Chromium induced developments of diseases and their inhibitions by cargos

ABSTRACT

The exposure of hexavalent chromium (Cr-VI) mainly through the inhalation, skin contact or oral administration by the environmental chromium industrial processes causes the major toxicity-induced health hazardous mortality throughout the world. Though reduced chromium (Cr-III), as an essential micronutrient, is utilized to maintain the normal blood glucose, lipid and protein profiles through the metabolism in the body to activate the action of the hormones such as insulin, the high concentration exposures of Cr-VI and Cr-III cause oxidative stress-induced DNA oxidation and adducts, DNA strand breaks and mutations, DNA-protein cross-links, membrane-lipid peroxidation and reduced/altered antioxidant/immune response activities, leading to cellular damage-related various diseases and the development of malignant cancer. Applications of different cargos may inhibit Cr-VI or its intermediates-induced cytotoxicity, immunotoxicity, or genotoxicity through chromium-chelating and scavenging free radical reactive species, restoring antioxidant and immune response activities or arresting disease-oriented signal transductions or other pathways at cellular and molecular levels. This review mainly demonstrates the development of various diseases by chromium exposure and their inhibitions by cargos.

Key Words: Hexavalent chromium; Oxidative stress; Diseases; Cargos; Inhibition

1. INTRODUCTION

Chromium (Cr), the most abundant element in the Earth's crust, is found in the chromite ore combined with iron and oxygen ($FeCr_2O_4$), mineral crocoites ($PbCrO_4$), or others used in the Cr-manufacturing industries in the forms of sodium chromate (Na_2CrO_4), sodium dichromate ($Na_2Cr_2O_7$), chromium oxide (CrO_3), potassium chromate (K_2CrO_4), and potassium dichromate

($K_2Cr_2O_7$), when exposed to humans mainly through the inhalation, dermal contact, and drinking water, causes the development of various diseases [1-11]. Cr normally exists in two stable oxidative states of hexavalent Cr-VI and trivalent Cr-III. Kinetically inert octahedral Cr-III compounds forming primarily positively charged complexes such as $[(Cr(OH)]^{2+}$ react with biological molecules such as DNA, RNA, and proteins, leading to interferences with their normal activities, whereas tetrahedral Cr-VI complexes form oxyanions such as CrO_4^{2-} or $Cr_2O_7^{2-}$, which show their biological consequences but do not interact with macromolecules [1]. Cr-VI composites, upon exposure, can penetrate the cells through the sulfate-anion channels and are reduced to Cr-III compounds with the formation of highly reactive Cr-V and Cr-IV transitionals by the cellular reductants such as glutathiones (GSH) and ascorbates [11]. During the reduction procedures, molecular oxygens are activated and reduced to super oxide anions ($O_2^{\cdot-}$) generating hydrogen peroxides (H_2O_2), hydroxyl radicals, singlet oxygens and superoxides via the Fenton and Haber-Weiss type reactions [11,2]. Consequently, excessively generated reactive oxygen species (ROS) give rise to oxidative stress, overpowering body antioxidant and immune defense system, leading to DNA damage, including the formation of Cr-III-DNA adducts, DNA single and double -strand breaks, DNA-protein cross-links, cellular and sub-cellular damages as well as membrane lipid injuries affecting DNA replication, transcription, and translation, and resulting in genetic mutations and altered gene expressions -oriented carcinogenesis [11,2,8,12]. Though Cr-III compounds at lower concentrations, act as insulin enhancers for the type 2 diabetes, at higher concentrations, they can lead to DNA damage through their accumulations around the cells, altering the cellular surface morphology and entering into the cells via the reactions with the cellular reduction, which finally are converted to Cr-V by oxidation [11,1]. In general, Cr-VI is eliminated through the kidney (<5 nm) as low molecular Cr-III compounds and to a lesser extent

by billiary excretions into feces, while bigger compounds (>6 nm) are secluded within the tissues until reduced or decomposed in size [13].

The application of external antioxidants, antibiotics, and anti-carcinogens may inhibit oxidative stress and disease progression by donating electrons, neutralizing free radicals, and blocking different pathways of disease-promotion. Some cargos such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), quercetin, curcumin, melatonin, luteolin, vitamins C and E, selenium, propyl thiouracil, α-lipoic acid, pterostilbene, and boron compounds have been used to treat as recognized antioxidant, anti-inflammatory, and anti-cancer activities against Cr-VI-induced toxicities-related various diseases [14,15,2,7-11]. This review chiefly elucidates the mechanistic development of various diseases by the exposure of chromium and the inhibitions of diseases by different cargos.

2. MODE OF ACTION OF CHROMIUM

The main probable cellular and molecular mechanisms of Cr-VI-induced disease developments demonstrate the intracellular Cr-VI metabolic process, Cr-VI-induced oxidative and nitrative stresses / damages, Cr-VI-inducted DNA damage and mutagenesis, Cr-VI-induced inflammations, Cr-VI-induced different signaling pathways and genes / proteins expressions, and cancer formations [1-4,16-18] (Figure 1).

