ROS

REVIEW ARTICLES

Imbalance of Oxidative and Reductive Species Involved in Chromium(VI)-Induced Toxic Effects

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ABSTRACT | Hexavalent chromium [Cr(VI)] is a common environmental pollutant which can be exposed via digestive tract, respiratory tract, and skin contact, and directly or indirectly cause adverse health effects in humans. Extensive research indicates that generation of reactive oxygen species (ROS) from the reduction of Cr(VI), an important characteristic of Cr(VI) metabolism, is the major mechanism underlying the toxic effects induced by Cr(VI) treatment, such as apoptosis, DNA damage, and carcinogenesis. ROS production is increased in a time-dependent and dose-dependent manner during the reduction of Cr(VI) by various biological systems. Meanwhile, positive regulation of antioxidative defenses also plays important roles in balancing ROS levels. This review summarizes the recent progress on the ROS and antioxidative system induced by Cr(VI) exposure. Some representative signaling cascades and molecules, including AP-1, NF-κB, p53, Nrf2, and Akt are discussed in depth with regard to their involvement in Cr(VI)-induced toxic effects.

KEYWORDS | Hexavalent chromium; Toxic effects; Reactive oxygen species; Antioxidants; Signaling pathway

ABBREVIATIONS | COX, cytochrome c oxidase; GSH, reduced form of glutathione; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, nuclear factor-erythroid 2 (NF-E2)-related factor 2; ROS, reactive oxygen species

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1. INTRODUCTION

Chromium (Cr) and its compounds, which are important industrial and agriculture materials and widely used in various fields, are known to be genotoxic and mutagenic to humans and animals based on a number of epidemiological and animal studies. In fact, hexavalent chromium [Cr(VI)] is classified as the class I carcinogen [1]. With increasing consumption and applications of chromium in metallurgy, electroplating, tanning process, and stainless steel industry, Cr(VI) contamination has become a global environmental problem [2, 3]. There are three possible exposure routes to Cr(VI) and its compounds: inhalation, dermal contact, and oral ingestion [4]. Accumulation of Cr(VI) exposure is associated with increased risk of allergic dermatitis, skin ulcers, perforation of nasal septum, damage to the liver, kidneys, heart, and reproductive organs, and even cancer development [5, 6].

Trivalent chromium [Cr(III)] and Cr(VI) are two major forms of chromium. The fate of chromium in the environment is dependent upon its oxidation state. Cr(III) is considered an essential trace element which is involved in glucose and lipid metabolism, and in potentiating the action of insulin in the human body [7–9]. Cr(VI) closely resembles SO_4^{2-}/HPO_4^{2-} and can cross the cell membrane via an anion carrier [10, 11]. Once inside in the cell, Cr(VI) can be reduced to Cr(V), Cr(IV), and Cr(III) by low molecular weight molecules, enzymatic, and nonenzymatic reductants [12–14]. During the reduction process, these reactive chromium intermediates can generate a variety of reactive oxygen species (ROS), which is considered as an important step in the mechanism of Cr(VI)-induced DNA damage and characteristic of Cr(VI) metabolism [15–17].

ROS are a class of normal metabolic products including superoxide anion radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($OH^\bullet$), hydrogen peroxide (H_2O_2) and play an important role in biological systems [18]. It is found that ROS are essential for various biological processes and can act as second messengers in normal cells [19, 20]. However, the dynamic balance between ROS production and clearance could be destroyed by biological pathways after overexposure to Cr(VI), which leads to a number of harmful reactions including lipid peroxidation, oxidative tissue damage, cellular injury, and DNA damage [21, 22]. Many studies suggested that the toxic and carcinogenic effects of Cr(VI) may be partially associated with the overt production of ROS and associated oxidative stress [17, 21, 23]. However, the ROS-induced toxic effects and mechanisms of Cr(VI) toxicity are not fully understood. This paper summarizes the available evidence for the involvement of Cr(VI)-induced ROS and cellular antioxidants in the process of Cr(VI) reduction. The potential role of the redox signaling cascades and the antioxidant network in protecting against the deleterious action of Cr(VI)-induced ROS are also discussed in the article.

