

Review Article

Cytogenomics of hexavalent chromium (Cr⁶⁺) exposed cells: A comprehensive review

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The altered cellular gene expression profile is being hypothesized as the possible molecular basis navigating the onset or progress of various morbidities. This hypothesis has been evaluated here in respect of Cr⁶⁺ induced toxicity. Several studies using gene microarray show selective and strategic dysregulations of cellular genes and pathways induced by Cr⁶⁺. Relevant literature has been reviewed to unravel these changes in different test systems after exposure to Cr⁶⁺ and also to elucidate association if any, of the altered cytogenomics with Cr⁶⁺ induced toxicity or carcinogenicity. The aim was to verify the hypothesis for critical role of altered cytogenomics in onset of Cr⁶⁺ induced biological / clinical effects by identifying genes modulated commonly by the toxicant irrespective of test system or test concentrations / doses, and by scrutinizing their importance in regulation of the flow of mechanistically linked events crucial for resultant morbidities. Their probability as biomarkers to monitor the toxicant induced biological changes is speculative. The modulated genes have been found to cluster under the pathways that manage onset of oxidative stress, DNA damage, apoptosis, cell-cycle regulation, cytoskeleton, morphological changes, energy metabolism, biosynthesis, oncogenes, bioenergetics, and immune system critical for toxicity. In these studies, the identity of genes has been found to differ remarkably; albeit the trend of pathways' dysregulation has been found to remain similar. We conclude that the intensity of dysregulation of genes or pathways involved in mechanistic events forms a sub-threshold or threshold level depending upon the dose and type (including speciation) of the toxicant, duration of exposure, type of target cells, and niche microenvironment of cells, and the intensity of sub-threshold or threshold level of the altered cytogenomics paves way in toxicant exposed cells eventually either to opt for reversal to differentiation and growth, or to result in toxicity like dedifferentiation and apoptosis, respectively.

Key words Apoptosis - chromate carcinogenesis - chromium - epigenetics - genomics - microarray

Introduction

Hexavalent chromium (Cr⁶⁺) is a toxic metal known for its carcinogenic effect in humans. The lung cancer risk is prevalent in pigment chromate handlers, ferrochromium production workers, stainless steel

welders, and chromeplaters¹. Besides occupational cancer, risk of other kinds of adverse health effects are also reported in humans after short term/prolonged exposure through inhalation, ingestion, or topical contact²⁻⁵.

Chromate compounds are cytotoxic, genotoxic, and carcinogenic in nature⁶⁻¹⁹. Mechanism of action is proposed to involve reactive oxygen species (ROS) generation, oxidative stress, and DNA damage; a variety of other changes like increased formation of DNA adducts and DNA-protein cross-links, DNA strand breaks, chromosomal aberrations and instability²⁰⁻²⁶, disruption of mitotic cell division, chromosomal aberration, premature cell division²⁶⁻³⁴, S or G2/M cell cycle phase arrest³⁵⁻⁴³, and carcinogenesis⁴⁴ also occur in humans or experimental test systems. However, the molecular basis of these changes culminating into biological effects, tissue lesions, or cancers have not been examined. Cr⁶⁺ induced alterations in cellular gene expression forming the basis of these changes remain an unexplored probability. Several cytogenomic studies using gene microarray approach demonstrated a selective and strategic dysregulations of cellular genes and pathways by Cr⁶⁺. The cytogenomics studies gave toxicologists a comprehensive view of genomic changes occurring in the pathogenesis of Cr⁶⁺ toxicity.

The aim of this review was to derive a hypothesis on the critical role of altered cytogenomics and its intensity culminating into the elicited biological/clinical effects, and to verify this hypothesis by (i) identifying genes and pathways modulated commonly by Cr⁶⁺ irrespective of test system or exposure conditions, and (ii) by scrutinizing their importance in regulation of the flow of mechanistically linked intracellular events that may be crucial for Cr⁶⁺ induced toxicity or carcinogenicity.

Oncogene activation, DNA adduct formation, select gene expression, and epigenetic changes

Earlier non-microarray based studies investigated the role of mutations in oncogene like *ras*, *p53*, *Bcl-2*, *cyclin-D1* or their altered expression in Cr⁶⁺ carcinogenesis; these studies were conducted in experimental test systems or cancer tissues of Cr⁶⁺ exposed workers. Activated *ras* oncogene was seen in Cr⁶⁺ lung cancer, however, considered a rare event and not involved in Cr⁶⁺ carcinogenesis⁴⁵. Changes in *Bcl-2* and *p53* expression level were noted although these were found to be unspecific to Cr⁶⁺ carcinogenesis; the study was inconclusive as the levels were found to be similar in cancer tissue from ex-chromate workers as well as the non-exposed subjects and workers with pneumoconiosis⁴⁵. Further investigations revealed mutant *p53* gene in lung cancer of chromate exposed workers⁴⁶ illustrating *p53* mutation following Cr⁶⁺ exposure; the elevated serum levels of pan-tropic p53 (pan-p53) proteins in Cr⁶⁺ workers⁴⁷; and induction

of p53 level up to 6-fold in Cr⁶⁺ exposed human lung fibroblasts⁴⁸. The key role of *p53* gene in chromate toxicity or carcinogenesis was demonstrated using *p53* deficient transgenic mice^{49,50}; intervention studies showed that the loss of crucial gene *p53* increased the genomic DNA fragmentation⁴⁹.

Recently, the effect of short term high dose (0.05 and 0.25 µM) Cr⁶⁺ exposure on benzo alpha pyrene (B(a)P) (DNA damage) directed gene alteration in mouse hepatoma cells was investigated⁵¹. RT-PCR based analysis showed upregulation in genes related to apoptosis (*Aifm*, *Bid*, *Bak*, *Bcl2*, *Fas*, *Apaf1*, *Tnf*, *Bax*), cell cycle control (*Rad17*, *Mdc1*), tumour suppression (*p15*, *p16*, *p18*, *p19*, *p21*, *p27*), DNA damage (*Brcal*, *Brca2*, *ATM*, *Gadd45*, *Mgmt*) and down-regulation in genes related to drug metabolism (*Cyp1b1*, *Cyp1z2*, *Gst1*, *Nqo1*, *Cyp1a1*, *Aldh3*). In an *in vivo* study using mice exposed to (0, 50, 500 and 5000 ppb) Cr⁶⁺ in drinking water for two months and co-exposed to B(a)P for 24 h, downregulation of all the genes except *Cyp1b1* gene in Cr⁶⁺ exposed mouse liver was seen⁵¹. In an earlier study, the co-exposure of Cr⁶⁺ and B(a)P was found to increase the carcinogen-DNA adduct formation in mouse hepatoma cells⁵². These observations indicated that Cr⁶⁺ exposure facilitated the carcinogen - DNA adducts formation causing DNA damage.

With respect to epigenetic changes, Cr⁶⁺ induced methylation of p16 promoter and repression of DNA-mismatch-repair or tumour suppressor genes mut L homologue 1 (*MLH1*) and *MLH2* has been reported^{53,54} besides the genetic instability in chromate lung cancer. Sun *et al*⁵⁵ reported an increase in protein as well as mRNA level of G9a, a histone methyl-transferase that methylated *H3K9* (histone H3 lysine 9) and accounted for global elevation of its dimethylated type and silencing of tumour suppressor gene *MLH1* transcription. Others showed that Cr⁶⁺ inhibited the transcription co-activators^{56,57}. Klein *et al*⁵⁸ showed methylation of genes and modulation of gene cyclin-D1 by Cr⁶⁺ in transgenic cells; study revealed the responsiveness of cell cycle regulation to the toxic metal. A crucial role of *cyclin D1* in Cr⁶⁺ toxicity was noticed in a study on ex-chromate workers affected with lung cancer wherein cyclin-D1 expression was found to be more as compared to non-exposed subjects harbouring other disease like pneumoconiosis⁴⁵. The altered expression of ATM (ataxia telangiectasia mutated) gene⁵⁹, aneuploidy and dysregulation in spindle assembly checkpoint bypass⁶⁰ were reported

in Cr⁶⁺ exposed cells; these changes normally support apoptosis, cell cycle regulation, as these are requisites of cells responding to DNA damage and to genomic instability.

