

Research Article

Identification of Human Cell Responses to Hexavalent Chromium

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Hexavalent chromium [Cr(VI)] is a recognized environmental toxin with ubiquitous distribution in industrialized societies. Its concentration in ambient air derives from several sources including but not limited to chemical processes, the burning of fossil fuels and the production of cement. It is a food contaminant because of its deposition into bodies of water. The majority of published studies on the effects of Cr(VI) concern animal models and these studies have shown that it can induce a variety of cytotoxic and genotoxic reactions that affect the immune system. In order to identify the specific cellular impact of Cr(VI) on humans, we studied its effect on protein production and gene expression in human peripheral blood mononuclear cells (PBMC) obtained from both men and women of each major ethnic group including Caucasians, Hispanics, Asians and African-Americans. High-throughput protein profiling using bead-based

protein arrays showed a concentration-dependent biphasic effect of Cr(VI) on the expression of many cytokines and chemokines by activated PBMC. High-density oligonucleotide microarray analysis identified several functional families of genes including those involved in immune response, intracellular signaling, cell cycle, apoptosis, RNA transport and binding, organelle organization and biogenesis that were strongly affected by Cr(VI). Cr(VI) suppressed many cellular receptor genes involved in immune response and activated many cell cycle-related and proapoptotic genes. These results defined responses that were unique to Cr(VI). This methodology defined an effective manner for identifying injurious/toxic human exposures to Cr(VI) at the cellular level that may facilitate the identification and monitoring of efficacious treatments for Cr(VI)-related maladies. *Environ. Mol. Mutagen.* 48:650–657, 2007. © 2007 Wiley-Liss, Inc.

Key words: cytokines; chemokines; gene expression profiling; peripheral blood mononuclear cells; immune response

INTRODUCTION

Chromium (Cr) is a heavy metal that exists primarily in trivalent Cr(III) and Cr(VI) forms. Cr(VI) is a widely used industrial chemical and is a recognized occupational carcinogen that is released by many industries including chrome plating, stainless steel production, welding, chrome pigmenting, and leather tanning [Grevatt, 1998]. High doses of Cr(VI) or chronic exposures can induce a variety of cytotoxic and genotoxic effects that affect the immune system. Chromium enters the body through inhalation, the gastrointestinal tract, and the skin [Costa and Klein, 2006]. Inhalation is the most common route of occupational exposure of Cr(VI) and is associated with an increased risk of lung cancer. Dermal exposures to chromium may induce irritant and allergic contact dermatitis [Shrivastava et al., 2002]. Upon entering the cell, Cr(VI)

is reduced to Cr(III) resulting in the formation of reactive intermediates, which contribute to cytotoxicity, genotoxicity, and carcinogenicity of Cr(VI) compounds [Shrivastava et al., 2002; O'Brien et al., 2003]. In contrast, Cr(III) compounds are essential micronutrients involved in carbo-

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hydrate and lipid metabolism. They have been shown to promote the action of insulin [Vincent, 2004; Guerrero-Romero, 2005].

It is widely accepted that the central and unifying factor in regard to the toxicity and carcinogenicity of transition metals is the generation of reactive oxygen species by the Fenton reaction leading to oxidative stress, DNA lesions, DNA-protein, and DNA-DNA cross links [Valko et al., 2005]. The extensive DNA damage results in the activation of the p53 pathway, cell-cycle arrest, and apoptosis [Carlisle et al., 2000a,b; Bagchi et al., 2001; Wang and Shi, 2001]. Although this concept regarding the mechanism of toxicity and genotoxicity of Cr(VI) compounds has received much attention, a number of findings have suggested that non-Fenton-like pathways, such as the formation of chromium-DNA adducts and the generation of reactive carbon-based radical species may also produce secondary toxicological effects [O'Brien et al., 2003; Valko et al., 2005].

