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Gene expression profile in response to chromium-induced cell stress in A549 cells

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Abstract

Chromium (Cr) is a trace element required for life. Biological activities of Cr are complicated and remain to be fully investigated. It is known that the valence of Cr plays an important role in the biological activities of Cr. For example, Cr (VI) is classified as a metal carcinogen [1], but Cr (III) is widely used as a nutritional supplement [2, 3]. Establishment of a gene expression profile for Cr-induced cellular response is necessary to facilitate investigation of the biological activities of Cr. In the present study, a large-scale gene expression analysis was conducted using RNA of human lung epithelial cells after *in vitro* exposure to Cr (VI). Utilizing high-density oligonucleotide arrays representing 2400 genes, we observed that expression of 150 genes was up-regulated, and that of 70 genes were down-regulated by Cr (VI). Functional analysis of these responsive genes led to an outline of potential biological activities of Cr in six aspects. The gene expression profile reveals that Cr may involves in redox stress, calcium mobilization, energy metabolism, protein synthesis, cell cycle regulation and carcinogenesis in the cell. The results provide a critical clue for understanding molecular mechanisms of the biological activities of Cr. (Mol Cell Biochem **222**: 189–197, 2001)

Key words: chromium (VI), gene chip, redox stress, cell cycle regulation, carcinogenesis

Introduction

Chromium is one of the trace elements in human body that have double properties. It is a carcinogen when its valence becomes VI. It is an essential nutritional element if its valence is in III. Chromate (Cr (VI)) compounds are widely used in industry for chrome plating, chrome pigment, leather tanning, and stainless steel production. It has been proposed that chromium-induced DNA damage, such as chromosome aberration, DNA breakage, and gene mutation, is an important mechanism through which Cr (VI) induces cancer. Studies from our laboratory and other groups have demonstrated that reactive oxygen species (ROS) can mediate the biological activity of Cr (VI) [1]. Cr (VI) is able to generate ROS both intra-cellularly and extra-cellularly. ROS generation is required for several biological activities of Cr (VI), such as induction of apoptosis [4], and activation of NF-κB [5].

Cr (III) compounds are nontoxic and non-carcinogenic as being demonstrated in experimental studies. Chromium picolinate, a Cr (III) compound, is able to improve glucose metabolism, serum lipid parameters, and to reduce body fat [2, 3]. As a result, chromium picolinate has been promoted widely as a health aid for the general population. Unlike Cr (VI), trivalent Cr has a very large safety range and is not toxic at levels up to 1 mg per day in nutritional studies. It remains unclear how Cr (III) exerts its biological effects in the body [2, 6]. One barie in Cr (III) study is that Cr (III) can not enter the cell in cell culture. Since Cr (VI) is able to enter the cell and to be reduced to Cr (III) in the cell, a study of the cellular response to Cr (VI) may facilitate our understanding of nutritional mechanism of chromium picolinate. In this respect, establishment of a gene expression profile for Cr (VI)-induced cellular responses will provide critical guidance for the study of chromium in both carcinogenic and nutritional aspects.

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Advances in high-throughput screening technology has made it possible to analyze expression of thousands of genes at a time. Oligonucleotide-based micro-array represents a state of the art approach in large-scale screening technology. This technology has been used to study HIV polymorphisms [7], aging [8] and mitochondrial function. In this study, a genechip of 2400 genes was used to analyze differential gene expression in response to Cr (VI)-induced cellular stress.

Materials and methods

The human lung type II epithelial cell line (A549) was used as a model in the study. Potassium dichromate was utilized as a source of Cr (VI). The cells were plated in a 100 mm cell culture dish in 10 ml culture medium, which is formed by RPMI-1640 supplemented with 5% fetal bovine serum and 2 mM glutamine. After a 2 h treatment with 300 μM potassium dichromate *in vitro*, the cells were harvested for RNA extraction using Trizole. Rest of the experiment was conducted through a contract service at Alpha Gene, Inc. (Woburn, MA, USA). The messenger RNA (mRNA) was purified from total RNA (400 μg) with an affinity column. Then, the mRNA (4 μg) was reverse transcribed into cDNA that was labeled with fluorescence, Cy3 and Cy5. The fluorescence-labeled cDNA was used to hybridize the genechip that represents 2388 human genes and 12 negative controls (Alphagene, Woburn, MA, USA). The fluorescence data were collected using ScanArray 3000 and analyzed with program ImaGene (BioDiscovery, Los Angeles, CA, USA). The data presented in this study represent mean values of 2 individual hybridizations. Only those genes that exhibited at least a 2 fold change were evaluated in this study.