Studies demonstrated alterations in cellular pathways after Cr⁶⁺ exposure. In cell signalling (MAPK) pathway, activation of (Extra cellular signal regulated kinase) *ERK*, (C-Jun-N-terminal kinase) *JNK*, (mitogen activated protein kinase) *p38* (regulators of cell growth, proliferation, apoptosis, and differentiation.) was observed; the activation of change depended on toxicant's concentrations, resultant ROS generation or oxidative stress⁶¹⁻⁶⁶. Their activation was also reported in Cr⁶⁺-exposed mouse embryonic stem cells⁶⁷; lower level of toxicant activated *JNK* (c-Jun-N-terminal kinase) via *LCK* (leukocyte C-terminal Src kinase, a member of the Src family of protein tyrosine kinases) or the *Fyn-Cas-Crk* (*FAK/Src-Yes-Fyn/p130 CAS/CRK*) signalling cascade; *LCK* could activate *STAT3* (signal transducer and activator of transcription) and (interleukin-6) *IL-6* which contributed to inflammation and cancer⁶⁸. Others studies investigating ROS dependent changes found that Cr⁶⁺ exposure activated nuclear factor kappa β (*Nfkb*) and *p38* (mitogen activated protein kinase 14) pathway; *Nfkb*, important for apoptosis, was also considered an indicator of Cr⁶⁺ induced cytotoxicity⁶⁹.⁷⁰ Using cultured cells, investigators also showed activation of activator protein-1 (AP-1) but *HOGG1* (8-oxoguanine DNA glycosylase) gene was found to be uninfluenced. It is inferred that *Nfkb* does not participate in tumorigenesis; it is rather associated with a decrease in cell proliferation and induction of apoptosis⁷¹. Overexpression of inflammation specific *COX-2* via *Nfkb* / *c-Jun* / *AP-1* dependent pathway was observed in normal human bronchial epithelial cells and mouse embryonic fibroblasts after Cr⁶⁺ exposure⁷². The signalling molecule (*VEGF*) vascular endothelial growth factor was found to be overexpressed by Cr⁶⁺. *VEGF*, involved in angiogenesis, is usually overexpressed in lung cancer, and used as prognostic marker⁷³⁻⁷⁷; one study⁷⁸ on the contrary showed the suppression of *VEGF* expression by Cr⁶⁺. In signalling pathway, other types of genes that are activated in response to Cr⁶⁺ are *Fyn* and *LCK* and the initiation of an interferon signalling mechanism^{69,79}. Activation of *AKT* (α serine-threonine protein kinase) was also noticed by Cr⁶⁺ in human lung fibroblast transformation. *AKT* is known to override G1/S checkpoint bypass, prevent Cr⁶⁺ induced decrease in localization of retinoblastoma protein and p27 (cyclin dependent kinase inhibitor 1B) the key factors of G1/S checkpoint, and contribute to

toxicant induced genomic instability⁸⁰. Levels of *ApoJ* / *CLU* (a senescence biomarker apolipoprotein J and an oxidative stress responsive gene protein clusterin) in serum were noted to be high in shipyard welders during the oxidative stress and were found to be lower after worksite intervention⁸¹.

The sporadic studies on oncogene activation, gene expression with or without DNA adduct formation, and epigenetic changes provided only a limited knowledge on the role of mutagenic events, oncogenes and tumour suppressor genes, and the concurrent changes in expression of assorted genes in Cr⁶⁺ carcinogenesis. To elucidate comprehensive information on the change in cytogenomics after Cr⁶⁺ exposure, the investigators used gene microarray based approach. These efforts were made in conjunction with the hypothesis that the mechanism of Cr⁶⁺ carcinogenesis was not limited only to Fenton-reactions, or the resultant genotoxic effects, or the oncogenes / tumour suppressor genes, but also involved critical alterations in global gene expressions. Both *in vivo* and *in vitro* studies elucidated epigenetic and gene expression changes that logically seemed crucial for shaping sub-clinical effects of Cr⁶⁺ like inflammation, apoptosis, and cell transformation. These exploratory studies used the limited gene-microarray or whole genome microarray, different test systems / test concentrations / test compounds / exposure durations. These investigations yielded an explosion of information for its utility to understand the key gene expression changes that contribute to toxicity after Cr⁶⁺ exposure or form the molecular basis of its toxic effects.

This review reveals the identity of dysregulated genes and pathways that could form the molecular basis of Cr⁶⁺ toxicity, and be useful in elucidating the mechanism of action of this toxicant.

Genomic studies

The microarray-based *in vivo* / *in vitro* studies⁸²⁻⁸⁹ are summarized in this section. In these investigations, sodium or potassium dichromate, or sodium chromate were used in test systems of human cell *i.e.* peripheral blood mononuclear cells (PBMC), A549, BEAS-2B, dermal fibroblasts or in rat. The test concentrations ranged from 9-300 μ M in case of cells; a dose of 0.25 mg/kg body wt was used for rat. The size of microarrays included 216, 1200, 2400, 12000, 22000, 28000 or 44,000 probes. These studies examined genes and relevant pathways. Irrespective of the duration of toxicant exposure, stress and apoptosis were found to

be the most influenced pathways; energy metabolism, DNA repair / metabolism, biosynthesis, and oncogene were moderately influenced; immunoregulation and the cell and focal adhesion / gap and tight junction / extracellular matrix / cytoskeleton were mildly influenced pathways.

In vitro studies

A549 cells, 300 μM potassium dichromate, 2 h exposure: In the first study on Cr⁶⁺ induced cellular gene expression modulation, Ye and Shi⁸² examined genomics in human lung type II epithelial A549 cell using microarray of 2400 genes and potassium dichromate as a source of Cr⁶⁺. They investigated the molecular basis of Cr⁶⁺ provoked ROS generation and the resultant oxidative stress, and found a significant dysregulation of 220 genes that were part of the pathways of oxidative stress, Ca²⁺ mobilization, energy metabolism, protein synthesis, cell cycle regulation, apoptosis, and carcinogenesis.

In stress response pathway (Table I), an upregulated transcription was seen in Cu / Zn superoxide dismutase (SOD), glutathione peroxidase, MT-IIA, MTF-1 (metal-regulatory transcription factor), p53, heat shock proteins (*HSP60*, *HSP70*, *HSP75*), and activating transcription factor-3 (ATF-3). The oxidative stress responsive proteins protected the correct conformation of newly formed proteins; their main function was to protect cells from ROS / oxidative stress and preserve the vitality of cells.

In apoptosis pathway Ye and Shi⁸² reported upregulation of only the *hSLAH1* (Siah E3 ubiquitin protein ligase 1) gene (Table II). Functionally, this apoptotic gene facilitated to label the protein for proteasomal degradation and the programmed cell death through induction of p53 signalling during Cr⁶⁺ induced stress.

In cell cycle regulatory pathway (Table III), the upregulated genes transcribed protein products important for cell survival, cell polarization, cell growth and differentiation, G1 cell cycle arrest, and tumour suppressor function. By underexpressing the respective proteins, the downregulated genes dysregulated cell cycle control via slowdown of cyclin dependent kinase activation causing cell cycle arrest, and challenging epithelial cell integrity for apoptosis.

In DNA repair and metabolism pathway (Table IV), only three genes were found to be dysregulated. The upregulated gene encoded photolyase that was involved

in DNA repair. The downregulated *CK2* (casein kinase 2) and cell division cycle (*CDC47*) decreased the function of serine / threonine kinase and also required for DNA replication. The binding of *CDK4* (cell cycle dependent kinase), *CDK5* with *CK2/CDC45* encoded a protein to regulate the signalling mechanisms in cell proliferation and growth.

Among oncogenes (Table V), the upregulation of intracellular kinase, G-protein, Src, and MAPK showed their involvement in cell proliferation and differentiation. MAPK signalling regulated the proto-oncogene; activation of oncogenes by Cr⁶⁺ would support carcinogenesis.

In pathways of cellular energy metabolism and biosynthesis, a great majority of genes were found to be dysregulated. In energy metabolism pathway (Table VI), the upregulated genes would facilitate proton pumps for ATP synthesis, osmoregulation and active transport of molecules across cell membrane, nerve/muscle electrical excitability, and bioenergetics regulation. In biosynthesis pathway (Table VII), the upregulated genes would aid proteasomal degradation, biosynthesis dependent programmed cell function, and cell matrix homeostasis.

In summary, these studies revealed that 300μM Cr⁶⁺ influenced the global cytogenomics and in particular regulated the stress, DNA repair, signalling system (Ca⁺⁺, G-protein), Src kinase, MAPK and CDK, oncogenes, bioenergetics, and cell cycle. Specificity of oncogene expression to Cr⁶⁺ exposure may be predisposed by the adenocarcinomas cell line based test system.

BEAS-2B cells, 10 μM sodium dichromate, 4 h exposure: Andrew *et al*⁸³ investigated the cellular gene expression profile of human bronchial epithelial BEAS-2B cells using a limited microarray of 1200 genes. They examined changes in gene expression after acute exposure to 10 μM dose of the toxicant. Cr⁶⁺ was found to modulate a cluster of 44 genes (Tables I-V).

In stress pathway (Table I), none of the genes showed upregulation; only heat shock protein genes (*hsp40*, *hsp60*, *hsp90*) recorded downregulation. The molecular basis seemed to be the protein transport and chaperon like functions. Their downregulation indicated irregular trafficking of deformed proteins and the risk of apoptosis.

Downregulation of anti-apoptotic proteins (Table II), DIF-2, was also in line with above observation.