Regardless of the mechanism, Cr(VI) exposure has pleiotropic effects on the cell. Besides mutagenic and transforming effects, chromium treatment inhibits DNA replication, transcription, translation, mitochondrial respiration, and other cellular processes. [O'Brien et al., 2003]. Evidence shows that at least some of these effects are independent of DNA damage. Workplace and indirect exposures from chromium have been documented to cause numerous health effects, including allergic skin reactions, skin irritation, skin ulcers, and irritations of the mucous membranes that line the upper respiratory tract and lungs. The determination of a body burden or injurious effect from chromium has, in the past, relied upon serum chromium measurements, which are imprecise for documenting long-ago or chronic exposures to chromium. Moreover, because the injurious effects of Cr(VI) are often not recognized until the disease has advanced, the need for identifying subclinical effects as well as the effects of low-dose exposures to chromium is vital.

Advances in high-throughput gene microarray technology have made it possible to analyze the expression of many genes in the human genome thereby allowing systematic analyses of cellular responses to chemical agents. Statistical analyses of large data sets generated in such studies may identify cellular processes and pathways that can lead to the comprehensive understanding of the biological effects of environmental hazards. It is well established that the mutagenic and transforming effects of chromium are observed under conditions that are toxic to the cells. However, exposure doses, which occur at below cellular toxicity levels, can remain hazardous to humans, as were proven by particular epidemiological studies [Costa and Klein, 2006]. Gene expression analyses of the cells of the immune system, which were exposed to low levels of toxicants, may detect early changes in the affected cells and identify "molecular signatures" of such

exposures. Therefore, we analyzed gene expressions in peripheral blood mononuclear cells (PBMC) exposed to low doses of Cr(VI).

MATERIALS AND METHODS

Isolation and Culture of Peripheral Blood Mononuclear Cells

We collected 20–30 ml of blood from eight healthy volunteers by venipuncture and diluted it with equal volumes of Hank's balanced salt solution (HBSS, GIBCO-BRL). Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. Blood samples were layered on top of Ficoll (Histopaque, Sigma) and centrifuged at 1,200 rpm at room temperature for 20 min. PBMC were harvested from the interphase. Cells were washed in HBSS and their viability was determined using Trypan-blue staining. Cells were cultured in triplicate at a density of 1×10^6 cells/ml in complete RPMI medium supplemented with antibiotics and 10% fetal bovine serum. We added 1 μ g/ml phorbol-12-myristate-13-acetate (PMA, Sigma) in combination with 1 μ g/ml of ionomycin (Calbiochem) and/or various concentrations of sodium bichromate (Sigma) to some samples. After culturing PBMC for 18 h, cells were centrifuged at 2,000 RPM for 5 min and the supernatant was removed and placed at -80°C for storage. The cells were either immediately lysed for RNA extraction or placed at -80°C for storage.

Collection of Cells for RNA Isolation and Cell Lysis

RNA was isolated using Trizol Reagent (Invitrogen) according to the manufacturer's instructions. Briefly, 500 μ l of Trizol was added to the tube and the cells were lysed by pipetting. We added 100 μ l of chloroform to the lysates, and the samples were shaken for 15 s. The tubes were centrifuged at 15,000g for 10 min, and the aqueous phase was collected. RNA was precipitated from the aqueous phase with 250 μ l of isopropanol, the RNA pellets were washed with 500 μ l of 70% ethanol, dried, and dissolved in 100 μ l of RNA-free water. Total cellular RNA was isolated using RNeasy columns (Qiagen, Valencia, CA), according to the manufacturer's instructions. The RNA concentration was determined by the absorbance at 260 nm and its quality was assessed by agarose gel electrophoresis. RNA samples were stored at -80°C .

Cytokine Assay

Concentrations of 15 cytokines and chemokines in blood plasma as well as in culture supernatants were determined by a multiplex bead immunoassay using a BioPlex fluorescence bead reader (BioRad) and following manufacturer's instructions. Three panels of antibody-conjugated beads (BioSource) for measuring GM-CSF, IL-1 β , IL-6, IL-8, and TNF- α as proinflammatory molecules, IFN- γ , IL-2, IL-4, IL-5, and IL-10 as Th1/Th2 cytokines, and MIP-1 α , MIP-1 β , MCP-1, Eotaxin, and RANTES as proinflammatory chemokines were used in the experiments.