Results

Induction of redox gene expression

The study reveals that Cr-induced oxidative stress occurs at the gene expression level (Table 1).

Copper-zinc superoxide dismutase (Cu/ZnSOD) and glutathione peroxidase (GPx) were induced by Cr (VI). Cr (VI)-induced oxidative stress was reported previously, but molecular events in the response to oxidative stress remain unclear [4, 5, 9]. Cu/ZnSOD is located in the cytosolic fraction of the cell. Its function is to specifically catalyze conversion of O^{-}_{2} into H_2O_2. A protective role of SOD against oxidative stress has been observed in many biological processes including aging [10]. SOD expression was induced 2.45 fold by Cr (VI), indicating that Cr (VI) may induce O^{-}_{2} production in the cell. The induced SOD expression may be responsible for removal of the extra $O^{-}_{2.}$ GPx is a major enzyme responsible for conversion of H_2O_2 into H_2O through consumption of GSH. GPx is located in the cytosolic fraction of the cell. The result in Table 1 shows that GPx is induced 4.33 fold by Cr (VI), indicating that GPx may take care of H_2O_2 induced by Cr (VI). These data provide another piece of evidence that Cr (VI) induces ROS in the cells. Increased expression of both SOD and GPx indicates that the cellular antioxidant systems are activated in response to Cr (VI) to remove extra O^{-}_{2} and H_2O_2.

Expression of metallothionein IIA (MT-IIA) and metal-response transcription factor 1 (MTF-1) were induced by Cr (VI). Metallothionein (MT) is a small protein whose primary function is to protect cells against toxic metals [11]. Other functional roles of MT include maintaining zinc homeostasis and protecting cells from ROS. A hallmark of MT-I and MT-II genes is their rapid transcriptional induction by metals, such as zinc and cadmium. Transcriptional induction of MT is mediated by MTF-1 at the metal response elements (MREs) in the gene promoter [12]. In the human body, MT-IIA is the major MT molecule. In this study, both MT-IIA (3.99 fold) and MTF-1 (3.04 fold) were induced by Cr (VI), suggesting that MTF-1 may be responsible for the increased expression of MT-II.

Photolyase is a group of enzymes responsible for DNA repair in response to ultraviolet light (UV) [13]. UV-induced DNA damage is associated with the activation of photolyases which repair damaged-DNA in a light-dependent manner. Two types of photolyases are known, one specific for cyclobutane pyrimidine dimers (CPD photolyase) and another specific to pyrimidine (6-4) pyrimidone photoproducts ((6-4) photolyase) [14]. Data in Table 1 show that the human homolog of the Drosophila (6-4) photolyase gene was induced 5.17 fold by chromium. This is the first evidence of induction of photolyase in response to agents other than UV light.

In a previous study, we observed that cells undergo programed-cell death (apoptosis) and p53 activation in response to Cr (VI) in the same experimental system [4]. This study further supports apoptosis and p53 activation induced by chromium [15–19]. An apoptosis-related gene, hSIAH (the human homologue of the Drosophila seven in absentia gene), was induced 7.69 fold by Cr (VI) (Table 1). hSIAH is activated during apoptosis in the intestinal epithelium [20]. This gene serves as a target of the p53 tumor suppressor, and it transduces the signaling of p53 during development of apoptosis [21].

Other stress-related genes that were induced by Cr (VI) include heat shock protein 75 (hsp75) and activating transcription factor 3 (ATF-3). Hsp75 belongs to the Hsp90 family whose function is to protect newly synthesized proteins, and regulate their functions through protein folding [22, 23]. Data presented in Table 1 show that hsp75 was induced 3.63 fold by Cr (VI). Association of ATF3 with stress responses has been reviewed [24]. The ATF3 mRNA increases greatly when the cells are exposed to stress signals. It has been sug-