Table I. Dysregulated genes of stress response pathway in Cr⁶⁺ exposed cells

Gene	Function	Upregulated (U) / downregulated (D)	Ref
Activating transcription factor 3,4 (<i>ATF3, 4</i>)	Stress response	U	82,89
Cellular glutathione peroxidase I	Stress	U	88
Cu/Zn superoxide dismutase (<i>SOD</i>)	Catalyzing conversion of superoxide into hydrogen peroxide	U	82,88
Glutathione peroxidase (<i>GPXI</i>)	Removal of hydrogen peroxide	U	82, 83, 89
Glutathione S-transferase subunit 5 theta	Cellular stress	U	88
Heat shock protein (<i>Hsp 60, Hsp 70</i>)	Involved in protein transportation and conformation change	U	
Heat shock protein 75 (<i>hsp75</i>)	Involved in protein transportation and conformation change	U	82
Heat shock proteins (<i>Hsp 40, Hsp60, Hsp 90</i>)	Involved in protein transportation and conformation change	D	83
Metal-regulatory transcription factor 1 (<i>MTF-1</i>)	Controls gene expression of MT-II	U	82, 89
<i>MT-II</i>	Protection of cells from metal toxicity and oxidative stress	U	82
Plasma glutathione peroxidase precursor	Stress	U	88
Glutathione peroxidase 1,2 (<i>Gpx1, 2</i>)	Removal of hydrogen peroxide	U	89
Glutathione reductase (<i>Gsr</i>)	Maintains high levels of reduced glutathione in the cytosol	U	
Nuclear factor (<i>Nrf2</i>)	Transcription activator that binds to antioxidant response (ARE) elements in the promoter regions of target genes.	U	
Peroxiredoxin 1 (<i>Prdx1</i>)	Involved in redox regulation of the cell	U	
Stress induced phosphoprotein (<i>Stip1</i>)	Mediates the association of the molecular chaperones HSC70 and HSP90	U	
Ribonucleotide reductase M2 (<i>Rrm2</i>)	Provides the precursors necessary for DNA synthesis	U	

Similarly, downregulation of anti-death protein ephrin type-A receptor 2 ECK (tyrosine kinase receptor) demonstrated predisposition to apoptosis.

The trend of apoptosis was also noticed by the downregulation of genes in cell cycle pathway (Table III). *ERF1* (EGF response factor-1) encoded the transcriptional activator C2H2-type zinc-finger nuclear protein that was functionally tumour suppressor and important for cell growth and differentiation. Cyclin K protein regulated cyclin dependent kinase and RNA polymerase. *LUCA 2* (lysosomal hyaluronidase 2) transcribed the hyaluronidase, a cell surface protein, associated with tumour suppression function and involved in cell growth, differentiation and migration. For cell cycle regulation in Cr⁶⁺ exposed cells, *TNKI* (tyrosine kinase 1) encoded the non-receptor type

of tyrosine kinase that phosphorylated proteins downstream of Src kinase in intracellular signalling (Table III). *YWHA1* (tyrosine 3-monooxygenase / tryptophan 5-monooxygenase activation protein) encoded the signal transducers; *FGFR1* (fibroblast growth factor receptor 1) encoded the fibroblast growth factor receptor type of protein to modulate growth and differentiation. Their underexpression seemed to be in consonance with a shift of cell cycle into apoptosis mode or the dedifferentiation mode.

Cr⁶⁺ induced predisposition of cells to apoptosis or transformation in tumour phenotype was perceptible by the sluggish DNA repair and metabolism (Table IV) through downregulation of genes involved in DNA template elongation, DNA binding and replication, G₀ to G₁/S phase of cell cycle, the cell growth, senescence,

Table II. Dysregulated genes of apoptosis pathway in Cr⁶⁺ exposed cells

Gene	Function	Upregulated (U) / downregulated (D)	Ref
Caspase4	Activation cascade of caspases responsible for apoptosis	U	86,84
Cytoplasmic FMR1 interacting protein 2 (<i>CYFIP2</i>)	p53/TP53- dependent induction of apoptosis	U	84
Prolyl endopeptidase (<i>Prep</i>)	Expressed in apoptotic cell in p53 manner	U	
Serine/threonine protein kinase <i>MST4</i>	Mediator of cell growth. Modulates apoptosis	U	
Ring finger protein 144B (<i>RNF144B</i>)	Induces apoptosis via a p53/TP53-dependent but caspase-independent mechanism	U	
Mitochondrial ribosomal protein S30 (<i>MRPS30</i>)	Pro-apoptotic gene	U	
NUAK family SNF1-like kinase 2 (<i>NUAK2</i>)	Expression induced by NF-kappa-B activation	D	
Semaphorin (<i>SEMA3A</i>)	Pro-apoptotic gene control fas-mediated apoptosis	D	
Ras homolog gene family, member B (<i>RHOB</i>)	Mediates apoptosis in neoplastically transformed cells after DNA damage.	D	
Bcl-XL	Potent inhibitor of cell death. Inhibits activation of caspases	U	85,88
B-cell CLL/lymphoma 2 (<i>Bcl-2</i>)	Suppresses apoptosis		88
Immediate early response 3 (<i>DIF-2</i>)	Anti-apoptotic	D	83
P ²¹ (<i>WAF1/CYP1</i>)	Apoptotic	U	85
Death receptor 6 (<i>DR6</i>)	Induce apoptosis	U	
Collagenase type 4	Breakdown of extracellular matrix	U	
Cell death protein (<i>RIPK1</i>)	Activation of NF-kappa-B.	U	86
Baculoviral IAP repeat-containing 1 (<i>BIRC1</i>)	Anti-apoptotic	D	
Apoptosis inducing serine/threonine protein kinase (<i>STK17B</i>)	Pro-apoptotic	U	
Death inducer obliterator gene (<i>DIDO1</i>)	Pro-apoptotic	U	
Phorbol-12-myrestate-13-acetate induced protein 1 (<i>PMAIP1</i>)	Pro-apoptotic	U	87
Oxidative stress induced growth inhibito (<i>OKL38</i>)	Proliferation of normal cells through the regulation of cell death	U	
Transcription factor 1 (<i>E2F1</i>)	Cell cycle regulation and p53-dependent apoptosis	U	
B Cell lymphoma6 (<i>BCL6</i>)	Anti-apoptotic	U	
Tumour protein p53 inducible nuclear protein 1 (<i>TP53INP1</i>)	Induces apoptosis	U	
Tumor necrosis factor receptor (<i>TNFRS10</i>)	Activation of NF-kappa-B	U	
BCL2/adenovirus E1B 19kDa interacting protein 3 (<i>BNIP3</i>)	Apoptosis-inducing protein	U	
Death effectors domain containing 2 (<i>DEDD2</i>)	May play a critical role in death receptor-induced apoptosis	U	
Cyclin dependent kinase (<i>CDK9</i>)	Anti-apoptotic	D	
Protein kinase C, alpha (<i>PKCA</i>)	Anti-apoptotic	D	
Fas apoptotic inhibitory molecule (<i>FAIM</i>)	Anti-apoptotic	D	
Kruppel-like factor 2 (<i>KLF2</i>)	Anti-apoptotic	D	

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Gene	Function	Upregulated (U) / downregulated (D)	Ref
Tumour necrosis factor receptor (<i>TNFRS10</i>)	Activation of NF-kappa-B	U	86,87
Siah E3 ubiquitin protein ligase 1 (<i>hSLAH1</i>)	Apoptosis	U	82
DIF-2 IEX-1L anti-death protein	Anti-apoptotic	D	83
Ephrin type-A receptor 2 ECK	Induces apoptosis in a p53/TP53-independent	D	

and differentiation. In DNA repair group of genes, only *HATB2* (histone acetyl-transferase B subunit) (aka *RBBP7/ RBBP46*) was upregulated; it was functionally involved, as histone acetyltransferase, to import the acetylated histone into nucleus and deposit on up-coming DNA chains for chromatin assembly. Its overexpression would support DNA synthesis and possibly also the cell growth and differentiation.

Among oncogenes (Table V), *c-myc* (myelocytomatosis), *FRA1* (Fos like antigen 1), *PKB/akt* (murine thymone viral oncogene), PP-1a (protein phosphatase 1) and *TNK1* (tyrosine kinase 1) were found to be downregulated. However, MAPKAP kinase was found to be upregulated. This protein helped in protein transport; its upregulation favoured transition of cells into S phase. The underexpression of *c-myc* (the transcription factor) seemed to down play the cell cycle progression and thus favoured apoptosis. Its erratic transcription is described in haematopoietic tumorigenesis^{90,91}. Gene *FRA1* encoded the fos-like antigen. This protein zipped up with JUN proteins to form AP-1 transcription factor for regulation of cell proliferation, differentiation, and transformation. Downregulated *PKB* (AKT1) was a Ser/Thr protein kinase. Usually inactive in G₀ phase of cell cycle; it is activated by (platelet derived growth factor) PDGF through phosphatidylinositol 3-kinase and in this form it negatively regulated apoptosis by phosphorylation of the participating proteins. The downregulated *TNK1* catalyzed phosphorylation of proteins downstream the membrane bound kinase. It is involved in negative regulation of cell growth and has tumour suppressor function.

Cr⁶⁺ exposure influenced the genes⁸⁴ controlling cytoskeleton and cell junction boxes through adhesion/extracellular matrix. All the examined genes were downregulated. *COLa2* (collagen *a2*) encoded pro-collagen to maintain the matrix integrity. *CTNNA1* (catenin alpha 1) encoded the cadherins that in association with actin participated in cell differentiation. *ITGB4* (integrin beta 4) encoded integrins to form cell-matrix or cell-cell adhesions. These proteins regulated

cell integrity and shape. In aberrant expression state, integrins are key players in invasive carcinomas. Gene *ZYX* (zinc binding protein) encoded zyxin (the zinc binding protein) that accumulated in phosphorylated form at the cell surface making focal adhesion sites along the actin cytoskeleton and thus participating in signal transduction mechanisms. *UPAR* is a urokinase type plasminogen activator which regulates migration and invasion of cells and *CI-B18* gene participates in cell adhesion process. Low expression of this gene could dysregulate the adhesion-molecule dependent changes favouring cell transformation.