Gene Expression Analysis

An oligonucleotide-based Affymetrix Human U133A GeneChip Array was used for the gene expression analysis. The array, which contains over 22,000 probe sets, represents 18,400 transcripts, which are derived from $\sim 14,500$ well-characterized human genes. All labeling reactions and hybridizations were carried out according to the Affymetrix GeneChip[®] eukaryotic target labeling protocol. Test hybridizations were performed using "Test3" arrays (Affymetrix, Santa Clara, CA) to insure the quality of the biotinylated probe sets. Bound probes were detected by laser excitation of the fluorescent markers and scanning of the resultant

TABLE I. Effect of Cr(VI) on Secretion of Extracellular Cytokines and Chemokines by PBMC

	Plasma	Media	Cr(VI)		
			0.2 μ M	1 μ M	10 μ M
GM-CSF	132 \pm 15	<20	<20	<20	<20
IFN- γ	20.2 \pm 1.2	<10	<10	<10	<10
IL-1 β	<100	<50	<50	<50	<50
IL-2	<10	<5	<5	<5	<5
IL-4	81.5 \pm 4.1	<10	<10	<10	<10
IL-5	<5	<3	<3	<3	<3
IL-6	11.2 \pm 0.6	4.3 \pm 0.2	<5	<5	<5
IL-8	17.7 \pm 1.1	144 \pm 28	97.1 \pm 51.7	73.8 \pm 17.3	100 \pm 27
IL-10	21.7 \pm 1.8	<3	<3	<3	<3
TNF- α	<20	<20	<20	<20	<20
MIP-1 β	79.0 \pm 3.9	41.6 \pm 3.0	32.5 \pm 15.8	23.5 \pm 10.2	21.4 \pm 1.1
MCP-1	173.2 \pm 9.6	80.8 \pm 30.6	36.2 \pm 10.0	19.1 \pm 1.0	32.9 \pm 5.4
Eotaxin	143.6 \pm 7.2	6.8 \pm 0.3	7.4 \pm 0.8	9.6 \pm 0.8	25.1 \pm 2.2
MIP-1 α	160.5 \pm 7.6	38.4 \pm 1.9	37.3 \pm 1.9	40.3 \pm 5.7	72.7 \pm 5.0
RANTES	13087 \pm 654	383 \pm 19	448 \pm 50	555 \pm 73	1958 \pm 98

Concentrations of proteins (pg/ml) were determined in the plasma as well as in the supernatants of PBMC cultured in the presence of sodium bichromate at indicated concentrations. "Media" corresponds to un-stimulated PBMC cultures. Means and standard deviations from at least three independent experiments are shown.

emission spectra was accomplished using a scanning confocal laser microscope (Probe Array Scan, Agilent Technologies). Data acquisition was performed using GCOS (Affymetrix GeneChip Operating Software Package). Data normalization, background correction, and all subsequent statistical tests for significant differential expressions were performed using an S+Array Analyzer statistical software package (Insightful). CEL files were exported from GCOS into the ArrayAnalyser module of the package and used for calculation of the Affymetrix expression summary (RMA method, quintiles normalization). Student's t-test was applied to calculate statistically significant differentially expressed genes. Raw *P* values were corrected for FDR (false discovery rate) using the Benjamini and Hochberg procedure [Benjamini and Hochberg, 1995]. Only genes with FDR < 0.05 were used for further analysis.

Differentially expressed transcripts were initially annotated by using the NetAffx Analysis Center (<http://www.affymetrix.com>) according to the most up to date version of the Gene Ontology Database (<http://www.geneontology.org/>). Further biological annotation was performed using the DAVID web-based functional annotation tool (<http://david.abcc.ncifcrf.gov>) and the PathwayStudio functional annotation software package (Ariadne Genomics).

RESULTS AND DISCUSSION

Effect of Cr(VI) on Expression of Extracellular Cytokines

Cr(VI) is an industrial and environmental compound with many recognized adverse health effects. It is vital to determine when injurious Cr(VI) exposures have occurred long before the appearance of overt clinical signs and symptoms in humans. Because these injurious effects most certainly develop in a discrete cellular manner, developing a methodology to find and categorize these cellular effects is crucial.