2. Cr(VI) REACTIONS AND ROS SOURCES

Extracellular Cr(VI) iron itself is not a cytotoxic agent and cannot react with DNA in vitro or in isolated nuclei. But, it can induce a wide variety of DNA lesions including Cr-DNA adducts, DNA-protein crosslinks, DNA-DNA crosslinks, and oxidative damage by producing a series of reactive intermediates and ROS in cells [23, 24]. ROS were increased in a time-dependent and dose-dependent manner in the reduction of Cr(VI) by various biological systems, in particular, microsomes, mitochondria, and ascorbate [25, 26].

The oxidation-reduction system and some reduction molecules contribute significantly to the maintenance of cellular redox balance when Cr(VI) is taken up by cells [13]. For instance, glutathione and ascorbate rapidly form a complex with Cr(VI), followed by a slow reduction of Cr(VI) to yield Cr(V) (Reactions 1 and 2 in **Figure 1**). Then, Cr(V) was found to cause DNA damage by reacting with H_2O_2 forming $OH^\bullet$ via the Fenton reaction (Reaction 3 in **Figure 1**). Additionally, the thiol radical ($GS^\bullet$) can further react with other thiol molecules in oxygenated tissues to

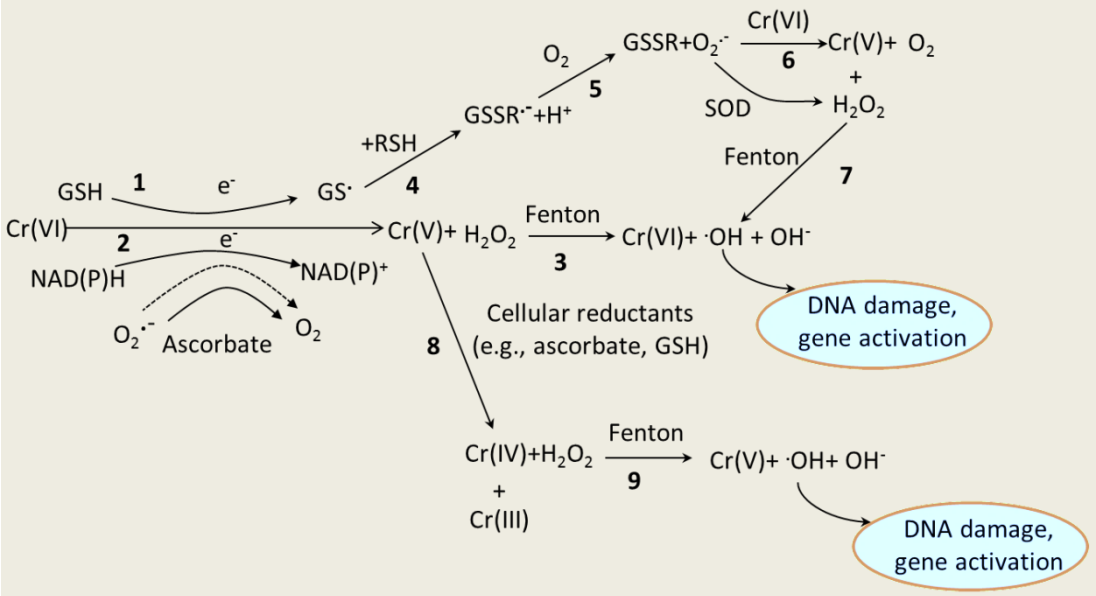

FIGURE 1. Biological reductants of Cr(VI) and ROS reactions. As illustrated, reduction of Cr(VI) by biological reductants results in the formation of ROS, especially hydroxyl radicals (OH˙ or ˙OH) via the Fenton-type reaction, leading to biological damage. This scheme is based on Refs. [17, 23].

produce $O_2^{\bullet-}$ to cause cellular damaging effects (Reactions 4 and 5 in **Figure 1**). In addition, $O_2^{\bullet-}$ can further reduce Cr(VI) to Cr(V) (Reaction 6, in **Figure 1**) which then can react with H_2O_2, leading to the DNA damage via the Fenton reaction (Reaction 7 in **Figure 1**). Meanwhile, Cr(V) can also be reduced by cellular reductants, such as ascorbate and reduced form of glutathione (GSH), to Cr(IV) (Reaction 8 in **Figure 1**), which enters the Fenton reaction to generate OH˙ (Reaction 9 in **Figure 1**) [17, 23].