The examination of genes regulating energy metabolism (Table VI) revealed the increased expression of glucose transport gene *GLUT1* in Cr⁶⁺ exposed cells; it appeared to support major transport of aldoses including pentoses. *AIATR* encoded the serine protease inhibitor that controlled inflammation; its downregulation would support inflammation essential for Cr⁶⁺ toxicity. Commensurate with apoptosis, downregulation of *CI-B18* indicated the loss of cell differentiation potential and bioenergetics to regulate S phase of cell cycle.

Cr⁶⁺ exposure was shown to result in downregulation of several transcription regulators and suppressors (Table VII). A tone-down in level of these proteins could affect the S phase of the cell cycle requiring biosynthesis of proteins and thus influence cell proliferation and differentiation. This study⁸³ suggested again the role of test dose-linked threshold switch which is crucial for Cr⁶⁺ toxicity in cells. However, a paucity of information on the observed cytogenomics *vis-a-vis* toxicity was notable. Cr⁶⁺ induced changes in transcription regulators and suppressors seen in the test system that lacks functional *p53* and *Rb* gene showed the responsiveness of collateral genes and not the target gene to the toxicant.

BEAS-2B cells, 0.25 & 0.5 μM potassium dichromate, 4 week exposure: In BEAS-2B cells, Sun *et al.*⁸⁴ for the first time reported the altered gene expression profile with respect to Cr⁶⁺ toxicity. They established

Table III. Dysregulated genes of cell cycle pathway in Cr⁶⁺ exposed cells

Gene	Function	Upregulated (U) / downregulated (D)	Ref
CyclinD1	Regulates the cell-cycle during G (1)/S transition	U	84,88
Transforming growth factor, beta 2 (<i>TGFβ2</i>)	Regulation of cell growth	D	84
Hedgehog interacting protein (<i>HHIP</i>)	Regulation of cell development	D	
Cdc42-interacting protein 4 (<i>CIP4</i>)	Cell polarization and cell growth	U	82
Cyclin-dependent kinase 1 (<i>p34CDC2</i>)	Cell growth	D	
Proliferation associated protein (<i>Pag</i>)	Cell proliferation	U	
Pigment epithelium differentiation factor (<i>PEDF</i>)	Cell differentiation	U	
Cdk5 activator isoform <i>p39</i>	Cell growth	U	
<i>INK4p19</i>	Induces G1 cell cycle arrest	U	
<i>CROC-1A</i>	Cell growth	U	
Retinoblastoma binding protein 2 (<i>RBP2</i>)	Tumour suppressor	U	
<i>trk E</i>	NGF receptor kinase	U	
Cell division cycle 25 homolog B (<i>CDC25B</i>)	Promote cell growth	D	
Keratin 6 isoform K6e (<i>KRT6E</i>)	Growth marker	D	
<i>Cyclin E</i>	Control of the cell cycle at the late G1 and early S phase	U	86,88
<i>Cyclin G</i>	G2/M phase arrest in response to DNA damage	U	86,88
<i>Cyclin T2</i>	Cell growth	U	86
<i>Cyclin L1</i>	Transcriptional regulator participates in pre-mRNA splicing	U	
<i>Cyclin D2, 3</i>	Regulates the cell-cycle during G(1)/S transition	U	86,88
Cell division cycle 5-like (<i>CDC5L</i>)	Involved in cell cycle control	U	86
Cell division cycle 14 (<i>CDC14A</i>)	Required for centrosome separation	U	
<i>CDC28</i>	Essential for their biological function	U	
Cyclin-dependent kinases regulatory subunit 2 (<i>CKS2</i>)	Binds to the catalytic subunit of the cyclin dependent kinases and is essential for their biological function	U	
Cyclin-dependent kinase inhibitor 1B (<i>CDKN1B</i>)	Involved in G1 arrest	U	
Cyclin B1 interacting protein (<i>CCNB1IP1</i>)	Control of the cell cycle at the G2/M (mitosis) transition	U	
CDC-like kinase 3(<i>CLK4</i>)	Role in the formation of spliceosomes	U	88
CDC-kinase 1,3,4	Involved in pre-mRNA processing	U	86
CDC2-related protein kinase (<i>CRKRS</i>)	Involved in RNA splicing	U	
Cyclin D binding myb-like transcription factor 1 (<i>DMTF1</i>)	Growth arrest	U	
Retinoblastoma 1 (<i>RBI</i>)	Negative regulator of the cell cycle	D	
Growth arrest-specific 7 (<i>GAS7</i>)	Maturation and differentiation of cerebellar neurons	D	
Growth arrest-specific 2 like 1 (<i>GAS2L1</i>)	Involved in the cross-linking of microtubules and microfilament	D	

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Gene	Function	Upregulated (U) / downregulated (D)	Ref
Tubulin alpha-1 (<i>TUBA3</i>)	Major constituent of microtubules	U	88
Cell division-control protein 25B (<i>CDC25B</i>)	Tyrosine protein phosphatase which functions as a dosage-dependent inducer of mitotic progression.	U	
Cyclin dependent kinase 4 (<i>CDK4</i>)	Regulate the cell-cycle during G(1)/S transition	U	
<i>Cyclin K</i>	Transcriptional regulation	D	83
EGF response factor1 (<i>ERF1</i>)	Regulates cell proliferation and differentiation	D	
Lysosomal hyaluronidase 2 (<i>LUCA2</i>)	Cell proliferation, migration and differentiation	D	
Tyrosine kinase (<i>TNK1</i>)	Involved in negative regulation of cell growth ,tumour suppressor	D	
YWHA1 14-3-3n protein eta; protein <i>ASI</i>	Signal transduction, apoptosis and cell cycle regulation	D	
Fibroblast growth factor receptor 1 (<i>FGFR1</i>)	Roles in angiogenesis, wound healing, cell migration	D	
Growth arrest and DNA damage inducible 45 alpha (<i>Gadd45a</i>)	Stimulates DNA excision repair <i>in vitro</i> and inhibits entry of cells into S phase	D	89
Proliferating cell nuclear antigen (<i>PCNA</i>)	Involved DNA replication	U	
Cyclin-dependent kinase 1 (<i>Cdk1</i>)	Control of the eukaryotic cell cycle	U	
Transcription factor Dp1 (<i>Tfdp1</i>)	Can stimulate E2F-dependent transcription	U	
GrpE-like1 (<i>Grpel1</i>)	Essential component of the PAM complex	U	
Tetratricopeptide repeat domain (<i>Ttc27</i>)	Important to the functioning of chaperone, cell-cycle, transcription and protein transport complexes	U	
Heat repeat containing 1(<i>Heatr1</i>)	Involved in nucleolar processing of pre-18S ribosomal RNA	U	
Trefoil factor 1(<i>Tff1</i>)	Stabilizer of the mucous gel overlying the gastrointestinal mucosa that provides a physical barrier against various noxious agents	U	
E2F transcription factor (<i>E2F2</i>)	Control of cell cycle and action of tumor suppressor proteins	U	

Cr⁶⁺ transformed cell lines using 0.25 or 0.5 µM dose and 4-week long exposure condition and investigated the gene expression profile; the experimental conditions simulated the occupational exposure and the associated lung cancer. Microarray analysis using 28,869 gene array revealed the differential expression of genes recording >1.5 fold change in >1200 genes. A major group of genes was found to be commonly dysregulated in 0.25 or 0.5 µM Cr⁶⁺ transformed cell; and functionally associated to biosynthesis, apoptosis, cell junction desmocollin 2 (*DSC2*), desmocollin 3 (*DSC3*), prolyl endopeptidase (*Prep*), extracellular matrix *ADAM* (ADAM metalloproteinase domain 12), TIMP metalloproteinase inhibitor (*TIMP3*), matrix metalloproteinase-2 (*MMP2*), cysteine-rich secretory

protein (*CRISPLD2*), cell adhesion (*CDH6*), claudin 1 (*CLDN1*), L1 cell adhesion molecule (*LICAM*), latrophilin 2 (*LPHN2*), absent in melanoma (*AIM1*), integrin alpha (*ITGA*), collagen type 4 alpha 1 (*COL4A1*), (*COL5A1,2*) and biglycan (*BGN*), laminin beta 1, gamma 2 (*LAMB1*, *LAMC2*), fibrillin (*FBLN1*, *FBLN2*). Mostly upregulated genes associated with cell junction, cell adhesion and extracellular matrix in Cr⁶⁺ transformed cells. Four genes namely latrophilin 2 (*LPHN2*), absent in melanoma 1 (*AIM1*), matrix metallo peptidase 2 (*MMP2*), and cysteine-rich secretory protein LCCL domain containing 2 (*CRISPLD2*) were downregulated. Downregulated genes signified acquisition of carcinoma phenotype in Cr⁶⁺ exposed cells. Upregulation of cyclins in

Table IV. Dysregulated genes of DNA repair, metabolism pathway in Cr⁶⁺ exposed cells