Although much data has been gained on the effects of Cr(III) on the immune system, very little information on the effects of Cr(VI) has been accumulated [Shrivastava et al., 2002]. In this study, our aim was to identify bio-

markers for chronic exposure to chromium. This has several advantages over looking at only the early markers. For example, unlike in vivo where the cytokine levels dramatically decrease because of their binding to cognate receptors expressed on various cells, dilution in the body fluids or degradation, in the current approach, we can identify a range of cytokines that will be released and accumulated in the culture supernatant allowing for a more comprehensive analysis. To better understand how chromium exposure impacts the immune system, we investigated the effects of Cr(VI) on PBMC. To determine the long-term effects of chromium exposure, we identified changes in the gene expression patterns and selected cytokine and chemokine expressions after 18 h of incubation. The cells were cultured in the medium alone or in the presence of 0.2, 1, and 10 μ M levels of sodium bichromate. Cell viability was monitored by propidium iodide staining followed by FACS analysis, and we consistently found ~90% of the cells to be healthy (not shown). The cellular response was analyzed by measuring the secretion of 15 cytokines and chemokines using a multiplex bead array system. Table I shows the concentrations of individual cytokines and chemokines in the media after incubation with or without Cr(VI). No change in the production of inflammatory cytokines was observed upon the addition of chromium (the concentrations of most of those cytokines were below the detection limit). In contrast, the secretion of chemokines varied. Three chemokines, Eotaxin, MIP1- α , and RANTES, showed dose-dependent increases in secretion, while MIP-1 β secretion was inhibited by Cr(VI) in a concentration-dependent manner. The changes in concentrations of two chemokines, IL-8 and MCP-1, showed a biphasic pattern. Although a decrease in chemokine production at lower Cr(VI) (0.2 and 1 μ M

TABLE II. Effect of Cr(VI) on Mitogen-Stimulated Expression of Extracellular Proteins

	Media	PMA/ionomycin			Cr(VI)	
		Media	0.2 μ M Cr(VI)	<i>P</i> value	1 μ M	10 μ M
GM-CSF	<20	1224 \pm 94	1162 \pm 64		779 \pm 43	308 \pm 26
IFN- γ	<10	4446 \pm 301	4506 \pm 272		4022 \pm 351	1799 \pm 177
IL-1 β	<50	277 \pm 14	310 \pm 12	0.037	236 \pm 16	160.4 \pm 8.0
IL-2	<5	22870 \pm 1140	21650 \pm 1180		21780 \pm 1090	13512 \pm 734
IL-4	<10	98.7 \pm 5.0	132.2 \pm 6.6	0.003	100.3 \pm 3.6	62.8 \pm 6.8
IL-5	<3	111 \pm 10	123.7 \pm 4.5		100.1 \pm 4.0	46.7 \pm 2.3
IL-6	4.3 \pm 0.2	986 \pm 102	960 \pm 48		696 \pm 127	171 \pm 24
IL-8	144 \pm 28	7261 \pm 545	8033 \pm 402		7357 \pm 755	1916 \pm 305
IL-10	<3	410 \pm 20	436 \pm 45		373 \pm 14	144 \pm 16
TNF- α	<20	12890 \pm 436	20390 \pm 3440	0.021	14370 \pm 1740	7330 \pm 450
MIP-1 β	41.6 \pm 3.0	14580 \pm 1970	16530 \pm 1110		17560 \pm 1570	7150 \pm 650
MCP-1	80.8 \pm 30.6	612 \pm 27	628 \pm 31		378 \pm 37	54.1 \pm 7.1
Eotaxin	6.8 \pm 0.3	47.7 \pm 2.4	49.6 \pm 2.5		50.6 \pm 1.4	43.1 \pm 2.2
MIP-1 α	38.4 \pm 1.9	6836 \pm 342	7297 \pm 365		7042 \pm 352	2285 \pm 118
RANTES	383 \pm 19	9592 \pm 480	11676 \pm 459	0.006	11650 \pm 580	11410 \pm 570

PBMC from a donor were stimulated with PMA and ionomycin and cultured in the presence of sodium bichromate as described under Materials and methods. *P* values correspond to the change in the expression of extracellular cytokines in cells treated with 0.2 μ M Cr(VI) compared to PMA/ionomycin alone. Only *P* values \leq 0.05 are shown. For other details, see Table I.

sodium bichromate) concentrations was noted, the levels were increased when exposed to a higher dose (10 μ M) of sodium bichromate.