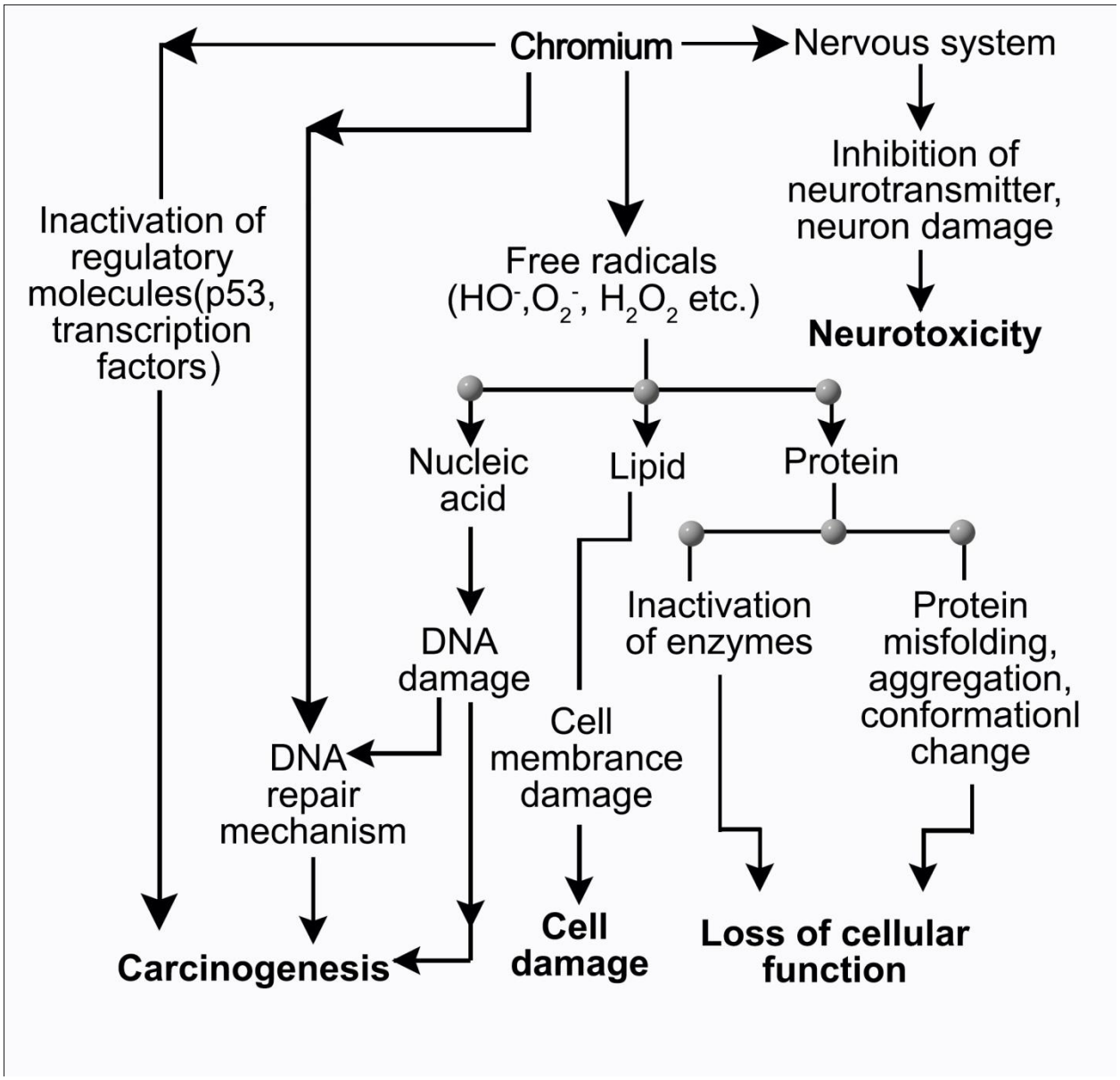

Figure 1. The effects of chromium exposure by several pathways.

2.1 Role of chromium in pathogenesis

It is reported that Cr-III is less toxic than Cr-VI for organisms as Cr-III is less soluble as well as poorly absorbed into the gastrointestinal (GI) cells [2]. However, long-term exposure of Cr-III can cause cellular genotoxicity as Cr-III can enter into the nucleus to react with DNA to produce

cellular mutagenic or clastogenic effects [2]. On the other hand, Cr-VI, due to its higher solubility and potent oxidizing characteristics, can enter into the cells through carrier-mediated transport across the plasma membranes following intracellular reductions to finally Cr-III causing highly cytotoxic effects to trigger cancer and other diseases by various complex mechanisms [2].

2.2 Permeability of chromium into the cell membrane

In the presence of oxygen (O_2), Cr-VI can form chromate (CrO_4^{2-}) in the basic state and dichromate ($Cr_2O_7^{2-}$) in the acidic state [2]. The tetrahedral divalent anion Cr-VI can pass through the plasma membrane passively via chloride phosphate and sulfate anionic carriers against the concentration gradients of the divalent anions$^{(2-)}$ [2]. After absorption via the GI tract, Cr-VI is uptaken by the cells in various tissues and organs and is reduced to the maximal stable Cr-III intracellularly [2]. The extracellular reductions of Cr-VI also are taken primarily in saliva, followed by gastric juices of the stomach and in the intestine by microbes [2]. Generally, Cr-III, impermeable to the membrane, is retained in the area produced on the reduction of Cr-VI [2]. However, a small quantity of extracellular Cr-III can enter the cells by phagocytosis, and its toxic effect occurs in the mitochondria and nuclei [2].