Gene	Function	Upregulated (U) / downregulated (D)	Ref
Casein kinase 2 (<i>CK2</i>)	Cell proliferation, cell differentiation and apoptosis	D	82
Cell division cycle (<i>CDC47</i>)	DNA replication initiation and elongation	D	
Photolyase	DNA repair	U	
Apurinic/aprimidinic endonuclease (<i>APEX</i>)	DNA base excision repair	U	88,89
Deoxyribonuclease I precursor (<i>DNASE I</i>)	DNA cleaving	U	88
DNA topoisomerase II alpha (<i>TOP2A</i>)	DNA Repair	U	
DNA polymerase beta subunit (<i>POLB</i>)	DNA base excision repair	U	
DNA polymerase alpha (<i>POLA</i>)	DNA replication initiation	U	
DNA polymerase delta catalytic subunit	DNA replication and repair	U	
Telomerase protein component 1 (<i>TEP1</i>)	Addition of new telomeres on the chromosome ends.	U	
Replication factor C (<i>RFC37</i>)	Elongation of DNA template	D	83
Histone acetyltransferase B subunit 2 (<i>HATB2</i>)	Histone acetylation	U	
Minichromosome maintenance complex component 5 (<i>MCM5</i>)	DNA replication	D	
Id-1H DNA-binding protein inhibitor <i>ID-1</i>	DNA binding	D	
DNA excision repair protein (<i>XPF</i>)	DNA repair endonuclease	U	85
Growth arrest and DNA-damage-inducible (<i>Gadd45a</i>)	DNA damage repair	U	87, 89
Breast cancer1 (<i>Brcal</i>)	Maintain genomic stability and acts as a tumor suppressor	U	89
MutL homologue 1 (<i>Mlh1</i>)	Involved in DNA mismatch repair system	U	
MutS homologue 2,6 (<i>Msh 2,6</i>)	Involved in DNA mismatch repair system	U	
RAD51, RAD54 homologue (<i>Rad51, 54</i>)	Involved in DNA repair and mitotic recombination	U	
Exonuclease 1 (<i>Exo1</i>)	Functions in DNA mismatch repair	U	
XRCC6 binding protein (<i>Xrcc6bp 1</i>)	Has a role in chromosome translocation	U	
Ercc8	Involved in nucleotide excision repair	U	
BRCA1/BRCA2- containing complex, subunit 3(<i>Brc3</i>)	Metalloprotease that specifically cleaves 'Lys-63'-linked polyubiquitin chains	U	

Cr⁶⁺ transformed cells in contrast to downregulation of TGF β signalling system seemed to support cell transformation. Downregulated genes were related to integrins, collagens, laminin, and fibrillin components. *HHIP* (hedgehog interacting porotein) gene which antagonized hedgehog signalling pathways was underexpressed in these transformed cells. Study revealed the dysregulation of genes associated with cell adhesion, integrin receptor, cell matrix component, metalloproteinase indicating the loss of cell contact inhibition process of normal cells which is a cardinal change in cell transformation.

BJ-hERT cells, 0-6, 9 μ M sodium chromate tetrahydrate, 4-24 h exposure: In this study, researchers developed a sub-population of telomerase-transfected human fibroblast (BJ-hTERT) called as B-5Cr which survived the lethal dose of Cr⁶⁺. These transgenic and apoptosis resistant cells had an increased growth potential⁸⁵. A genotoxic dose of 0-6, 9 μ M Cr⁶⁺ induced apoptosis in BJ-hERT cells but B-5Cr cells, that were resistant to apoptotic dose of 0-6, 9 μ M Cr⁶⁺, ignored the apoptotic signal of secondary Cr⁶⁺ insult. In order to investigate the molecular basis of such a selective clonogenic cell survival response to Cr⁶⁺, the analysis of gene expression

Table V. Dysregulated genes of oncogene pathway in Cr⁶⁺ exposed cells

Gene	Function	Upregulated (U) / downregulated (D)	Ref
Cytochrome-c-like polypeptide	Mitochondrial electron transport	U	82
<i>v-src</i> sarcoma <i>Src</i> -kinase	Proliferation, migration, and transformation	U	
<i>Raf</i> oncogene	Involved in the transduction of mitogenic signals from the cell membrane to the nucleus.	U	
<i>Wnt-13</i> (Wingless-type MMTV Integration site family)	Involved in normal development and in carcinogenesis	U	
Checkpoint suppressor	Regulates cell cycle checkpoints	U	
Cystatin M	Inhibition of metastasis	U	
MAPKAP kinase	Regulates various cellular activities such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis	U	82,83
Carcino associated antigen GA733-2	Functions as growth factor receptors	U	82
Proto-oncogene <i>Jun-B</i>	Proto-oncogene	U	
Breast tumour autoantigen	Plays a role as a transcriptional regulator or as an oncogene	U	
Retinoid x receptor beta	Receptor for retinoic acid	D	
c-myc myelocytomatosis	Oncogenes	D	83
FOS-like antigen 1 <i>FRA1</i>	Transcription the primary growth factor response	D	
Murine thymoma viral oncogene <i>PKB/akt</i>	Regulating cell survival, insulin signalling, angiogenesis and tumour formation	D	
Protein phosphatase 1 <i>PP-1a</i>	Essential for cell division, regulation of glycogen metabolism, muscle contractility and protein synthesis	D	
Tyrosine kinases 1 <i>Tnk1</i>	Tumour suppressor	D	
Ras	Transforming protein	U	88
c-jun n-terminal kinase	Proto-oncogene	U	
Myelocytomatosis (<i>myc</i>)	Oncogene	U	89

was performed after exposure to secondary doses (0-6 and 9 μ M) of Cr⁶⁺ in B-5Cr and BJ-hTERT cells using human genome arrays. In apoptosis resistant transgenic B-5Cr cells, results revealed dysregulation of genes involved in cell cycle regulation, and apoptosis besides the dysregulation of genes in DNA repair irrespective of toxicant exposure period (Table IV). Cell cycle regulatory gene *p21* (*WAF1*, *i.e.* cyclin-dependent kinase inhibitor-1) was upregulated in both cell populations. Those genes that up-regulated in BJ-hTERT but not in B-5Cr were the genes of pathways regulating growth arrest, DNA-damage-inducible and apoptosis involving *GADD45*, *Caspase 3*, *MKP5*, *Myc*, *c-rel* oncogene, *VDAC* (voltage dependent ion channel) gene. Genes that upregulated only in B-5Cr cells and not in BJ-hTERT cells included DNA repair endonuclease gene *XPF* (xeroderma pigmentosum group F), *Collagenase type 4*, *Bcl-xL*, and ligand induced apoptosis signalling

receptor *DR6*. *UV-RAG* gene was upregulated in B-5Cr cells⁸⁵. Pritchard *et al* (2005)⁸⁵ revealed that most of the genes altered by Cr⁶⁺ in transgenic cells were involved in apoptosis, cell cycle and DNA repair pathways. The upregulation of *GADD45*, *caspase 3* in BJ-hTERT cells and *bcl-XL* in B-5cr cells demonstrated the molecular basis for acquisition of apoptosis or clonogenic transformation potential following Cr⁶⁺ exposure in respective type of cells⁸⁵. Upregulation of DNA repair gene in transgenic B-5cr cells indicated the possibility of clonogenic transformation of cells after DNA damage. The study⁸⁵ suggested that cells with competent or upregulated DNA repair mechanism can withstand apoptosis stimulus. Together with 'adequate-to-survive DNA repair' status, clonal expansion of cells can occur; these cells can escape the cell death signal in presence of the upregulated DNA repair mechanism and can thus be selected clonogenically for tumourigenesis.