3. RELATIONSHIP BETWEEN Cr(VI) EXPOSURE AND MITOCHONDRIAL DAMAGE

Mitochondria are the oxidation reaction center and provide most of the cellular energy as the site of adenosine triphosphate (ATP) generation by transmitting the electrons to oxygen through the respiratory chain [27]. Cr(VI) exposure can change the expression of mitochondria-related genes include mitochondrial respiratory chain complexes I (NADH1), IV(COX1), and V(ATP-6S), and nuclear-encoded genes such as hexokinase 2 (HK2), M2 pyruvate kinase (PKM2), mitochondrial voltage-dependent anion channel (VADAC1), and adenine nucleotide translocator 1(ANT1). For example, after Cr(VI) treatment for 12 h in L-02 hepatocytes, COX1 gene expression decreased obviously. However at 24 h treatment, NADH1 and ATP-6S genes increased distinctly, while COX1 gene expression was still low. These results indicated that Cr(VI) could affect the electron transfer on the respiratory chain and energy metabolism, further leading to the apoptosis of hepatocytes by altering the mitochondria-related genes [28, 29]. Furthermore, it is suggested that ROS induced by Cr_2O_3 can cause a decrease in mitochondrial membrane potential and an increase in the ratio of BAX/Bcl-2 leading to mitochondria-mediated apoptotic cell death [30]. When the mitochondrial DNA is lost, Cr(VI)-induced ROS production and apoptosis were significantly decreased [28, 29].

Mitochondria have long been known to generate a series of ROS in a dose-dependent manner with Cr(VI) exposure [30]. It is found that 50 μM Cr(VI) can induce an increase in liver mitochondrial electron leakage from the respiratory chain at the ubiqui-

none binding sites of complexes I and III, and the total ROS content and oxidative damage were significantly decreased by 200 μM GSH [31]. It is suggested that activation of autophagy could repair mitochondrial dysfunction to protect hepatocytes potentially by removing damaged mitochondria [32]. It is found that ROS are essential in Cr(VI)-induced caspase-3 activation, and Cr(VI) induces ROS-dependent caspase-3 activation by inhibiting mitochondrial respiratory chain complex (MRCC) I activity. Cr(VI) exposure could induce S and G2/M phase cell cycle arrest by targeting TOF1, Mrc1, CDK2, Cyclin E, BubR1, Mad2, Cyclin B, and CDC25 via p53, Akt, NF-κB, and MAPK pathways [33, 34].

4. Cr(VI)-INDUCED ACTIVATION OF ROS-MEDIATED SIGNALING PATHWAYS

The toxic effects of Cr(VI) may involve a number of cellular regulatory proteins or signaling proteins participating in cell growth, apoptosis, cell cycle regulation, DNA repair, and differentiation. Proteomic analysis revealed that most of expression alteration proteins are correlated with ROS-elicited responses, which are involved in the Cr(VI)-induced toxicity and carcinogenesis [33, 35]. The toxic effects of Cr(VI) are tightly linked with the activation of redox-sensitive factors including Akt, AP-1, NF-κB, Nrf2, mitogen-activated protein kinase (MAPK), and p53, among others by the antioxidant network signals via the electron chain transmission and intracellular redox molecules [36]. The functions encoded by these redox-sensitive factors have been involved in the expression of protective genes that repair damaged DNA, power the immune system, arrest the proliferation of damaged cells, and induce apoptosis.

4.1. AP-1

Activator protein 1 (AP-1) is localized in the nucleus and is important for cell growth and differentiation. It is assembled through dimerization of basic region-leucine zipper (bZIP) proteins including the Jun (c-Jun, JunB, JunD), Fos (FosB, Fra-1, Fra-2), Maf, and ATF subfamilies. Both c-Jun dimers and c-Jun–c-Fos heterodimers have a conserved Cys motif (KCR) and are the important transcription factors for DNA binding [37]. The redox regulation of c-fos is partially due to the redox modification of these residues, which influences AP-1 activity [38, 39]. The activation of AP-1 by Cr(IV) was dose-dependent [40]. Overexpression of TAM67 (a dominant-negative mutant of c-Jun) can dramatically inhibit the COX-2 induction by Cr(VI). It was indicated that c-Jun/AP-1 pathways were required for Cr(VI)-induced COX-2 expression [41].