2.3 Chromium-VI metabolism by intracellular / extracellular reductions

Cr-VI can enter the cells as oxyanions following metabolic reductions to Cr-V, Cr-IV, and finally trivalent Cr-III by intracellular enzymatic and non-enzymatic antioxidants, and its entry may be continued by lowering the intracellular Cr-VI concentration and allowing constant entry of the extracellular Cr-VI into the cells to maintain the balance of Cr-VI [4,2]. The main antioxidants, involved in Cr-VI reductions at pH 7.4, include glutathione (GSH), ascorbate

(Asc), thioredoxin, cysteine, lipoic acid, hydrogen peroxide (H_2O_2), fructose, ribose, mitochondrial electron transport complexes, microsomal cytochrome P450 / NADPH-cytochrome P450 reductase, $NADP^+$ oxidoreductase, glutathione reductase (GR), and ferredoxin [2,4]. As a transition metal, chromium may produce ROS through the indirect oxidization of flavin cofactors to semi-quinon radicals ($UQ^{\cdot-}$) and interacts either with the oxygen under the actions of cytochrome P450 or with the peroxides under the actions of hemoglobin, myoglobin, cyclooxygenase, catalase, and peroxidase, or transfers the oxens from the peroxides or oxygen to the metal ions [2]. The reduced forms of Cr may also induce genomic DNA damage, leading to inhibition of DNA replication [4]. Upon GSH depletion, Cr-VI may induce over-expression of cytotoxic heme-oxygenase 1 genes in dermal fibroblasts [4]. In general, extracellular reductions of Cr-VI to Cr-III detoxify the molecules as Cr-III cannot cross the cell membranes, while intracellular reductions of Cr-VI to Cr-III may trap Cr-III within the cells to enable formation of stable Cr-III complexes with nucleic acids and protein, leading to DNA damage [4].

2.4 Chromium-VI-induced oxidative and nitrative stresses / damages

Redox-active metallic chromium may produce ROS through redox cycling [2]. The reductions of Cr-VI involve the usage of H_2O_2 for generating $HO^{\cdot}$ radicals through Fenton-like reactions [2]. In the presence of Cr-VI, endogenous H_2O_2 and $O_2^{\cdot-}$ anions also lead to the generation of $HO^{\cdot}$ radicals through Haber-Weiss reactions [2].

$$Cr\text{-}VI + H_2O_2 \rightarrow HO^{\cdot} + Cr\text{-}V + OH^- \quad \text{.....1}$$

$$Cr\text{-}V + H_2O_2 \rightarrow HO^{\cdot} + Cr\text{-}IV + OH^- \quad \text{.....2}$$

$$Cr\text{-}IV + H_2O_2 \rightarrow HO^{\cdot} + Cr\text{-}III + OH^- \quad \text{.....3}$$

$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + HO^{\cdot} + HO^{-}$ ……..4

Superoxide anions, generated in mitochondria mostly, are also toxic as they react with nitric oxide (NO) to generate peroxynitrite ($ONOO^-$), the potent reactive nitrogen species (RNS) for cellular damage [2]. Several reports have implicated that Cr-VI-induced stress may cause oxidative damage in lipids and proteins by several mechanisms. H_2O_2 and $HO^{\cdot}$, generated by Cr-VI during Haber-Weiss and Fenton reactions, may attack membranous lipids to cause peroxidation and membrane injuries [2,4]. The reduced intermediates of Cr-VI may bind to amino acids, peptides, and proteins to form protein carbonyls in the presence of H_2O_2 [2]. Cr-VI may cause both functional and structural alterations of the plasma membranes through alterations of the proportions of cholesterol and phospholipids by the depletion of GSH [2]. The accumulations of Cr-III extracellularly and intracellularly may induce morphological alterations in the cell surfaces, causing the disruptions of protein-lipid structures of the cell membranes, leading to the loss of cellular integrities [2].

2.5 Cr-VI-induced DNA damage

Cr-VI acts as an inducer of DNA strand breaks and DNA-cross-links at pH 7.4 followed by its reduced Cr-III-form to produce genotoxicity as well as carcinogenicity [2]. Eventually, Cr-III creates oxidative DNA strand breaks-lesions, resulting in the formation of Cr-DNA-adducts, DNA-DNA inter-strand cross-links, and DNA-protein cross-links, succeeding in the inhibition of replication, mutagenesis, and cell death [2].

2.6 Cr-VI-induced DNA damage with GSH

GSH acts as an antioxidant as well as a metal chelator for its thiol (-SH) group, having a high affinity for metals [2]. Generally, GSH can reduce Cr-VI to form Cr-V, Cr-IV, and Cr-III

stepwise through the donation of one electron at a time, while Cr-V and Cr-III can break DNA double strands and activate DNA damaging signal [2]. Primarily, Cr-VI reacts with GSH to form Cr-V-GSH complex and GSH-thiyl radical. Cr-V-GSH complex then reacts with H_2O_2 to produce $HO^{\cdot}$ via a Fenton-type reaction to lead to DNA damage [2].

Cr-VI + GSH → Cr-V-GSH complex + $GS^{\cdot}$ ……..1

$GS^{\cdot}$, further reacts with one molecule of GSH to generate a di-sulfide radical, and the di-sulfide radical, in turn, reacts with molecular oxygen to form $O_2^{\cdot -}$ [2].

$GS^{\cdot}$ + GSH → $GSSG^{\cdot -}$ + H^+ ……..2

$GSSG^{\cdot -}$ + O_2 → GSSG + $O_2^{\cdot -}$ ……..3

The interactions between Cr compounds and DNA may cause strand-breaks along with the production of $HO^{\cdot}$ to cleave DNA, while guanine residues of DNA may react with $HO^{\cdot}$ to produce radical adducts such as 8-hydroxy-deoxyguanosine (8-OH-dG), the marker for oxidative damage in disease-development [2]. The glutathione-Cr-III-DNA cross-links may cause lesions in ternary Cr-DNA phosphotriester adducts owing to the coordination of Cr-III with DNA phosphates, leading to mutagenecity during replication or may cause principal polymerase arresting lesions to block DNA replication [2]. The cellular concentration of GSH is decreased owing to the reductions of Cr-VI and the inhibitory effect of Cr-VI on glutathione reductase, and the reduction-process may be accelerated in the presence of higher GSH synthesized via the γ-glutamyl cysteine pathway through the breakdown of the protein to get its precursor amino acid cysteine [2].