Table VI. Dysregulation genes of energy metabolism pathway in Cr⁶⁺ exposed cells

Gene	Function	Up regulated (U)	Down regulated (D)	Ref
Na ⁺ /K ⁺ ATPase	Exchange of ions through plasma membrane	U		82
Na ⁺ /K ⁺ ATPase	Exchange of ions through plasma membrane		D	83
A1ATR (Alpha-1-antitrypsin precursor)	serine protease inhibitor		D	
Bone metabolism <i>BMP4</i>	Induces cartilage and bone formation		D	
<i>C1-B18</i>	Involved in mitochondrial respiratory chain		D	
<i>GLUT1</i> (glucose transport gene)	Glucose transport	U		
<i>TRAP</i> (triiodothyronine receptor auxiliary protein)	Involved in cell transformation		D	
Pyruvate dehydrogenase kinase isoenzyme 2 (<i>PK2</i>)	Inhibition of ATP synthesis from glucose degradation	U		82
Na,K-ATPase beta-1 subunit	ATP-dependent Na, K pump	U		
AMP deaminase	Energy generation	U		
Medium-chain acyl-CoA dehydrogenase (<i>MCAD</i>)	Promoting degradation of saturated fatty acid	U		
H(+) ATPase	Energy transfer in mitochondria	U		
Obese gene (<i>ob</i>)	Enhancement of fatty acid degradation	U		
Muscle fatty-acid-binding protein (<i>FABP</i>)	Fatty-acid metabolism	U		
Dihydrolipoamide dehydrogenase	Glucose degradation	U		
ATP synthase subunit C	Biosynthesis of ATP	U		
Flavin-containing monooxygenase 1 and 4 (<i>FMO1</i> & <i>FMO4</i>)	Xenobiotics metabolism	U		88
Flavin-containing monooxygenase oxidase A and B	Xenobiotics metabolism	U		
Cytosolic epoxide hydrolase (<i>EPHX2</i>)	Xenobiotics metabolism	U		
NADH-cytochrome b5 reductase (<i>NB5R3</i>)	Desaturation and elongation of fatty acids	U		
NADPH-cytochrome P450 reductase (<i>NCPR</i>)	Electron transfer from NADP to cytochrome P450 in microsomes	U		
Long chain-specific acyl-CoA dehydrogenase (<i>ACADVL</i>)	Catalyzes the first step of the mitochondrial fatty acid beta-oxidation pathway	U		
Dimeric NADP-preferring aldehyde dehydrogenase (<i>ALDH3A1</i>)	Detoxification of alcohol-derived acetaldehyde	U		
Thiosulphate sulphurtransferase (<i>TST</i>)	Cyanide detoxification, the formation of iron-sulphur proteins and the modification of sulphur-containing enzymes	U		
Oxidized low density lipoprotein (lectin-like) receptor 1 (<i>OLR1</i>)	Internalizes and degrades oxidized low-density lipoprotein	U		84
Natriuretic peptide precursor B (<i>NPPB</i>)	This gene encodes a protein which functions as a cardiac hormone	U		
ELOVL family member 7 (<i>ELOVL7</i>)	Synthesis of saturated and polyunsaturated very long chain fatty acids	U		

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Gene	Function	Up regulated (U)	Down regulated (D)	Ref
Natriuretic peptide receptor C/guanylate cyclase C (<i>NPR3</i>)	Homeostasis of body fluid volume		D	
EPH receptor A4 (<i>EPHA4</i>)	Development of nervous system	U		
Chordin-like 1 (<i>CHRDLI</i>)	Antagonizes the function of bone morphogenetic protein 4 (BMP4)	U		

Human PBMC cells, 0.2-10 μM sodium dichromate, 18 h exposure: In a study to identify biomarkers for Cr⁶⁺ exposure using human PBMC, change in gene expression was probed as the early markers using human gene chip of 18,000 transcripts⁸⁶. Researchers selected PBMC as the test system and test dose of 0.2 μM Cr⁶⁺ to analyze gene expression alteration in immunoregulatory pathways. The test dose was biologically effective as it decreased chemokine secretion; the dose of 0.2 μM was preferred over 10 μM dose that elicited an increase in chemokine secretion and was comparatively higher in view of the investigators. A cluster of 1,659 genes was found to be significantly altered by 0.2 μM Cr⁶⁺. Genes pertaining to pathways of apoptosis, cell cycle regulation, and immune systems were found to be dysregulated (Tables II, III, VIII). Low expression of many immunoregulatory genes like *CD163*, *MRC*, *CD93*, *CD14*, *SLAMF8*, and *STAB1* was noticeable (Table VIII). Several pro-apoptotic genes cell death protein gene *RIPK1*, apoptosis inducing serine/threonine protein kinase *STK17B*, death inducer obliterater *DIDO1* were upregulated and the anti-apoptotic gene *BIRC1* was downregulated indicating predisposition to apoptosis after Cr⁶⁺ exposure (Table II). The upregulation of cyclins (also seen in humans⁴⁵), CDC proteins, cyclin dependent kinases, G2/M transition regulatory protein, and downregulation of growth arrest proteins supported observation of the role of thresholds in Cr⁶⁺ toxicity (Table III). The downregulation of genes encoding protein involved in vital functions like anti-inflammation process revealed the onset of inflammation after Cr⁶⁺ exposure, which strengthened the process of Cr⁶⁺ programmed cell death. The study⁸⁶ indicated the modulation of many vital pathways by Cr⁶⁺ including - the immune response, intracellular signalling, apoptosis, along with the cellular metabolism, RNA transport and binding, biogenesis and organelle organization, and transition metal binding. Cr⁶⁺ induced changes seen in immunoregulatory gene expression may be non specific to toxicant as the test system was immune cell.

Human fibroblast cells, 5μM Potassium dichromate, 16h exposure: Investigations, using human dermal fibroblast cells and human Ref-8V2 Satrix bead chip array⁸⁷, revealed the global gene expression profile of Cr⁶⁺ exposed cells. Relation between dermatitis and Cr⁶⁺ is well known but the gene expression study in this aspect was done for the first time by Sellamuthu *et al*⁸⁷. Dermal fibroblasts were cultured and exposed to 5μM (LC₅₀ value) Cr⁶⁺ concentration. Total RNA was isolated for microarray study. Several apoptosis linked genes involved in p53 signalling pathways were found to be overexpressed, suggesting that apoptosis was p53 dependent. The pro-apoptotic genes were noted to be upregulated and the anti-apoptotic genes downregulated. A cluster of 1153 genes was found to be significantly altered (>1.8 fold). More than 200 dysregulated genes showed relation to the programmed cell death. Besides apoptosis, differentially expressed genes also belonged to cell death, cell viability and survival. This study emphasized on genes involved in apoptosis. Interestingly 300 genes participating in cancer pathway were found to be differentially expressed. Apart from cancer, potential of Cr⁶⁺ to impact pathways of inflammation, immuno-regulatory system, endocrine system, metabolism, and genetic disorder of skin was also noticeable; genes were found to be involved in cell function of growth and differentiation, signalling mechanism, transport, cell cycle regulation, protein metabolism, and cell development. These results validated the development of threshold for apoptosis after Cr⁶⁺ exposure in human fibroblasts.

In vivo study

Sprague-Dawley rats, 0.25 mg/kg b. wt., sodium dichromate for 3 consecutive days: Izzotti *et al*⁸⁸ conducted a study on Sprague-Dawley rats after administering sodium dichromate; test dose was administered intratracheally and repeatedly. Gene expression profile was investigated in rat liver and lung using 216 gene nylon arrays. A cluster of 56 genes was found to be upregulated 3-fold in lung compared to liver (Tables II-VI); none of the examined genes was

Table VII. Dysregulated genes of biosynthesis pathway in Cr⁶⁺ exposed cells

Gene	Function	Upregulated (U) / downregulated (D)	Ref
Excitatory amino acid transporter 4	Glutamate transporter	U	82
Ubiquitin	Degradation of protein	U	82, 83
Putative transmembrane protein precursor (<i>B5</i>)	Glycosylation of protein	U	82
Ribosomal protein L38	Protein synthesis	U	
Translation initiation factor 5 (<i>eIF5</i>)	Promote protein synthesis	U	
Mevalonate pyrophosphate decarboxylase (<i>MPD</i>)	Biosynthesis of steroid	U	
Ribosomal protein L21	Protein synthesis	U	
Ribosomal protein S4 (<i>RPS4X</i>) isoform	Protein synthesis	U	
Ribosomal protein L41	Protein synthesis	U	
Ribosomal protein L34 (<i>RPL34</i>)	Protein synthesis	U	
Threonyl-tRNA synthetase	Activation of amino acid for protein synthesis	U	
Ribosomal protein S17	Protein synthesis	U	
Ribosomal protein S24	Protein synthesis	U	
Ribosomal protein S20 (<i>RPS20</i>)	Protein synthesis	U	
T-complex protein 1 alpha, eta subunit	Folding of proteins upon ATP hydrolysis	U	88
Calnexin precursor (<i>CANX</i>)	Calcium-binding protein	U	
Crystallin beta B1,B2,A1,A4	Components of the vertebrate eye lens	U	
Bleomycin hydrolase (<i>BLMH</i>)	Metabolic inactivation of the glycopeptide bleomycin	U	
Amphiphysin II (<i>AMPH</i>)	Associated with the cytoplasmic surface of synaptic vesicles	U	
Thiol-specific antioxidant protein	Involved in redox regulation of the cell	U	
Hepatoma-derived growth factor (<i>HDGF</i>)	Transcriptional repressor	D	83
Early growth response protein 1 (<i>hEGRI</i>)	Transcription factor	D	
Transcription factor <i>ETR101</i>	Transcription factor	D	
Putative transcription activator <i>DB1</i>	Transcription activator	D	
A1ATR Alpha-1-antitrypsin precursor	Serine /protease inhibitor	D	
Cytochrome P450 1B1 (<i>CYP1B1</i>)	Transcriptional regulator	D	
Cytochrome P450, family 1, subfamily B, polypeptide 1(<i>CYP1B1</i>)	Drug metabolism and synthesis of cholesterol, steroids and other lipids	D	84
Bone morphogenetic protein 2B,4 (<i>BMP2B, BMP4</i>)	Induces cartilage and bone formation	D	83
cAMP-dependent transcription factor 2 (<i>CREB2</i>)	Transcription factor	U	
Ribosomal protein S6 kinase RSK1	Protein synthesis	U	83
Eukaryotic translation termination factor (<i>ERFI</i>)	Regulates cell proliferation and differentiation	D	
Immediate early gene (<i>ETR101</i>)	Transcription factor	D	
Yeast mitochondrial protein import 1 homolog (<i>Fte1</i>)	Protein synthesis	U	
Early growth response protein <i>hEGRI</i>	Transcriptional regulator	D	