AP-1 activation is often mediated by Cr(VI) in the presence of H_2O_2 as well as several cytokines and other physical and chemical stresses [40, 42]. Van-Landingham et al. [43] found that this activation can be blunted by catalase, hydroxyl radical scavengers, and deferoxamine (an iron-chelator), which suggests that Cr(V)-mediated generation of hydroxyl radicals may be involved in the activation [40]. It was also reported that p38 MAPK cascades were involved in the activation AP-1 after Cr(VI) exposure. Similar to hydroxyl radical scavengers, the specific inhibitor for p38 MAPK also can attenuate AP-1 activation induced by Cr(IV) exposure [40]. In addition, the oxidation of nuclear thioredoxin-1 (Trx1), which can be used to assess the impacts of oxidants on the thiol redox status of the cytosol, can prevent the binding of AP-1 to DNA to decrease cell proliferation and enhance cell death [13]. Although Cr(VI)-induced activation of AP-1 is accompanied with ROS generation, a simultaneous examination of the regulating mechanism and AP-1 activity is still needed to better define these relationships in response to Cr(VI).

4.2. p53

p53 is a tumor suppressor protein which guards a cell-cycle checkpoint, and inactivation of p53 allows uncontrolled cell division. Under normal conditions, the redox-sensitive transcription factor p53 is repressed through its binding to mdm2, which targets p53 for degradation [44]. Cr(VI) treatment causes significant cytotoxicity and genotoxicity. To provide a chance for DNA damage repairing, more p53 protein could be produced to induce the cell cycle arrest at the junction of G1/S and G2/M phase and to control the progression of cell cycle [45, 46]. A hydroxyl radical scavenger can significantly suppress activation of p53. During Cr(VI) reduction by NAD(P)H in A549 cells, increased formation of hydroxyl radicals following Cr(V)-catalyzed decomposition of H_2O_2 could enhance p53 activation [17].

In addition, oxidation of nuclear Trx1 by Cr(VI) treatment would also be expected to interfere with

the binding of p53 to DNA through NF-κB, and the redox state of nuclear Trx1 may be a critical determinant of p53 binding upon Cr(VI) treatment [13]. Furthermore, studies also have shown that Cr(VI) may induce apoptosis through p53-independent mechanisms.

In vitro studies showed Cr(VI) induced apoptosis by activating p53 protein [34]. Moreover, it was shown that Cr(VI)-mediated ROS generation induced a mitochondria-mediated and caspase-dependent apoptosis in skin epidermal cells through activation of p53 [47]. However, some other studies have reported conflicting results regarding the effect of Cr(VI) on p53. For example, Cr(VI) could still induce DNA damage, mitochondrial injury, oxidative stress, and apoptosis in Hep3B cells in an ROS-dependent manner with the loss of functional p53 [48].

4.3. NF-κB

Nuclear factor κB (NF-κB) is a nuclear transcription factor that regulates the expression of a large number of genes that are critical for the regulation of apoptosis, viral replication, tumorigenesis, and inflammation, and involved in various autoimmune diseases. The enhancing domain of target genes was interacted in the configuration of a dimer of NF-κB /Rel/Dorsal (NRD) family, which is probably an important contributor to Cr(VI) carcinogenesis [49]. NF-κB can be activated by Cr(VI) reduction, and catalase and scavengers of hydroxyl radicals can inhibit the NF-κB activation to alleviate Cr(IV) toxicity [41, 50]. Furthermore, high expression of NF-κB was observed in club cells treated with Cr(IV), indicating NF-κB was upregulated in club cells during Cr(IV) reduction with repetitive Cr(VI) exposure [41, 51].

Chen and associates [40] demonstrated that p38 MAPK and IκB kinase were involved in Cr(VI)-induced activation of NF-κB signal pathway, and blockage of NF-κB signaling pathway can inhibit Cr(IV)-induced toxicity. Meanwhile, it is interesting that the NF-κB activity cannot be enhanced at larger Cr(VI) doses [52]. It has been revealed that NF-κB can activate the expression of TNF-α to enhance ROS production [53]. Then, TNF-α rapidly causes the oxidation of Trx, which could regulate NF-κB translocation activation, consequently blocking TNF-α-induced ROS generation and apoptosis with Cr(VI) treatment [54].