2.7 Cr-VI-induced DNA damage with ascorbate

Asc, the principal non-enzymatic reductant of Cr-VI, causes the damage of nuclear DNA [2]. The reactions of Asc with Cr-VI produce ascorbate radical (Asc.-), carbon dioxide anion radical ($CO_2^{\cdot-}$) and other carbon-based radicals to facilitate the Cr-III-binding with uncleaved DNA [2].

$Cr\text{-}VI + Asc^{H-} \rightarrow Cr\text{-}V + Asc^{\cdot-}$ ……1

$Cr\text{-}V + Asc^{H-} \rightarrow Cr\text{-}IV + Asc^{\cdot-}$ ……2

$Cr\text{-}IV + Asc^{H-} \rightarrow Cr\text{-}III + Asc^{\cdot-}$ ……3

The generated $Asc^{\cdot-}$ radicals may react with each other to form dimers [2]. The dimers further may react with H^+ to form ascorbate and dehydroascorbate (DHA), which are recycled into the Cr-VI reductions [2].

$Asc^{H-} + H^+ \rightarrow Asc + DHA$ ……..4

Asc can enhance the Cr-III complex by reduction of Cr-VI to increase DNA-protein crosslinks and cytotoxicity [2]. In general, Asc and Cr-VI can form Cr-DNA adducts via the multi-coordinated bindings of Cr-III to DNA, resulting in the resistance to dissociation by chelators and the crosslinking of DNA-Cr-DNA by checking the guanine specific region in DNA polymerases [2].

2.8 Cr-VI-induced DNA damage with antioxidant NADPH/NADH enzymes

The microsomal and cytosolic reductions of Cr-VI with quinine oxidoreductase are NADPH-dependent, which occur through the donation of two electrons directly to form Cr-III from Cr-VI without formations of Cr-V and Cr-IV -intermediates [2]. In the presence of NADPH, cytosolic Cr-VI is reduced to Cr-V through the transfer of one electron for forming Cr-V-NADPH complex utilizing GR. Cr-V can cause DNA single-strand breaks and inhibit GR activity, while

Cr-III can produce DNA-protein crosslinks [2]. In the presence of NADPH, the cytochrome P-450 electron transport chain may participate in the microsomal Cr-VI-reduction to form stable Cr-V-NADPH complexes such as the glucose-6-phosphate (G6P)-Cr-V complex [2]. Cr-VI with microsomes and NADPH may produce both the single and double -stranded DNA bindings through the interactions of DNA and enzymatically generated chromium intermediates forming polyribocytidylic acid, polyoxyriboadenylic acid, polyribouridylic acid and polyriboguanylic acid [2]. In the intracellular condition, both Cr-VI and Cr-V complexes may interact with guanine and adenine of DNA, leading to oxidative damage and transfigured to stable Cr-III-deoxyguanosine (dG)-DNA and Cr-III-deoxyadenosine (dA)-DNA adducts for producing bulky DNA implicated as less repairable and utilized to induce mutations [2].

2.9 Cr-VI-induced DNA damage with mitochondrial electron transport chain (ETC) enzymes

Mitochondrial ETC complexes I and IV may reduce Cr-VI to Cr-V and deplete a higher rate of oxygen, while complex-I may donate two electrons to Cr-VI [2]. Mitochondrial complex-II indicating succinate and glutamate serve as electron donors in the ETC complex, while succinate in the presence of ADP facilitates the reductions of Cr-VI and glutamate in the presence of respiratory-chain inhibitors facilitates the reductions of Cr-VI with NAD or FAD -linked substrates [2]. Cr-VI may also inhibit the mitochondrial ETC complexes I and II through the reactions with thioredoxin (Trx) / peroxi-redoxin (Prx) systems for damaging mitochondrial proteins, while the excessive Trx / Prx oxidations and thio-redoxin reductase (TrxR) inhibitions may lead to the loss of mitochondrial membrane potential [2]. The reductions of Trx with Cr-VI occur owing to the inhibition of TrxR to retain the Trx in the reduced state, and the activity of TrxR may not be reversed by the elimination of Cr-VI or the inclusion of electron donor NADPH

for TrxR [2]. Following the above mechanisms, the endogenous antioxidant defense systems are damaged or suppressed after Cr-VI-exposure in both the time and dose -dependent manners [2].

2.10 Cr-VI-induced epigenetic alterations

The epigenetic, mutagenic and heritable modifications on cellular DNA for the exposure of Cr-VI include DNA methylations, micro-RNAs and histone modifications [4]. The soluble sodium chromate induces aberrant DNA methylations of the gpt reporter genes, correlated with the silencing of the gpt genes' expressions and altered expressions of DNA repair and tumor suppressor genes [4]. The methylations of the hMLH1 genes are correlated to the repressions of hMLH1 protein expressions and to the diminished protein expressions in the p16 tumor suppressor genes in the chromate-exposed lung cancer cells [4]. Human lung carcinoma A549 cells exposed to Cr-VI exhibit enhancement of histone H3 Lys-9 dimethylations in the promoter zones of the hMLH1 genes related to the reduction in the mRNA expression [4].

2.11 Chromium-induced immune-responses and inflammations

The higher dosages of Cr-VI-inhalation decrease the phagocytic activities of alveolar macrophages and the humoral immune responses, whereas its lower dosages increase these activities and responses [16]. The proliferation of both the B and T cells and the generation of immunoglobulins by lipopolysaccharide-triggered B cells are restrained by the higher cobalt-chromium or their prosthesis-implant-exposure, indicating systemic immune-suppression [16]. Supplemental dietary chromium on immune responses may enhance anti-ovalbumin-antibody and mitogen-stimulated blastogenic responses of peripheral blood mononuclear cells (PBMC), whereas higher doses of Cr-VI as well as Cr-III, immunized with bovine serum albumin, may suppress the antibody responses [16].

