | | |
| XPC               | DNA-repair protein complementing XP-C cell line produced different complementing protein (p125) | 5.00 | | | | | |
| XPD               | Xeroderma pigmentosum group D complementing protein (XPD); DNA excision repair protein ERCC2 | 2.33 | | | | | |
| YWHA1             | 14-3-3 protein eta; protein AS1; YWHAH; YWHA1 | 2.89 | 1.50 | | | | |
| Zyxin + zyxin-2   | | 2.25 | 3.00 | | | | |

*Information from GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html).
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 arsenic exposure clearly indicated a stress response, whereas the other nonoverly 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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