Table 1. Stress response genes

	Acccession #	Gene	Function	Fold ↑	% ↓
1	U76247	hSIAH1	Apotosis	7.69	–
2	D83702	Photolyase	DNA repair	5.17	–
3	X58295	Glutathione peroxidase	Removal of hydrogen peroxide	4.33	–
4	X76717	MT-II	Protection of cells from metal toxicity and oxidative stress	3.99	–
5	AF043254	Heat shock protein 75 (hsp75)	Involved in protein transportation and comformation change	3.63	–
6	X78710	Metal-regulatory transcription factor 1(MTF-1)	Control gene expression of MT-II	3.04	–
7	L19871	Activating transcription factor 3 (ATF3)	Stress response	2.97	–
8	X02317	Cu/Zn superoxide dismutase (SOD)	Catalyzing conversion of superoxide into hydrogen peroxide	2.45	–

gested that the JNK/SAPK pathway is involved in the induction of ATF3 by stress signals. Data in Table 1 shows that ATF3 expression was increased 2.45 fold in response to Cr (VI).

Calcium mobilization

The present study indicates a role of Cr (VI) in the regulation of calcium signaling. Alterations in the expression of calcium-related genes are presented in Table 2. Calcineurin A [25] and calcineurin B were induced by Cr (VI). Calcineurin (also known as protein phosphatase 2B, PP2B) is a serine/threonine phosphatase whose function is to hydrolyze phosphorylated serine and threonine residues in the target proteins, such as nuclear factor of activated T cells (NFAT). This phosphatase contains two subunits: calcinuerin A (catalytic subunit) and calcineurin B (regulatory subunit). Calcinuerin is responsible for activating NFAT through dephosphorylation of serine or threonine in NFAT protein. Activity of calcineurin is regulated by calmodulin, which is a cytosolic protein serving as an intracellular receptor for calcium. The results indicate that expression of calcineurin A2 (an isoform of calcineurin A) was induced 76.91 fold, which is the maximal induction in this study. Calcinuerin B was also induced 2.43 fold by Cr (VI). Expression of both subunits of calcinuerin were activated by Cr (VI), indicating the induction of calcium mobilization in response to Cr (VI). Although it has been reported that other metals, such as cadmium (Cd^{2+}), can induce calcium mobilization, this appears to be the first evidence that Cr (VI) may regulate calcium signaling pathways. It is possible that Cr (VI) induces calcium mobilization through induction of oxidative stress, since oxidants can initiate calcium mobilization.

In accordance with the increased expression of calcineurin genes, cyclophilin (immunophilin) [26], an inhibitor of the calcineurin, was also induced. Cyclophilin is a member of prolyl isomerases, which serves as an intracellular receptor for cyclosporin A (CsA). CsA binds to cyclophilin and the resultant complex binds to calcineurin with a high affinity [27]. This interaction leads to inhibition of calcineurin activity and finally results in immunosuppression. Data in Table 2 indicate that chromium induced the expression of cyclophilin by 3.54 fold.

As shown in Table 2, three other calcium-related genes were induced by Cr (VI): caldesmon (18.76 fold), sorcin (6.27 fold) and PEP-19 (2.37 fold). Activities of these gene products are controlled by calcium.

Calcium-ATPase, encoded by gene ATP2C1, is an ATP-powered calcium pump that sequesters calcium into the Golgi in the cytosol [28]. Calcium-ATPase mutation leads to Hailey-Hailey disease (HHD) due to disregulation of cytoplasmic calcium in the keratinocytes [28]. As shown in Table 2, calcium pump expression was reduced by 59% after Cr (VI) treatment. This change might be responsible for a calcium increase in the cytoplasmic in response to Cr (VI).

Energy metabolism

Cr may play a significant role in the regulation of energy metabolism. Several energy metabolism-related genes, including the obese gene, were induced by Cr (VI) treatment

Table 2. Calcium signaling genes

	Acccession #	Gene	Function	Fold ↑	% ↓
1	M29551	Calcineurin A2	Activating NFAT in response to calcium mobilization	76.91	–
2	M83216	Caldesmon	Involved in control of cell morphology in response to calcium mobilization	18.76	–
3	L12387	Sorcin (SRI)	Related to drug resistance	6.27	–
4	Y00052	Cyclophilin	Cyclosporin A (CsA) receptor	3.54	–
5	M30773	Calcineurin B	Regulating calcinurine activity	2.43	–
6	X93349	PEP-19	Regulating cell movement in response to calcium mobilization	2.37	–
7	M23114	Calcium-ATPase (HK1)	Calcium pump	–	59.00