It is reported that the exposure of higher concentrations of cobalt-chromium particles to J774A.1 cells secretes cytokines such as IL-1α, TNF-α, IL-6 and IL-12, and cytokine mRNA expressions such as TNF-α, TGF-β, IL-1α, IFN-α, IL-6 and M-CSF [16]. The exposure of higher concentrated Cr-particles leads to acute inflammations and necrosis accompanying the chronic inflammatory responses with the significant enhancement of chemokine IL-8 homolog GRO-α, amphiregulin (EGFR ligand), cytokine IL-6 as well as matrix metalloprotease-9 resulting in cellular injuries, infiltration of macrophages, neutrophils and eosinophils, fibrosis and hyperplasia -associated cellular atypia [4].

2.12 Cr-VI-induced mutagenicity and tumor formation

A few studies have indicated that the exposure of Cr-VI compounds to hamster cells causes mutations at the hypoxanthine-guanine phosphoribosyltransferase (hgprt) locus and eventual chromosomal aberrations [1]. Another study has demonstrated that the exposure of Cr-VI to primary diploid human foreskin fibroblasts causes mutations to 6-thioguanine resistance [1]. The degree of mutagenicity of the ternary complexes, which occur among Cr, DNA and Cr-VI reducers, depends on the basis of reducers, while binary adducts are usually weakly mutagenic [1]. In the presence of higher Asc levels, the cells exposed to Cr-VI, produce the most mutagenic Asc-Cr-III-DNA complexes [1]. At a lower 2 mM concentration, GSH produces the weakly mutagenic GSH-Cr-III-DNA adducts on pSP189 plasmids, while at a higher concentration (upto 5 mM), GSH generates 4-times higher mutagenic GSH-Cr-III-DNA adducts by non-oxidative mechanisms [1]. Several investigators have reported that intra-tracheal instillations of potassium dichromate in transgenic mice have induced the time and dose-dependent enhancements of mutations at dosages above 3 mg/kg, while the depletions of tissue GSH with buthionine sulfoximine prior to Cr-III exposure have led to the decline in the mutations [1].

Several reports have indicated that the chronic exposures of slightly soluble to highly insoluble chromate particles are capable to produce higher toxicity leading to neoplastic transformation and tumor formation characterized by the aberrant $p16^{INK4A}$ methylations, low p53 mutation rates, loss of MLH1 expressions, enhanced microsatellite instability (MSI), and chromosomal aberrations / damages resulting in aneuploidy, triploidy and tetraploidy [4].

2.13 Cr-VI-induced gene expression

Several studies have exhibited that the Cr-induced regulation of gene expression is dependent on the dosages of Cr-compounds, duration of exposure, and the structures of cellular DNA and proteins [4]. Acute, short-term exposures (2-3 h) to high Cr-VI dosages cause different modes and mechanisms of clonogenic cell deaths compared to chronic exposures (12-48 h) to lower dosages (1-10 µM) [4]. A few investigators have performed experiments on 300 µM Cr-VI-induced gene regulation in human alveolar epithelial carcinoma A549 cells involving redox stress mechanisms, protein synthesis, cell cycle, energy metabolism, and carcinogenesis [4]. In this study, the Src-related genes such as HYL tyrosine kinases and cytoplasmic tyrosine kinase (Cy1) and, as well as MAPKAP kinases, Jun-B and the Raf oncogenes have been up-regulated [4]. The cell cycle and cell growth related genes such as Cdc42 interacting protein 4 (CIP4), proliferation related genes such as retinoblastoma binding protein 2 (RBP2), Cdk5 activator isoform p39 and INK 4p19 genes have been up-regulated by the exposure of Cr-VI [4]. The down-regulated cell growth and cell cycle associated genes include p34Cdc2, Cdc47, Cdc25b and casein kinase II alpha sub-unit by the exposure of Cr-VI [4]. A few researchers have shown that the exposures of 10 µM Cr-VI to A549 cells for 1 and 4 h have significantly decreased the expressions of the human epidermal growth factor receptor 2 (Her 2 / ErbB2) and the epidermal growth factor receptor (EGFR), while the exposures of Cr-VI for 24 h have up-regulated the

expressions of the ErbB2 receptor and the expression of EGFR receptor to the basal level, indicating the transient and selective regulation of Cr-VI for gene expression [4]. Other investigators have demonstrated that the exposure of 10 µM Cr-VI for 4 h to transformed human bronchial epithelial cells (BEAS-2B) and primary smooth muscle cells down-regulates the expression of cyclin k, c-myc, Cyp1b1, PP1A MAPKNPK-2, FGFR-1, Akt1 and HSP90 [4]. Moreover, the report in *in vivo* study has demonstrated the involvement of cell cycle regulatory gene expressions in human lung cancer exposed with chromate implicating the methylation of the cell cycle inhibitor gene p16 with the decline in p16 protein expression [4]. Furthermore, it is also reported that the exposure of Cr-VI (9µM) can enhance the gene expression of p21, p15 and GADD45 and diminish the gene expression of cyclin A, pro-survival Bcl-2, Bcl-$_{W}$ and Bcl-$_{XL}$ [4]. Other reports have indicated that the silent information regulator 1 (Sirt-1) used to regulate gene expression, oxidative stress response and energy metabolism, and participate in the anti-inflammatory and anti-apoptotic mechanisms, and peroxisome proliferation-activated receptor-g co-activator 1α (Pgc-1α) used to improve the ROS defense system, fatty acid metabolism, and regulate mitochondrial biogenesis and oxidative phosphorylation through interactions with particular transcription factors by the induction of SOD and GPx expressions in cells, are deactivated by the exposure of Cr-VI resulting in disorder of mitochondrial dynamics and oxidative stress [2]. On the other hand, nuclear erythroid 2-related factor-2 (Nrf-2) used to induce the expressions of glutathione and thioredoxin antioxidant components, and regulate the expressions of NADPH dehydrogenase quinone-1 (NQO1) for adapting cellular stress, and hemeoxygenase-1 (HO-1) used to stimulate cell proliferation, is deactivated with the exposure to Cr-VI leading to oxidative DNA damage and carcinogenesis [2].