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Gene	Function	Upregulated (U) / down regulated (D)	Ref
Pregnancy specific beta-1-glycoprotein 5 (<i>PSG 1,2,3,4,5,7,8</i>)	Proteins released into the maternal circulation during pregnancy	U	84
Zinc finger protein 711 (<i>ZNF711</i>)	Transcriptional activator	U	
Zinc finger protein (<i>ZFY</i>)	Transcription factor	D	
Keratin 34 (<i>KRT34</i>)	Involved in formation of nails and hairs	U	
Transcription factor AP-2 gamma (<i>TFAP2C</i>)	DNA-binding protein which regulate transcription of selected genes	U	
SATB homeobox 2 (<i>SATB2</i>)	Transcription regulation and chromatin remodelling.	U	
Ubiquitin specific peptidase 9, Y-linked (<i>USP9Y</i>)	Degradation of protein	D	
Carboxypeptidase E (<i>CPE</i>)	Biosynthesis of peptide hormones and neurotransmitters, including insulin	D	
ABI gene family, member 3 (NESH) binding protein (<i>ABI3BP</i>)	Inhibits metastasis of tumor cells as well as cell migration	D	
IGF-like family member 1(<i>IGFL1</i>)	Plays critical roles in cellular energy metabolism, growth, development	D	
Prostate transmembrane protein, androgen induced 1 (<i>PMEPA1</i>)	Ubiquitination and proteasome-mediated degradation of androgen receptor	D	
Delta/notch-like EGF repeat containing (<i>DNER</i>)	Neuron-glia interaction during astrocytogenesis	D	
Small nuclear ribonucleoprotein polypeptide N (<i>SNRPN</i>)	Pre-mRNA processing	D	
Eyes absent homologue 4 (<i>EYA4</i>)	Transcriptional activator involved in eye development	D	
Eukaryotic translation initiation factor 1A (<i>EIF1AY</i>)	Involved in protein biosynthesis	D	
Collagen, type V, alpha 2 (<i>COL5A2</i>)	Forms assembly of tissue-specific matrices	U	
DEAD (Asp-Glu-Ala-Asp) box polypeptide 3 (<i>DDX3Y</i>)	ATP-dependent RNA helicase	D	
Ribosomal protein S4 (<i>RPS4Y1</i>)	A component of the 40S subunit	D	

downregulated. Biological annotation analysis of the upregulated genes revealed their roles in pathways like stress response, DNA repair and metabolism, energy metabolism, biosynthesis, apoptosis, oncogene and cell cycle. In stress response genes (Table I), the observed upregulation in *Cu/Zn SOD*, *HSP-70*, and damaged protein degradation enzyme was similar to earlier observation⁸².

Amongst apoptosis related genes (Table II), overexpression of proteins catalyzing the pro-apoptotic activity was novel and included *Bcl-XL*, *Bcl-2* associated death promoter, cyclins, CDC-like kinase, CDC phosphatase, cyclin dependent kinase regulators, microtubule constituents, Ser/Thr protein kinase specific to G1 to S phase transition of cell cycle. Proteins interacting with *Rb* gene product to allow

mitosis, and protein kinase complex for progression of G1 phase of cell cycle were also overexpressed.

This study⁸⁸ revealed upregulation of several genes encoding DNA metabolizing enzymes (Table IV) like DNase, topoisomerases, multifunctional DNA repair enzyme, DNA polymerase alpha/beta/delta1, telomerase associated protein. The changes seemed to be crucial for DNA repair process through endonucleolytic activity, changing topology of DNA, forming constituent of the ribonucleoprotein complex responsible for telomerase activity, and identification and repair of apurinic/apyrimidinic sites.

Cr⁶⁺ upregulated two oncogenes - *ras* and *c-jun* terminal kinase gene (Table V), which encoded cell transforming proteins. Their increased levels

Table VIII. Dysregulated genes of immune system pathway in Cr⁶⁺ exposed cells

Gene	Function	Upregulated (U) / down regulated (D)	Ref
CD163 molecule	Anti-inflammatory	D	86
Mannose receptor, C type 1 (<i>MRC1</i>)	Endocytosis of glycoproteins by macrophages	D	
CD93 molecule	Role in intercellular adhesion	D	
SLAM family member 8 <i>SLAMF8</i>	Lymphocyte activation	D	
Stabilin 1 (<i>STAB1</i>)	Function in angiogenesis, lymphocyte homing, cell adhesion or receptor scavenging	D	
V-set and immunoglobulin domain containing 4 (<i>VSIG4</i>)	Negative regulator of T-cell proliferation and IL2 production	D	
CD36 molecule	Functions as a cell adhesion molecule	D	
Chemokine (C-C motif) receptor 1 (<i>CCR1</i>)	Involved in migration of immune cells	D	
Lymphocyte antigen 96 (<i>LY96</i>)	Cooperates with toll like receptor 4 in the innate immune response to bacterial lipopolysaccharide (LPS)	D	
Fc fragment of IgG (<i>FCGR1a</i>)	Functions in both innate and adaptive immune responses	D	
Colony stimulating factor 1 receptor (<i>CSF1R</i>)	Controls the production, differentiation, and function of macrophages	D	
CD14 molecule	Innate immune response to bacterial lipopolysaccharide (LPS)	D	
Leukocyte-associated immunoglobulin-like receptor 1 (<i>LAIR1</i>)	Plays negative regulatory role on cytolytic function of natural killer (NK) cells, B-cells and T-cells	D	
Leukocyte immunoglobulin-like receptor, subfamily B (<i>LILRB2</i>)	Receptor for class I MHC antigens	D	
leukocyte specific transcript 1 (<i>LST1</i>)	Role in modulating immune responses	D	
Chemokine (C-C motif) ligand 2 (<i>CCL2</i>)	Chemotactic factor that attracts monocytes and basophils	D	
MCP1 Monocyte chemotactic protein 1 precursor	Chemotactic factor that attracts monocytes	D	83
<i>MRP</i> macMARCKS; MARCKS-related protein	Recruitment of monocytes	D	
<i>IL-6</i> Interleukin-6 precursor	Inflammation and the maturation of B cells	D	
<i>NOL1</i> Proliferating cell nucleolar antigen	Regulation of cell cycle and cell proliferation	D	
Toll-like receptor 4 (<i>TLR4</i>)	Activation of innate immunity	D	84
CD24 molecule (<i>CD24</i>)	Modulates B-cell activation responses	D	
Calnexin (<i>Canx</i>)	Calcium-binding protein	U	89
B-cell linker (<i>Blnk</i>)	Plays a critical role in B cell development	U	
Interleukin 1 receptor like 1 (IL1rl1)	Receptor for interleukin-33 (IL-33)	U	
Interleukin 33 (<i>IL33</i>)	Cytokine that binds and signals through IL1RL1/ST2	U	
C-typr lectin domain family 7 (<i>Clec7a</i>)	Stimulates T-cell proliferation	U	
Transporter 2 (<i>TAP2</i>)	Transport of antigens from the cytoplasm to the endoplasmic reticulum	D	
Beta-2 (<i>B2m</i>) microglobulin	Component of the class I major histocompatibility complex (MHC)	D	
CD74 antigen	Plays a critical role in MHC class II antigen	D	