(Table 3). The major energy resources in the body are glucose and fatty acid. The energy-related gene expression profile supports the hypothesis that Cr induces fatty acid degradation, but reduces glucose consumption. Obesity is a common and complex disease that increases the risk of hypertension and diabetes. Both genetic and environmental factors play a role in the development and progression of human obesity [29, 30]. One of the genes that regulate fatty acid accumulation in the mouse is the obese (ob) gene [31, 32]. The ob gene product, leptin, functions as a hormone to regulate the body fat deposition. Animal experiments demonstrated that mutation of ob gene resulted in profound obesity and type II diabetes [31]. As shown in Table 3, chromium induced ob gene expression by 4.1 fold, indicating that chromium may reduce fat deposition and control body weight through the activation of ob gene expression. This mechanism may explain how Cr compounds act to control body weight.

Enhancement of fatty acid consumption by Cr is further supported by the increased expression of a fat metabolism enzyme, medium-chain acyl-CoA dehydrogenase (MCAD) (Table 3). MCAD is one of the three similar enzymes that catalyze the initial step of fatty acid beta-oxidation [33]. In the fatty acid oxidation process, the first step is the enzymatic dehydrogenation of saturated fatty acid to form a double bond in the carbon chain. MCAD is the enzyme responsible for catalysis of this step. In Cr-treated cells, MCAD was increased by 4.39 fold (Table 3), indicating an increase in the metabolism of saturated fatty acids.

On the other side, it is indicated that glucose consumption may be reduced by Cr (Table 3). Pyruvate dehydrogenase kinase 2 (PDK2) is a major enzyme responsible for the regulation of ATP generation from glucose metabolism. The key function of PDK is to reduce ATP generation from glucose by inactivating pyruvate dehydrogenase (PD). Data in Table 3 show that expression of PDK2 was induced 7.11 fold in Cr (VI) treated cells. This suggests that Cr (VI) may enhance PDK activity and lead to an inhibition of glucose degradation in the cell through Cr (III) which may promote glucose uptake in cells by enhancing insulin signaling [3, 6].

ATP synthesis may be enhanced by Cr because expression of several ATPases was enhanced by Cr (VI) (Table 3). These ATPases include: beta-1 subunit of sodium, potassium- transporting ATPase (Na, K-ATPase) (6.19 fold), the 115 kDa subunit of H^+-ATPase (4.18 fold), medium-chain acyl-CoA dehydrogenase (MCAD) (4.39 fold) and subunit c of ATP synthase (2.71 fold). Taken together, these data suggest that Cr (VI) enhances ATP synthesis from consumption of fatty acid, not glucose. Since Cr (VI) can be easily reduced to Cr (III) in cells, we propose that a net effect of chromium on energy metabolism is to increase the degradation of fatty acid, and at the same time promote synthesis of glycogen. This effect may be due to the combined biological activities of Cr (VI) and Cr (III).

Carcinogenesis

Several important molecules in the intracellular kinase cascades responded to Cr (VI). These include G-proteins, Src kinases and MAP kinase (Mitogen-activated protein) (Table 4). G-proteins and Src-kinase are very important proteins mediating receptor signals in the cell. In mammals, both G-proteins and Src-kinases are involved in the regulation of cell proliferation and differentiation. Crosstalk between these two families has become a new area of interest [34, 35]. G proteins are able to mediate signals from the receptors of hormones, neurotransmitters, and local mediators. A putative G protein was induced 3.33 fold by Cr (VI) [36]. Src kinases mediate receptor signals from cytokine, antigen or extracellular matrix receptors. Two Src-related genes were induced by Cr (VI). They were consensus tyrosine-lacking kinase (Cyl, 6.64 fold) [37] and hematopoietic consensus tyrosine-lacking kinase (HYL, 6.55 fold) [38]. These results indicate that Cr (VI) might be able to regulate cell growth or differentiation through G protein and Src kinases.