2.14 Cr-VI-induced survival signaling pathways

A few researchers have demonstrated that the exposure of Cr-VI exceeding 10 µM to human airway epithelial cells may activate MAPKs, and extracellular signal-regulated kinase (ERK) including ROS production [4]. A few other researchers have indicated that the exposure of Cr-VI (50 µM) to mouse embryonic stem cells may activate JNK ½, ERK ½ and p38, whereas at non-toxic concentration, Cr-VI in A549 cells may activate selectively c-Jun N-terminal kinase (JNK) and MAPK [4]. Another study has demonstrated that the exposure of Cr-VI (6 µM) to human lung fibroblasts may activate ERK which is not involved as a mediator of Cr-VI-induced cytotoxicity through non-involvement of MAPK [4]. A study has indicated that the exposure of Cr-VI to DU-145 human prostate carcinoma cells may stimulate VEGF secretion and expression for angiogenesis, while Cr-VI exposure may express signal transducer and activator of transcription-1 (STAT-1) as the rate limiting gene regulatory factor to suppress both the VEGF-A and SP-1 transcriptions [4]. Several studies have demonstrated that signal transducers of the Src family of kinases such as Src, Fyn, and Lck are involved in the progression of malignant cancer, while Cr-VI exposed to BEAS-2B cells may selectively activate Fyn and initiate an interferon-like signaling mechanism resulting in STAT-1-dependent interferon stimulated response element (ISRE) gene activation to transactivate interferon regulatory factor 7 (IRF 7) [4]. Other studies have indicated that *in vivo* chromate exposure in mice may activate serine / threonine kinase Akt (phosphorylated at ser-473) to produce lung airway immunoreactives for pulmonary fibrosis and cancer. Further studies have demonstrated that the inhibition of dysregulated protein tyrosine phosphatase (PTP) activity in human lung fibroblasts with the exposure of Cr-VI may lead to increased clonogenic survival, diminished expression of cell cycle inhibitory genes, enhanced expression of cell cycle promoting genes, and the enhancement in forward mutations at the HPRT locus through the Ras / c-Raf -dependent and the MEK/ERK-

independent signaling pathways [4]. A few investigators have also indicated that exposure to Cr-VI may promote tumorigenesis through the ROS-mediated Wnt/β-catenin signaling pathway in a colitis-associated colorectal cancer mouse model [18].

2.15 Cr-VI-induced DNA-lesions repair and side effects

Deficiencies in lesion-specific DNA repair systems exposed by chromium may be associated with the onset and progression of cancer owing to the enhanced frequency of mutations and subsequent activations of oncogenes and inactivations of tumor suppressor genes [1,19].

It is reported that the Cr-VI-induced oxidative DNA damage may activate base excision repair (BER) and the apurinic / apyrimidinic (AP) endonuclease repair system to excise damaged oxidized / alkylated bases through the usages of specific 8-oxo-guanine DNA glycosylase 1 (OGG1) resulting temporary formation of AP sites, while AP endonucleases (APE1) generate SSBs (5′-deoxyribophosphate) recognized by DNA polymerase β (Pol β) and the X-ray cross-complementing group 1 (XRCC1)-DNA ligase III-α (XRCC1-LigIII) complex to remove the 5′-deoxyribophosphate and correct deoxyribonucleotide, and seal the remaining nick in the DNA backbone [1]. Several studies have demonstrated that the exposure of A549 cells to Cr-VI (>25 μM), involved in ROS production and 8-oxo-2-deoxyguanosine formation, may inhibit OGG1 expression at the mRNA and protein levels, resulting in enhanced susceptibility to mutations of the cells [1]. The somatic mutations and the loss of heterozygosity in OGG1, the error-prone repair by BER and the dysfunction of the BER/APE axis, and the deficiency of XRCC1 protein to induce enhancement of chromatid exchanges (isochromatid lesions), all include Cr-VI-induced cancers [1].

Other studies have elucidated that the nucleotide excision repair (NER), the major repair mechanism for Cr-DNA adducts, excises the fragment of the damaged strand lesion following repair synthesis to utilize the intact strand as a template [1]. In mutant CHO cells, NER deficiency due to the loss of xeroderma pigmentosum complementation groups D or F may cause impaired removal of Cr-DNA adducts and enhanced sensitivity to Cr-VI lethality [1]. The removal of DNA lesions by NER may be error susceptible and mutagenic owing to the error-prone ligation and / or repair synthesis [1].

Several other studies have indicated that the loss or defects of the mismatch repair (MMR) system, utilized to correct single base mispairs and deletion/insertion errors during DNA replication, homologous recombination (HR), methylation, base oxidation, and other biological processes, are associated with high spontaneous mutagenesis, genome-wide instability, predisposition to a few types of cancer, resistance to chemotherapeutic components, and abnormalities in meiosis and mammalian systems owing to the loss of MLH1 expression and a high incidence of MSI for chromate-exposed cancers [1]. Few investigators have demonstrated that the ternary DNA adducts are processed aberrantly by DNA-MMR (MSH2 and MLH1 proteins) to form DNA double-strand breaks (DSBs) evaluated by the tumor suppressor p53 binding protein 1 (53BP1) foci formation, implicating genotoxicity [1]. Several researchers have indicated that in the presence of Asc in cells exposed to Cr-VI may inactivate p53 and its Ser15 phosphorylation with the inactivation of ataxia-telangiectasia mutated (ATM) / ataxia-telangiectasia and Rad3-related (ATR) kinase in the S-phase of the cell cycle, resulting in toxicity to DNA-protein cross-links (DPCs) [1,19].