Biphasic Effect of Cr(VI) Exposure on Cytokine Secretion by Mitogen-Activated Cells

Earlier studies have demonstrated that Cr(VI) ions have a biphasic effect on the function of the cells of the immune system both in vitro and in vivo, inducing stimulation at low doses and inhibition at high doses. For example, Cr(VI) ions stimulated the phytohemagglutinin-induced blastogenesis of human lymphocytes at low concentrations (0.01–1 μ M) and inhibited it at concentrations higher than 1 μ M [Borella et al., 1990]. Low chromium (VI) concentrations also stimulated the phagocytic activity of alveolar macrophages in exposed rats and increased the humoral immune responses; while higher Cr(VI) doses depressed both functions [Yucesoy et al., 1999]. Therefore, we investigated the effects of chromium exposure on leukocyte function. We treated mitogen-activated cells with hexavalent chromium and monitored the response. PBMC activation by phorbol-12-myristate-13-acetate (PMA) in combination with ionomycin stimulates all types of cells found in PBMC and causes an increase in the production of most cytokines and chemokines [Jason and Larned, 1997; Kurtzhals et al., 1995; Tsuchida and Sakane, 1988]. Thus, we activated PBMC by PMA-ionomycin mixture in the presence of sodium bichromate and measured the cytokine production. As shown in Table II, the addition of these agents stimulated the secretion of all cytokines and chemokines tested resulting in a dramatic increase in cytokine levels in the medium by 10 to several

thousand-fold. The presence of 0.2 μ M sodium bichromate had stimulatory effects on the extracellular expression of proinflammatory cytokines IL-1 β , TNF- α , Th2-type cytokine IL-4, and chemokine RANTES. An increase in the chromium concentration to 1 μ M decreased the expression of most cytokines and chemokines with the exception of IL-2, MIP-1 β , Eotaxin, and RANTES. Chromium (10 μ M) strongly inhibited, by 2- to 10-fold, the secretion of all cytokines and most chemokines. Eotaxin and RANTES were the only chemokines whose expression remained unchanged. The observed dose-dependent changes in cytokine levels are consistent with the biphasic effect of Cr(VI) on PBMC. They correlated with the previous studies [Borella et al., 1990; Yucesoy et al., 1999]. Similar biphasic effects of Cr(VI) on mitogen-mediated lymphocyte proliferation and cytokine production by PBMC was recently observed in a group of industrial workers [Mignini et al., 2004]. In addition, this approach allowed us to determine the effects of exposure to lower doses of chromium that were not apparent when directly tested against unstimulated PBMC (Table I).

Gene Expression Profiling in Cr(VI)-Exposed Cells

To develop a more comprehensive understanding of the effects of low doses of Cr(VI) on the immune system, we performed a large-scale gene expression profiling in chromium-exposed PBMC using high-density microarrays. We determined the gene expression profile in PBMC cultured in the presence of 0.2 μ M sodium dichromate and identified genes that were differentially expressed relative to those in untreated cells. Gene expression was analyzed using Affymetrix GeneChip Human Genome U133A 2.0

arrays representing 18,400 transcripts including those representing 14,500 well-characterized human genes. Significantly altered gene expression in PBMCs, due to their exposure to chromium, was identified by the Student's t-test. A total of 1,659 genes with significantly altered expression (FDR < 0.05) were identified in five PBMC samples, treated with 0.2 μ M sodium dichromate, and compared to eight untreated samples, which represented 11.4% of the total number of genes in the array. Of those genes, 867 were downregulated and 792 were upregulated (for a complete list of the genes, see the online supplementary material).