G-protein activation often leads to an increase in intracellular cyclic AMP (cAMP) by activating adenylyl cyclase that synthesizes cAMP from ATP. cAMP is an intracellular signaling molecule in all procaryotic and animal cells that

Table 3. Energy metabolism genes

	Acccession #	Gene	Function	Fold ↑	% ↓
1	L42451	Pyruvate dehydrogenase kinase isoenzyme 2 (PDK2)	Inhibition of ATP synthesis from glucose degradation	7.11	–
2	U16799	Na,K-ATPase beta-1 subunit	ATP-dependent Na, K pump	6.19	–
3	D12775	AMP deaminase	Energy generation	5.30	–
4	M16827	Medium-chain acyl-CoA dehydrogenase (MCAD)	Promoting degradation of saturated fatty acid	4.39	–
5	U73006	H(+) ATPase (115 kDa subunit)	Energy transfer in mitochondria	4.18	–
6	U43653	Obese gene (ob)	Enhancement of fatty acid degradation	4.10	–
7	X56549	Muscle fatty-acid-binding protein (FABP)	Fatty-acid metabolism	3.20	–
8	J03620	Dihydrolipoamide dehydrogenase	Glucose degradation	3.18	–
9	D13118	ATP synthase subunit C	Biosynthesis of ATP	2.71	–

Table 4. Carcinogenesis and kinase genes

	Acccession #	Gene	Function	Fold ↑	% ↓
1	X60114	Cytoplasmic tyrosine kinase (Cyl)	Src-related kinase	6.64	–
2	X77278	HYL tyrosine kinase	Src-related kinase	6.55	–
3	AF001434	NEN-1	Tumor suppressor	6.03	–
4	X94612	Type II cGMP-dependent protein kinase	Ion transport and fluid secretion	5.99	–
5	U02882	cAMP phosphodiesterase	cAMP regulation	5.74	–
6	U62800	Cystatin M (CST6)	Inhibition of metastasis	4.24	–
7	Y14391	Putative GTP-binding protein	G-protein	3.33	–
8	U09578	MAPKAP kinase (3pK)	MAP kinase p38 substrate	3.27	–
9	X51345	Jun-B	Nuclear protein	3.27	–
10	S80864	Cytochrome c-like polypeptide	Tumor marker	2.79	–
11	Z71621	Wnt-13	Tumor marker	2.73	–
12	M33011	Carcinoma-associated antigen GA733-2	Tumor marker	2.70	–
13	X03484	Raf oncogene	Kinase	2.63	–
14	U68723	Checkpoint suppressor 1	Tumor marker	2.57	–
15	U24576	Breast tumor autoantigen	Tumor marker	2.18	–
16	M84820	Retinoid X receptor beta (RXR-beta)	Tumor suppressor	–	79.00

have been studied. To maintain cell homeostasis, cAMP is continuously destroyed by one or more cAMP phosphodiesterases, which hydrolyze cyclic AMP to adenosine 5′-monophosphate (5′-AMP). In response to the increase in cAMP, cAMP phosphodiesterases activity should be enhanced. As shown in Table 4, cAMP phosphodiesterase was induced 5.74 fold by Cr (VI) [39], indicating a role of Cr (VI) in the regulation of cAMP signaling pathways.

G-proteins and Src-kinases regulate many signal pathways, including MAP kinases (through Ras and Raf). Raf is the immediate downstream substrate of Ras in the Ras/Raf signaling pathway. After activation, Raf passes a signal to its downstream mediators, such as MAP kinases. Table 4 shows that Raf expression was induced 2.63 fold by Cr (VI). MAP kinase p38 signal is mediated by its downstream kinase, such as MAPKAP2 (MAP kinase-activated protein kinase 2) [40]. Like MAPKAP2, 3pK (Mitogen-activated protein kinase-activated protein kinase 3) is another downstream substrate of p38 [41]. Table 4 shows that 3pK expression was induced 3.27 fold by Cr (VI). In addition, MAP kinase p38 is able to induce expression of c-jun and c-fos, two protooncogenes. Data in Table 4 demonstrate that one of the c-Jun family members, Jun B, was induced 3.27 fold by Cr (VI). Taken together, these data suggest that Cr (VI) may regulate MAP kinase signaling at multiple levels, including G protein, Src and Raf.