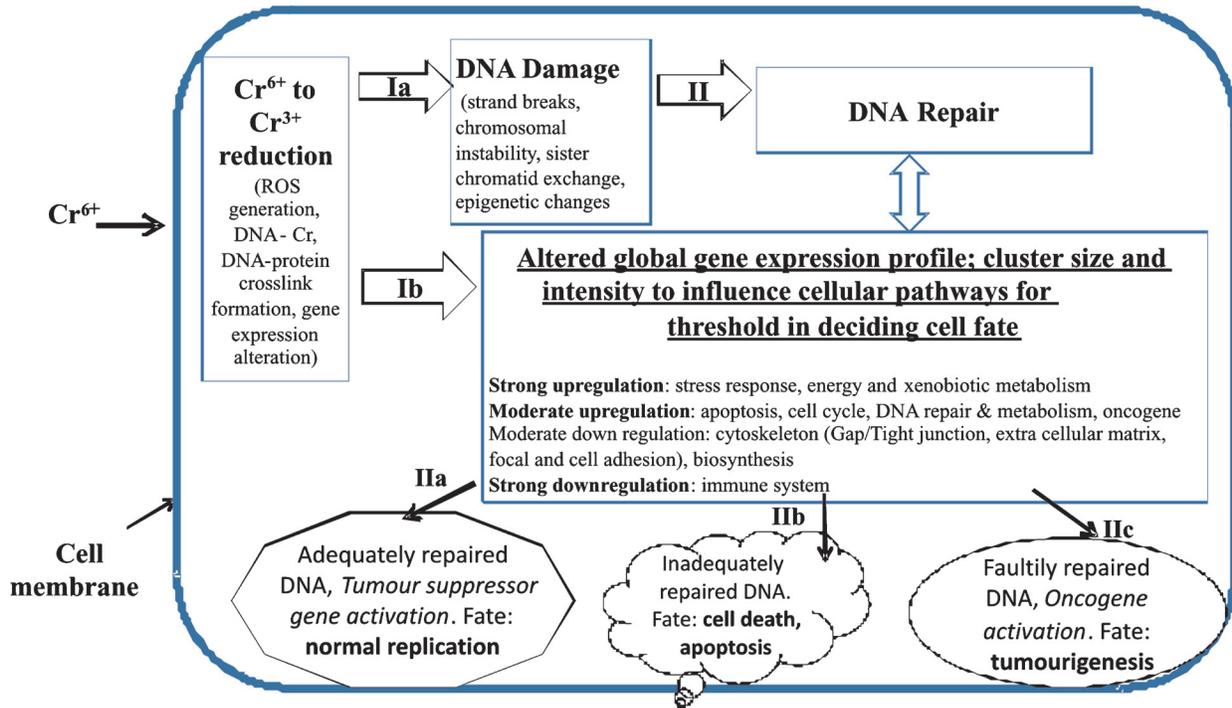


Fig. Sequentially, Cr⁶⁺, after the cellular uptake, can undergo metabolic reduction causing ROS generation, DNA damage (via route '1a'), and/or the altered gene expression in exposed cells (via route '1b'). In absence of 'adequate DNA repair' and the persistence of DNA damage, there could be limited options for the exposed cells. With 'adequate-to-survive DNA repair' option, toxicant exposed cells may either restore the normal process of cell growth & differentiation (via route 'IIa'); or with 'unrepaired or faultily-repaired-DNA option, cells may proceed to toxicity like cytotoxicity, necrosis, apoptosis (via route 'IIb') or to transformation into tumour phenotype (via route 'IIc'). Hypothetically, this is a critical and decision-making step in Cr⁶⁺ exposed cell for cell fate decisions that can be accomplished by the dose specific change in cytogenomics profile, gene expression intensity, status of DNA repair, and pathways for navigation of Cr⁶⁺ toxicity.

could help in growth of transformed cells. The upregulation of genes involved in energy metabolism was also noted (Table VI). Their protein products (namely flavin containing monooxygenases, epoxide hydrolases, Cytochrome b5 reductase, acyl-coenzyme A dehydrogenase, aldehyde dehydrogenase, thiosulphate sulphurtransferase) played critical role in influencing the metabolism of xenobiotics, fatty acid (long chain and very long chain), cholesterol, various aldehydes, lipid peroxides, corticosteroids, neurotransmitters, and sulphur containing proteins. In summary, this study revealed that Cr⁶⁺ administration caused selective changes locally at the site of administration albeit similar as seen in cultured cells⁸². Altered gene expression was seen in Cr⁶⁺ metabolism, stress response, DNA repair, signalling pathways, apoptosis and cell cycle regulation. The study, although limited, was contributory in understanding the mechanisms of Cr⁶⁺ toxicity and suggested an involvement of thresholds in Cr⁶⁺ toxicity.

B6C3F1 mouse, (0, 0.3, 4, 14, 60, 170 or 520 mg/l) sodium dichromate dehydrate for 7 & 90 days: Recently, Kopec *et al*⁸⁹ conducted the study on B6C3F1 female mice to investigate the key events of Cr⁶⁺ induced tumour formation *in vivo*. Dose dependent gene expression profile was examined after 7 and 90 days of regular exposure to Cr⁶⁺ in drinking water. Mouse intestinal epithelial gene expression was investigated using mouse 4X44K whole genome oligonucleotide microarray containing 21307 genes. After seven days, the differentially expressed genes exhibited the comparable expression profiles at ≤ 14 or ≥ 60 mg/l dose. A cluster of 6562 unique differentially expressed genes was identified having >1.5 fold change at one or more doses in duodenum at 8th day. Using the same data filtering criteria, cluster of 4448 unique differentially expressed genes in intestinal samples was noticed at 8th day, and clusters of 4630 and 4845 were detected in duodenum and jejunum, respectively at day 91. In long term exposure study, the

differentially expressed genes exhibited the dissimilar expression profile. Genes in duodenum and jejunum, responding to range of Cr⁶⁺ concentrations (0.3-150 mg/l) participated in functions, e.g. immunoregulation, oxidative stress, cell cycle, growth, proliferation, DNA damage / repair: only the selected intestinal genes that were differentially expressed following exposure to 0.3-520 mg/l sodium dichromate dehydrate have been listed in Tables I, III, V, VIII. Activation of oxidative stress responsive genes *MT2*, *MTF-1*, *Gpx* (glutathione peroxidase) and *Sod* was seen; Ye and Shi⁸² also observed changes in similar genes. In DNA repair pathway, base and nucleotide excision repair gene *Apex1* (base & nucleotide excision repair gene) *Mlh1*, *Msh2* (Mut S protein homology 2), and *Msh6* (Mut S protein homology 6). A comparison of changes in gene expression after 7 or 90 days of exposure to toxicant revealed overlaps of gene expressions. Taken together, the study showed that oxidative stress and the cytotoxicity were the early effects of Cr⁶⁺ exposure and that the differentially expressed genes were associated with oxidative stress, cell cycle and immuno-regulation pathways.

Summary and conclusion

It is apparent that exposure to Cr⁶⁺ results in dysregulated expression of a large group of genes; and the differences in gene identity are related to Cr⁶⁺ test doses / concentrations and test systems. Dysregulated genes are not associated with any specific pathway; however, these may participate in specific cellular function. A few studies^{84,85,89} have revealed the pattern of dysregulation and intensity of changes suitable for apoptosis, cell transformation, or carcinogenicity. The dysregulated genes are uncommon however, the dysregulated pathways are common; and their functions support Cr⁶⁺ toxicity or *in vitro* cell transformation. These studies show a strong upregulation of genes with respect to stress response, energy metabolism, DNA repair, cell cycle regulation; a moderate upregulation of genes with respect to biosynthesis, oncogene, apoptosis; and a strong downregulation of genes linked to immunoregulation, Gap/tight junction, focal/cell adhesion, extracellular matrix, cytoskeleton pathways.

The commonly upregulated genes seen in microarray based studies are related to stress response (*ATF*, *Cu/Zn SOD*, *GPX*, *MTF*), apoptosis (*caspase4*, *Bcl-xl*, *Tnfrs10*), cell cycle regulation (*Cyclin D1*, 2, 3, *Cyclin E & G*), DNA repair (*Apex*, *Gadd45a*), oncogene (*MAPKAP kinase*) and biosynthesis (*Ubiquitin*). The studies on gene expression using assorted genes also

show similar results such as overexpression of *p53*^{38,45-51,69-71,83,84,87}, activation of oxidative stress responsive genes *MTF-1*^{82,89}, induction of histone alkylation^{53,54,83}, upregulation of cyclins^{86,45}. The common genes and pathways dysregulated by Cr⁶⁺ exposure indicate the resultant dynamics of cytogenomics, its intensity, and the possible flow of key mechanistic events irrespective of the toxicant exposure conditions and the test systems to culminate rationally into the expressed biological / clinical effects. These commonly upregulated genes can serve as biomarkers for biomonitoring Cr⁶⁺ exposure; however, more studies using different doses and test systems are needed to validate these logical conclusions.

A critical role of cytogenomics, intensity of altered gene expression, and the flow of crucial mechanistic events in Cr toxicity has been shown by various studies. Thus, a hypothesis can be drawn that the cytogenomics profile and its intensity forms the sub-threshold or threshold level of gene expression to navigate Cr⁶⁺ toxicity as illustrated in the Figure. Sequentially, Cr⁶⁺, after the cellular uptake, can undergo metabolic reduction causing ROS generation, DNA damage (Figure: route '1a'), and/or the altered gene expression (Figure: route '1b') in exposed cells. In absence of 'adequate DNA repair' and the persistence of DNA damage, there could be limited options for the exposed cells. With 'adequate-to-survive DNA repair', cells either may restore the normal process of cell growth and differentiation (Figure: route 'IIa'); or with 'unrepaired or faultily repaired DNA, cells may proceed to toxicity like cytotoxicity, necrosis, apoptosis (Figure: route 'IIb') or to transformation into tumour phenotype (Figure: route 'IIc'). Hypothetically, this may be a critical and decision making step by the altered transcription in Cr⁶⁺ exposed cell. It is hypothesised that the decision making step is governed by the threshold of those altered biochemical reactions or the related interactions catalyzed by abnormally expressed genes or by those respective abnormally functioning pathways which critically manage stress, DNA damage, apoptosis, cell-cycle regulation, cytoskeleton, cell morphology, energy metabolism, biosynthesis, oncogenes' expression, immune system, bioenergetics; even the cross-talks of these dysregulated pathways can be crucial for the onset of toxicity. To conclude, the strong, moderate, or feeble intensity of dysregulation and reversibility of gene-expressions or pathways may depend upon several factors like dose and type (including speciation) of toxicant, duration of toxicants' exposure, type of target cells, their niche microenvironment, and

bioavailability of cellular antioxidants⁹². The resultant differential intensity of dysregulation may become the decision maker to pave way eventually either to opt for reversal to normal differentiation and growth, or to result in toxicity like dedifferentiation or apoptosis commensurate to the exposure conditions in exposed cells of tissues or organs. The hypothesis however, needs more investigations and validation in different test systems to elucidate the affiliation of the critical changes with Cr⁶⁺ toxicity.

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