Once the affected genes were identified, those that showed statistically significant differential expression were further analyzed to elucidate biological and molecular functions most affected by the treatment. A list of differentially expressed transcripts was exported into the web-based application DAVID (Database for Annotation, Visualization, and Integrated Discovery at <http://david.abcc.ncifcrf.gov>), annotated, and grouped according to Gene Ontology (level 3 of Biological Process and Molecular Function) and KEGG Pathway-based terminology. Modified Jackknife Fisher exact probability scores were calculated to assess the statistical significance of the enrichment of the functional groups with differentially expressed genes. A selected list of functional groups identified as significantly enriched for differentially expressed genes, and the corresponding *P* values are shown in Table III.

Several functional families of genes were strongly affected by Cr(VI) including cellular metabolism, immune response, intracellular signaling, apoptosis, RNA transport and binding, organelle organization, and biogenesis, as well as transition metal binding. Cr(VI) ions have a dramatic effect on genes related to the immune response. We observed a significant suppression of many cellular receptor genes including *CD163* (hemoglobin scavenger), *MRC1* (pathogen recognition), *CD93* (complement binding), *SLAMF8* (B-cell activation), *STAB1* (pathogen binding), *VSIG4* (complement binding), *CD36* (microbial binding), *CCR1* (MIP1- α /RANTES receptor), *LY96* (TLR4 cofactor), *FCGR1A* (IgG receptor), *CSF1R* (M-CSF receptor), *CD14* (binding of apoptotic cells), *LAIR1* (leukocyte-associated immunoglobulinlike receptor), *LILRB2* (leukocyte immunoglobulinlike receptor), *LST1* (activating NK receptor), and others. Of the 60 genes that were downregulated by more than 3.5-fold in all Cr(VI) samples, at least 19 genes represented cellular receptors and receptor cofactors, and at least 10 other genes coded for other proteins involved in the cellular immune response. For example, the *CCL2* gene encoding chemokine MCP1 was the second in the list of most downregulated genes. Although MCP1 secretion was suppressed by only 20% in Cr(VI)-treated cells (Table I), the expression of the corresponding gene was suppressed almost 40-fold. A significant number of downregulated genes belonged to the GTPase-mediated signal transduction and protein kinase family of genes.

Cr(VI) treatment had a stimulatory effect on the expression of certain cell-cycle genes, including cyclins E2, G1, T2, L1, and D3; cell division cycle genes *CDC5L* and *CDC14A*; and gene encoding CDC-kinases 1, 3, and 4 as well as CDC2-related kinase CRKRS. Expression of many other genes encoding cell-cycle regulatory proteins, such as CDC28 protein kinase regulatory subunit CKS2, cyclin-dependent kinase inhibitor CDKN1B, cyclin B1 interacting protein CCNB1IP1, cyclin D binding myb-like transcription factor DMTF1, and others also increased. In contrast, suppression of cell-cycle regulator RB1 and genes encoding growth-arrested cell-specific proteins GAS7 and GAS2L1 was observed. An increase in the expression of many cell-cycle-related genes was accompanied by changes in the expression of apoptotic-related genes and might reflect an early onset of apoptosis. For example, we observed the activation of caspase 4 gene *CASP4* and *TNFRSF10B*—a gene that encodes a member of the tumor necrosis factor receptor (TNFR) family. We noted upregulation of two other genes that encode the Map-kinase activating death domain MADD [Schievella et al., 1997] and the RIPK1 serine/threonine kinase [Hsu et al., 1996]. These two proteins interact with TNFR. We also observed the induction of other proapoptotic genes, such as the *STK17B* gene, encoding apoptosis-inducing serine/threonine protein kinase [Sanjo et al., 1998] as well as the death inducer-obliterator gene *DIDO1* [Garcia-Domingo et al., 2003]. These findings as well as suppression of the gene encoding apoptosis inhibitory protein BIRC1 correlate well with the Cr(VI)-mediated induction of apoptosis observed previously [O'Brien et al., 2003].