Activation of oncogenes by Cr (VI) may lead to carcinogenesis as several tumor-related genes were up-regulated (Table 4). They are: NEN-1 (6.03 fold), Cystatin M (4.24 fold), carcinoma-associated antigen GA733-2 (2.7 fold), breast tumor autoantigen (2.18 fold), checkpoint suppressor 1 (2.57 fold), Wnt-13 (2.73 fold), and cytochrome c-like polypeptide (2.79 fold). In addition, the anticancer molecule, retinoid X receptors beta (RXR beta), was reduced by 79% after Cr (VI) treatment, further implying a possible carcinogenic role of Cr (VI).

Biosynthesis

Metal-induced cell stresses often result in damage to a variety of proteins such as enzymes. This type of protein damage can lead to inactivation of an enzyme. Stressed cells respond to damage with an increase in protein synthesis in order to replace the inactivated or damaged protein, and an enhancement in protein-recycling process to eliminate the damaged proteins. Data in Table 5 provide a global view of genes that are associated with these processes.

New protein synthesis is carried out by ribosomes that are composed of two subunits, the large subunit (60S subunit) and small subunit (40S subunit). Each ribosome subunit contains two types of components: ribosomal protein and ribosomal RNA (rRNA). The eukaryotic ribosomes contain over 70 different proteins. The results show that the gene expression of key proteins in both the large ribosomal subunit and small ribosomal subunit were induced (Table 5). The induced-large subunit ribosomal proteins include: L21 (3.47 fold), L34 (2.8 fold), L38 (5.13 fold), and L41 (2.86 fold). The induced-small subunit ribosomal proteins include S4 (3.12 fold) [42], S17 (2.56 fold), S20 (2.25 fold) and S24 (2.39 fold). The increased expression of ribosomal proteins indicates that Cr-treated cells are initiating a reconstruction process through up-regulation of protein synthesis. In line with this hypothesis, expression of the eukaryotic translation initiation factor 5 (eIF-5) [43] and threonyl-tRNA synthetase [44] were induced 4.34 and 2.73 fold, respectively, in Cr-treated cells.

The ubiquitin-dependent proteolytic pathways are the major mechanisms responsible for removal of damaged pro-

Table 5. Biosynthesis and metabolism genes

	Acccession #	Gene	Function	Fold ↑	% ↓
1	U18244	Excitatory amino acid transporter 4	Glutamate transporter	16.75	–
2	X63237	Ubiquitin 80	Degradation of protein	17.47	–
3	L38961	Putative transmembrane protein precursor (B5)	Glycosylation of protein	6.52	–
4	Z26876	Ribosomal protein L38	Protein synthesis	5.13	–
5	U49436	Translation initiation factor 5 (eIF5)	Promote protein synthesis	4.34	–
6	U49260	Mevalonate pyrophosphate decarboxylase (MPD)	Biosynthesis of steroid	3.82	–
7	X89401	Ribosomal protein L21	Protein synthesis	3.47	–
8	M58458	Ribosomal protein S4 (RPS4X) isoform	Protein synthesis	3.12	–
9	AF026844	Ribosomal protein L41	Protein synthesis	2.86	–
10	L38941	Ribosomal protein L34 (RPL34)	Protein synthesis	2.80	–
11	M63180	Threonyl-tRNA synthetase	Activation of amino acid for protein synthesis	2.73	–
12	M13932	Ribosomal protein S17	Protein synthesis	2.56	–
13	M31520	Ribosomal protein S24	Protein synthesis	2.39	–
14	L06498	Ribosomal protein S20 (RPS20)	Protein synthesis	2.25	–

teins in the stress response. This proteolytic pathway contains two components: proteasome and ubiquitin. The proteasome is a large protein complex whose function is to degrade ubiquinized proteins. Ubiquitin is a small protein whose function is to mark the unwanted proteins and bring them to the proteasome for degradation. Ubiquitin 80 is an oxidation response protein [45, 46], which covalently attaches to oxidatively damaged protein and activates ubiquitin-dependent proteolytic pathways [45]. As shown in Table 5, the expression of ubiquitin 80 was increased 17.47 fold by Cr (VI), indicating an increased activity in protein recycling.

Cholesterol is an important component of cell membranes and plasma lipoproteins. It is also the precursor of many other biologically important steroids, such as various steroid hormones. Biosynthesis of cholesterol may be increased in the response to chromium (Table 5). This is supported by an up-regulated expression of cholesterol enzyme. Mevalonate pyrophosphate decarboxylase (MPD) is a unique enzyme in the cholesterol biosynthetic pathway [47]. This enzyme was induced 3.82 fold in response to Cr (VI). The increased expression of this enzyme may reflect a cellular mechanism for the repairing of cell membranes that are damaged by stress-induced lipid oxidation.