2.16 Cr-VI-induced neurotoxicity

Several studies have demonstrated that the nervous system exposed to chromium has shown the neurotransmitter inhibition and neuronal cells damage, resulting in neurotoxicity [20-22]. It is reported that chromium-exposure may inhibit acetylcholinesterase (AChE) activity, leading to cholinergic and dopaminergic neuronal cells damage, astrogliosis, and oligodendroglioma [20,22]. A few researchers have exhibited that the exposure of chromium may up-regulate the stress response genes such as Nqo1, Nrf2, Ucp2 and HO-1, and the apoptotic genes such as p53, p51, Bax and caspases 9 and 3 [20]. Few other studies have indicated the enhanced levels of metallothionein isoform-1(MT1) and JAK-STAT genes (JAK-1 and STAT-3) induced neuro-inflammation after chromium exposure [20]. Several investigators have also demonstrated that the exposure of chromium may up-regulate early neurogenesis related genes such as elavl3, sox19b, gap43, α-tubulin, neurod-1 and neurogenin-1 with low levels of DNA methylation and down-regulate dnmt-1 and dnmt-3 genes [21].

3. APPLICATIONS OF THERAPEUTIC MOLECULES / CARGOS ON CHROMIUM-EXPOSED CELLS

The reactive nitrogen and oxygen species are neutralized by the non-enzymatic antioxidants and antioxidant enzymes through the removal of free radicals, while antioxidants quench particularly free radicals, chelate redox metals, and regenerate other antioxidants. The main effective enzymatic antioxidants include SOD, CAT, and GPx, while non-enzymatic antioxidants include vitamins C and E, flavonoids, carotenoids, thiols (thioredoxin, glutathione, and lipoic acid), melatonin, and other compounds [2,5-11,14,15,18,23-27].

It is reported that SOD can catalyze the dismutation of $O_2^{\cdot-}$ to O_2 and shorter reactive H_2O_2 as effective anti-tumor antioxidant activity, while CAT can convert H_2O_2 to water (H_2O) and

oxygen (O_2) [2,4,15,18,23]. On the other hand, GPx can react with GSH and H_2O_2 to produce GSSG and H_2O or alcohol with simultaneous GSH production to protect against oxidative stress-induced cellular damage [2,4,23].

Several reports have revealed that vitamin C (ascorbic acid) in the form of $AscH^-$ can react with radicals to produce tricarbonyl ascorbate free radical ($AscH^{\cdot}$) leading to the formation of a poorly reactive terminal semidehydroascorbate radical ($Asc^{\cdot-}$) used to protect cell death as an antioxidant activity, while vitamin E (α-tocopherol) can protect lipid peroxidation through the conversion of α-tocopherol to α-tocopherol radical by the donation of labile hydrogen to lipid or lipid peroxyl radical and recycling to reduced α-tocopherol by ascorbic acid, which is used to inhibit free radical formation and activate endonucleases as antioxidant activity [11,23]. Several reports have also demonstrated that vitamins C and E can reduce the incidence of cancer through triggering apoptosis of cancer cells [8,11,23].

A few reports have elucidated that reduced glutathione (GSH) has the antioxidant capability to maintain the redox state of the protein sulphydryls (protein-SH) for DNA repair and expression, while under an oxidative environment, protein sulphydryls are converted to sulphenic acids (protein-SOH) and thiyl radicals with two electron oxidation and one electron oxidation respectively. These partial oxidized yields react with GSH to form S-glutathiolated protein (protein-SSG), which is reduced again to GSH and restored to protein-SH by the glutathione cycle via glutathione reductase and small glutaredoxin and thioredoxin proteins [23]. The other thiol antioxidants such as lipoic acid and its reduced form-dihydrolipoic acid have the capability to quench ROS, chelate redox metals, regenerate endo and exo-genous antioxidants, and repair oxidized proteins against HIV infection, diabetes mellitus, ischemia-reperfusion injury, and cardiovascular and neurodegenerative diseases [23].

Several reports have shown that carotenoids (β-caratene) may act as antioxidants for their conjugated double-bonded structure used to delocalize unpaired electrons through quenching singlet oxygen with free radicals such as $O_2^{\cdot-}$, $^{\cdot}OH$ and peroxyl radicals ($ROO^{\cdot}$), and pro-oxidant against apoptotic cancerous cell death, atherosclerosis and age-related muscular degeneration [23].

Few reports have demonstrated that selenium, associated with its presence in the thioredoxin reductase and GPx, may protect DNA and other cellular components from oxidative damage and regulate oxygen metabolism, cell division, detoxification, induction of apoptosis in cancerous cells, and activation of oncogenes [11,23].

Several reports have revealed that flavonoidal phenolic antioxidants (PhOH) such as quercetin and luteolin can interfere with the oxidation of lipid and other molecules through the donation of hydrogen atoms to radicals ($ROO^{\cdot} + PhOH \rightarrow ROOH + PhO^{\cdot}$), while the 5-hydroxy group of ring-A and the hydroxyl groups of ring-B may chelate redox-active metals, preventing catalytic breakdown of H_2O_2 (Fenton reaction) [23]. At higher concentrations, they may behave as pro-oxidants utilized for the treatment and prevention of cardiovascular disease, stroke, cancer, and other pathological disorders [23]. Several investigators have reported that flavonoids like quercetin and luteolin have the capability to inhibit DNA topoisomerase II catalytic activity for the induction of DNA and chromosome damage, cell cycle arrest, and caspase-3-induced apoptosis of cancer cells [26,27]. A few researchers have also demonstrated that quercetin and luteolin are able to inhibit Cr-VI-induced malignant cell transformation by targeting miR-21-PDCD4 signaling or multiple signaling pathways [14,15].