Cr(VI) also has a dramatic effect on cellular metabolism, particularly nucleobase, nucleoside, nucleotide and nucleic acid metabolism, and adenylyl nucleotide binding. Cr(VI) also affects phosphorus metabolism including kinase and phosphotransferase activity as well as phosphoanhydride hydrolase activity. Other previous reports have found similar effects of Cr(VI) on gene expression in human epithelial cell lines [Ye and Shi, 2001; Andrew et al., 2003].

Cluster Analysis of Gene Expression

Recent studies have demonstrated that the PBMC response to Cr(VI) treatment depends on the genotype of individuals, particularly on the presence of a specific haplotype of human leukocytes antigen (HLA) [Mignini et al., 2004]. We utilized a diverse group of donors, of both genders representing four major ethnic groups including, Caucasian, African-American, Hispanic, and Asian. To understand the impact of genetic diversity on cellular responses to Cr(VI) treatment, we performed hierarchical clustering of Cr(VI)-treated and untreated samples based on the expression of the top 500 genes identi-

TABLE III. Functional Groups Over Represented with Differentially Expressed Genes

Term	Number of genes	P value
BIOLOGICAL PROCESS		
Response to pest, pathogen or parasite	79	2.28E-08
Intracellular signaling cascade	123	2.83E-06
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	317	1.90E-05
Programmed cell death	68	3.49E-05
Inflammatory response	33	6.02E-05
Organelle organization and biogenesis	92	8.43E-05
Establishment of RNA localization	12	3.95E-04
Humoral immune response	24	0.001795
Vesicle-mediated transport	43	0.002031
Nucleobase, nucleoside, nucleotide and nucleic acid transport	12	0.002165
Intracellular transport	64	0.002275
Regulation of cell cycle	52	0.002286
Establishment of cellular localization	64	0.003033
Immune response	107	0.004685
Protein metabolism	292	0.005473
Regulation of programmed cell death	40	0.006619
Generation of precursor metabolites and energy	71	0.007046
Regulation of caspase activity	7	0.009006
Regulation of protein metabolism	26	0.013035
Phosphorus metabolism	88	0.014252
Hydrogen transport	14	0.016729
Cellular defense response	15	0.017986
Hemostasis	14	0.022964
Organic acid transport	12	0.029816
Detection of pest, pathogen or parasite	4	0.03034
Wound healing	14	0.030777
Positive regulation of hydrolase activity	6	0.046895
MOLECULAR FUNCTION		
Adenyl nucleotide binding	147	1.17E-05
Hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	68	3.25E-04
IgG binding	5	7.01E-04
Transcription cofactor activity	34	9.83E-04
Guanyl-nucleotide exchange factor activity	19	0.004399
SH3/SH2 adaptor activity	10	0.0047
RNA cap binding	4	0.005812
RNA splicing factor activity, transesterification mechanism	6	0.006586
Kinase binding	12	0.008904
Transition metal ion binding	210	0.010399
Nuclease activity	20	0.020505
SH2 domain binding	5	0.021973
Single-stranded RNA binding	4	0.028233
Rho GTPase activator activity	5	0.033067
Kinase activity	89	0.033943
Interferon binding	3	0.042408
Phosphotransferase activity, alcohol group as acceptor	71	0.045489
Chemokine binding	6	0.04843
MOLECULAR PATHWAY		
Fructose and mannose metabolism	11	0.005118
Antigen processing and presentation	15	0.007199
Natural killer cell mediated cytotoxicity	21	0.009147
T cell receptor signaling pathway	16	0.018889
Aminosugars metabolism	8	0.020728
Leukocyte transendothelial migration	18	0.030802
ER-associated degradation (ERAD) Pathway	5	0.045234

The analysis is based on the total number of 1,659 genes that showed altered expression in cells treated with 0.2 μ M sodium dichromate. See online supplemental material for the complete and detailed list of differentially expressed genes.

fied by the Student's t-test. As shown in Figure 1, the samples were grouped into two clusters showing good separation from each other, one containing all treated

samples and the other containing all untreated samples. Within each of the two clusters, sample subgroups were closely related. Although more individual variability in