Cell cycle regulation

Our other experiments demonstrated that Cr (VI) inhibited cell proliferation, and the inhibitory effect of Cr (VI) was dose-dependent (data not shown). In a previous study, we observed that Cr (VI) elevates p53 protein levels in the cytoplasm [4], indicating a molecular mechanism of the cell growth inhibition. The microarray data provides more molecular evidence for cell growth regulation by chromium. First, Cr (VI) induced expression of an inhibitor of cyclin-dependent kinase (CDK), INK4 p19 (Table 6). INK4 p19 is a CDK inhibitor [48, 49]. An increased INK4 activity leads to cell cycle arrest. INK4 p19 selectively inhibits CDK4 and CDK6 and results in G1 cell cycle arrest [49]. Expression of INK4 p19 was induced 2.65 fold by Cr (VI). Second, Cr (VI) induced expression of a cell growth inhibitor, retinoblastoma binding protein (Rbp), which is a cofactor of retinoblastoma (Rb) [50]. Rbp acts as a suppressor of the transcription factor E2F and leads to cell growth inhibition. Deficiency of Rbp 2 is a feature of sporadic human melanoma skin cancer [51]. Rbp 2 was induced 3.45 fold by Cr (VI) (Table 6). Third, as shown in Table 6, Cr (VI) reduced expression of several cell cycle or DNA replication mediators, such as CDC25B , CDC2, CDC47 and casein kinase II by 87, 98, 73 and 52%, respectively.

CDC25 is a phosphatase and a potential human oncogene [52]. CDC25 is responsible for activation of CDKs. CDC25 phosphatase removes inhibitory phosphate from tyrosine and threonine residues of CDKs and activates kinase activities of CDKs. In the human cells, CDC25 proteins are encoded by a multigene family, consisting of CDC25A, CDC25B, and CDC25C. While CDC25A plays a crucial role at the G1/S phase transition, CDC25C is responsible for the activation of the mitotic kinase, CDC2/cyclinB. Loss of CDC2/cyclinB activity leads to G2 arrest. CDC25B phosphatase is essential for the G2/M phase transition in human cells [53, 54]. CDC25B is overexpressed in 32 percent of human primary breast cancers tested. Expression of both CDC2 and CDC25B were reduced by 98 and 87%, respectively, by Cr (VI) (Table 6).

CDC47 and casein kinase II (CK2) are two kinases closely related to DNA replication. CDC47 is a member of the MCM protein family [55], which has been implicated in the regulatory machinery of DNA replication in the S phase. Expression of CDC47 mRNA is decreased in quiescent cells, but increased at the late G1 or S phase by growth factor stimulation [56]. CDC47 was reduced 73% by Cr (VI) (Table 6).

Table 6. Cell growth and cell cycle genes

	Acccession #	Gene	Function	Fold ↑	% ↓
1	AJ000414	Cdc42-interacting protein 4 (CIP4)	Cell growth	11.86	–
2	X67951	Proliferation-associated gene (pag)	Cell proliferation	11.77	–
3	M24398	Parathyrmosin	Zinc binding	6.90	–
4	L07758	IEF SSP 9502	Cell growth	6.75	–
5	U39360	CROC-1A	Cell growth	6.47	–
6	M76979	Pigment epithelium-differentiation factor (PEDF)	Cell differentiation	6.11	–
7	AF026547	Neurocan (CSPG3)	Cell motility	5.63	–
8	U32315	Syntaxin 3	Protein secretion	5.49	–
9	S66431	Retinoblastoma binding protein 2 (RBP2)	Tumor suppressor	3.45	–
10	U34051	Cdk 5 activator isoform p39	Cell growth	3.00	–
11	U40343	INK4 p19	Induce G1 cell cycle arrest	2.65	–
12	X74979	trk E	NGF receptor kinase	2.68	–
13	X66360	p34 CDC2	Cell growth	–	98.00
14	L42611	Keratin 6 isoform K6e (KRT6E)	Growth marker	–	88.00
15	Z68092	CDC25B tyrosine phosphatase	Promote cell growth	–	87.00
16	D55716	CDC47	DNA replication	–	73.00
17	J02853	Casein kinase II alpha subunit	DNA replication	–	52.00

CK2 is a serine/threonine kinase overexpressed in many human tumors and rapidly proliferating tissues. CK2 is composed of two catalytic subunits (alpha) and two regulatory subunits (beta). CK2 is required for DNA replication and over-expression of CK2 induces cell transformation. CK2 alpha, a catalytic subunit of CK2 [57], was reduced by 52% after Cr treatment.