It has been reported that the hydrophobic polyphenol curcumin has chelating and antioxidant characteristics owing to its enol-form for chelating positively charged metals and the presence of phenolic β-di-ketone and the methyl groups for free-radical scavenging have been utilized to chelate chromium, scavenge ROS including $O_2^{\cdot -}$, $HO^{\cdot}$, $ROO^{\cdot}$, nitrogen dioxide radicals ($NO_2^{\cdot}$), N, N-dimethyl-p-phenylenediamine dihydrochloride radical ($DMPD^{\cdot +}$), 1,1-diphenyl-2-picryl-hydrazyl free radical ($DPPH^{\cdot}$) and 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\cdot +}$), and protect cells from oxidative stress by inducing Nrf2/keap1/ARE pathway [10]. A few other investigators have reported that curcumin may protect mitochondrial function from Cr-VI-induced oxidative renal damage in rats [11].

It is reported that melatonin as an antioxidant can scavenge ROS ($^1O_2$, $ROO^{\cdot}$, hypochlorous acid (HOCl), $HO^{\cdot}$, H_2O_2, $O_2^{\cdot -}$) and carbon centered free radicals, and up-regulate the expression of antioxidant enzymes through its membrane receptors (MT1 and MT2) and nuclear receptor activation by protecting the cell membrane against lipophilic and hydrophilic oxy-radicals, attenuating the chain reacting lipid peroxidation, and inhibiting the gene expression of lipo-oxygenase and protein oxidation caused by the exposure of Cr-VI [2]. It is also reported that melatonin and its metabolite (AMK) can up-regulate the gene expressions of GPx, SOD, CAT, and GR, reduce the expressions of nitric oxide synthases (iNOS and mtNOS) through the decrement of NO and $ONOO^-$ and chelate the transition chromium forming di, tri, and tetra dentate ligands for detoxification [2]. Few investigators have demonstrated that melatonin can up-regulate antioxidant enzymes to maintain cellular antioxidant defense through enhancing Sirt-1/Pgc-1α signaling and translocating Nrf-2 transcription factor from cytosol to nucleus to increase gene expressions of phase-2 anti-oxidative enzymes such as heme oxygenase-1 (HO-1), c-glutamyl cysteine synthetase (c-GCS) and NADPH: quinine dehydrogenase-1 (NQO1),

suppress the expressions of pro-inflammatory NF-kB/COX-2 signaling, and reduce the formation of Cr-III-DNA adduct and DNA single strand break up caused by the Cr-VI exposure [2]. Several researchers have elucidated that melatonin can increase the activity of complexes-I and IV and ATP production by enhancing the activity of the mitochondrial electron transport chain (ETC) and shielding the apoptotic signaling cascade through restriction of the mitochondrial membrane permeability pores to enhance mitochondrial membrane potential caused by Cr-VI-induced mitochondrial damage [2].

A few reports have shown that propyl thiouracil is capable of reducing Cr-VI promoted oxidative stress with the enhancement of toxic malondialdehyde, protein carbonyl and protein oxidation products in adult mice [11]. Several other reports have shown that the boron compounds (boric acid and borax) are capable to bind the catalytic site of the 26S-proteasome to block its activity used to protect against Cr-VI-induced oxidative stress -promoted lipidemia, neurotoxicity and tumor [9]. A few investigators have also reported that pterostilbene as an anti-oxidative and anti-inflammatory agent is able to protect cells against free radical and endoplasmic reticulum stresses through scavenging free radicals and inhibit pro-inflammatory cytokine production through regulation of the p38MAPK/MK2 signaling and IL-1β-induced NLRP-3-inflammasome activation and apoptosis against Cr-VI-induced dermatitis [7].

4. CONCLUSIONS AND FUTURE PERSPECTIVES

The exposure of Cr-VI causes free radical stress, Cr-DNA adducts, damage of biological molecules such as proteins, enzymes, nucleic acids, DNA, lipids, and apoptosis, mutagenesis, and alteration of various survival signaling pathways, leading to immune responses, genotoxicity, neurotoxicity, and carcinogenesis, while its chronic exposure may lead to damage

of several organs such as the liver, kidney, brain and lungs, causing the development of various diseases in the body [1-5,8,10,11,14,16,20,23]. The antioxidant characteristics of drugs (at lower concentration) have been utilized to protect cells from free radical stress-induced damage, apoptosis or various signaling pathways, mainly through toxic radical scavenging and metal-chelating, while the pro-oxidant features of drugs (at higher concentration, including duration of exposure) have been used to induce apoptotic death of cancer cells or modulate signaling pathways induced by Cr-VI exposure [2,7-10,14,15,23]. However, detailed studies are required for long-term exposure of Cr-VI-induced cytotoxicity, genotoxicity, chelation, metabolism, immunotoxicity, and carcinogenicity, including its levels in cells, tissues, or organs with the evaluation of drug-toxicity *in vivo*. In this regard, vesicular / nanoparticulated drugs / cargos for *in vivo* application may be another approach to reduce drug toxicity, insolubility, and drug-resistance, and to target formulated low quantities of drugs to specific site/s of interest maximally to get higher biological efficiency with the evaluation of administrative routes, bio-distribution, pharmacokinetics, and elimination of drugs against chromium exposure before going to clinics [28-33].

COMPETING INTERESTS

Author has declared that no competing interests exist.

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