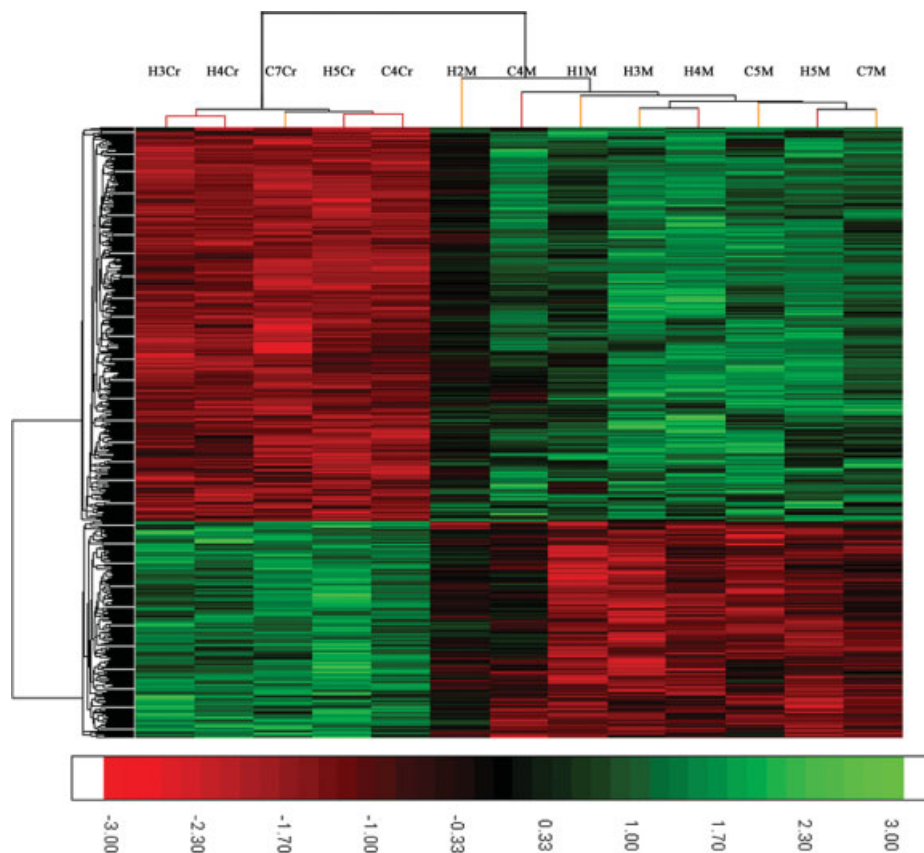


Fig. 1. Hierarchical clustering of Cr(VI)-treated and untreated samples. Cluster analysis was based on the top 500 most significant differentially expressed genes identified by the Student's *t*-test in five Cr(VI)-treated samples H3Cr, H4Cr, C7Cr, H5Cr, and C4Cr compared to eight untreated samples: H3M, H4M, C7M, H5M, C4M, H1M, H2M, and

C5M. H1Cr, H2Cr, and C5Cr samples did not pass the quality control tests and were excluded from the analysis. Red color denotes down-regulated genes; green color depicts the upregulated genes in a particular sample. Numbers indicate fold change in gene expression.

the gene expression was observed in the cluster of untreated samples, little variation was observed within the group of treated samples suggesting very similar responses to Cr(VI) treatment from cells obtained from different individuals.

CONCLUSIONS

We have detailed the discrete and unique responses of human cells to low doses of Cr(VI). These serve as a guidepost for ascertaining when exposures to Cr(VI) may become injurious. These cellular markers categorize when such exposures have induced human health effects, which may equate to an accurate and definite diagnosis. We utilized a spectrum of human cells taken from both genders and from multiple ethnicities to accurately identify responses. Detection of injurious consequences at a cellular level will allow us to establish the toxic characteristics of a chemical. Our approach has been to find and reproduce those cellular markers. Similar to this study, in an earlier study, we have studied the cause-and-effect relationships

of exposure to benzene and benzene metabolites [Gillis et al., 2007]. This DNA-based approach relies on the most sensitive of detection techniques. We consequently conclude that this approach could replace a past reliance on detection methods such as serum measurements, which are incapable of detecting low-dose exposures.

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