On the other hand, some of the cell cycle promoting kinases were induced by Cr (VI). They are CIP4 (CDK42-interacting protein 4, 11.86 fold), and CDK5 (3 fold). In line with these changes, some cell proliferation markers were also induced by Cr (VI). They are PAG (proliferation-associated gene, 11.77 fold), IEF SSP 9502 (a human homologue of the yeast PWP1 protein, 6.75 fold), IEF SSP 9502 (6.75 fold), CROC-1A (6.47 fold), and trk (2.68 fold). It is not clear why these genes were induced by Cr (VI). Their expression might be a part of the cellular stress response.

Discussion

Chromium has dual effects on the human body. The molecular mechanisms of the biological effects of chromium has been dominated by the free radical theory [1, 16, 58]. However, there has been no report on how Cr regulates gene expressions as a result of generation of ROS. In this study, genechip technology was used to explore differential expressions of more than two thousand genes after Cr treatment. The results show that many oxidative stress-related genes are induced in response to Cr treatment. These include Cu/Zn SOD, GPx and MT-II. Expression of these ROS-related genes provides a new evidence in favor of the free radical theory. Calcium mobilization is associated oxidative stress [59]. Although there have been many reports about oxidative stress in response to chromium, it is not clear whether calcium mobilization is involved in biological mechanisms of chromium. This study provides evidence about a role of calcium mobilization in chromium-induced signal transduction. Several calcium-related genes were induced by chromium. Products of these genes play different roles in calcium signal transduction pathways. Since calcium signal is coupled with ROS generation, induction of calcium-related genes become an indirect evidence that chromium induces oxidative stress in the cells.

It is believed that ROS is involved in many metabolism pathways [60–62]. The gene expression profiles from this genechip study provide a detail map for the chromium-responsive pathways. The study suggests that Cr-induced ROS may regulated energy metabolism through induction of gene expression. The induced-genes may enhance energy metabolism. The energy resource will mainly be fatty acid as several genes induced by chromium can promote degradation of fatty acid, and at the same time reduce glucose degradation. This effect will lead to a control of body fat deposition, and therefore may explain why chromium can reduce body fat as well as enhance muscle strength [3, 63]. Intracellular signaling components are targets of ROS [60–62]. These signaling components may extend ROS activities in carcinogenesis and cell cycle regulation. This study suggests that signaling molecules including G-protein, Src kinase, MAP kinases and cyclin-dependent kinases are activated by Cr-induced ROS. These signaling molecules are distributed all over in the signaling cascades from cell membrane to nucleus. These ROS-responsive genes are responsible for ROS responsiveness of different signal transduction pathways. In addition, many cell cycle-related genes are regulated by chro-

mium. Some of them are enhanced by chromium, such as INK p19 and Rb binding protein 2. Some of them are decreased by chromium, such as CDC25B and CDC47. Changes in these genes expression may explain the cell cycle rest activity of chromium [16, 17, 64]. These results together may provide a new basis for hypothesis of chromium carcinogenesis.

ROS oxidize proteins and leads to protein damage [62]. This study shows that the stressed cells may enhance protein synthesis to replace the damaged proteins. At the same time, the stressed cells may accelerate recycling process to remove the damaged proteins. Chromium exposure resulted in expression of genes responsible for both processes. This suggests that biosynthesis is activated in response to Cr-induced ROS.

In summary, this study provides a global view of differential gene expression in response to chromium treatment. Cr regulates expression of genes involved in oxidative stress, calcium signaling, fatty acid degradation, carcinogenesis and cell growth. This information may prove useful not only in understanding molecular mechanisms in Cr toxicity, but also in advancing our knowledge in a number of fields, such as neurology, toxicology, immunology, physiology, biochemistry and oncology.

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