4.4. Nrf2

Transcription factor nuclear factor-erythroid 2 (NF-E2)-related factor 2 (Nrf2) is one of the important regulators in cell defense against chemical/oxidative stress. It can upregulate a plethora of genes under the control of the antioxidant-response element (ARE), including glutathione S-transferases, NAD(P)H: quinone oxidoreductase 1 (NQO1), γ-glutamylcysteine ligase, heme oxygenase-1 (HO-1), UDP-glucuronyltransferases, glutathione peroxidases, and others [55, 56]. Acute Cr(VI) treatment in human bronchial epithelial cells increased inflammatory responses and expression of Nrf2 [55, 56]. The absence or activation disturbance of Nrf2 can directly change the oxidative susceptibility of the cells to Cr(VI) exposure. The activation of Nrf2 provided protection against Cr(VI) exposure, which correlated with induction of cytoprotective genes HO-1 and NQO1 [55, 56]. Nrf2 induction by Cr(VI) may depend on the cell types, animal species, and other specific conditions [13]. He and coworkers [57] found that induction of hydroxyl radicals was partially Nrf2-dependent, and lacking Nrf2 could elevate ROS production and apoptosis, which were further increased markedly by Cr(VI), indicating a protective role of Nrf2 against Cr(VI) toxicity. While studies suggested that ROS, rather than Nrf2, play a critical role in Cr(VI)-induced inflammation, a constitutively high level of Nrf2 is also important for Cr(VI)-induced cell transformation [58].

4.5. Akt

Akt (v-Akt murine thymoma viral oncogene)/PKB (protein kinase-B) is a serine/threonine kinase that is involved in mediating various biological responses, such as inhibition of apoptosis and stimulation of cell proliferation [54]. It has been demonstrated that ROS formation can activate the Akt pathway, which is upstream of the NF-κB pathway, and can, therefore, induce the activation of NF-κB [59]. The activation of Akt is generally triggered by the interaction of receptor tyrosine kinases, growth factors and cytokines. Cr(VI) can activate the Akt pathway thereby increasing IL-1α and TNF-α production, and this can inhibit ROS-induced cell death and cytokine expression by *N*-acetylcysteine (NAC) in vitro and in vivo studies [36]. In lung epithelial cells and rat hepatoma cells, it was found that, via the effects of ROS, Cr(VI) could

activate cell signaling, including the Akt pathway [60]. Son et al. [61] found that ROS is a key mediator of Cr(VI)-induced carcinogenesis through the activation of PI3K/AKT-dependent GSK-3β/β-catenin signaling and the promotion of cell survival mechanisms via the inhibition of apoptosis and autophagy. It is postulated that activated Akt in lung airway epithelium in vivo may be an early response to genotoxic exposure [61].

4.6. Other Possible ROS Related Signaling Pathways

Accumulating evidence reported the function of ROS in Cr(VI)-induced toxicity and carcinogenesis. Activation of the Cr(VI)-related signaling pathways is closely bound up with the generation of ROS. Despite the ROS-regulated redox sensitive signal proteins, such as AP-1, p53, Akt, and NF-κB, Nrf2 and MAPK pathways might also affect the release of cytokines, such as TNF-α. The mechanisms of oxidative and reductive species involved in Cr(VI)-induced toxic effects are complicated. Many factors or signaling pathways can regulate the cell apoptosis, mutation, carcinogenesis induced by Cr(VI). For instances, Ca^{2+} influx was significantly increased after Cr(VI) exposure for 6 h, which was closely related to Cr(VI)-induced red blood cells damage [62] . It is suggested that Cr(VI) can induce human lung bronchial epithelial cell malignancy via ROS-dependent activation of miR-21-PDCD4 signaling [63]. Furthermore, Bcl-2 protein negatively regulated superoxide-induced apoptosis in response to Cr(VI) exposure through the ubiquitin-proteasomal pathway [64]. Moreover, it is found that the carcinogenicity of Cr(VI) may occur partly through ROS-mediated Wnt/β-catenin signaling pathway, which has a critical role in carcinogenesis [65].

5. ANTIOXIDANT DEFENSE MECHANISMS IN Cr(VI) TOXICITY

As a strong oxidant, Cr(VI) can induce a whole spectrum of reactive intermediates and ROS to cause multi-system disorders, which is an important characteristic of Cr(VI) metabolism [66]. To defend Cr(VI)-induced toxic effects, ROS are balanced by the enzymatic and non-enzymatic antioxidants to prevent damage to DNA, lipids, proteins, and other biomolecules [67, 68]. Ascorbate, GSH, cysteine, lipoic acid, and NADPH can reduce Cr(VI) at physiological conditions. As ascorbate and GSH are abundant in mammalian cells and possess relatively lower reduction potentials, they may be the main non-enzymatic factors for the reduction of Cr(VI) [69]. When both reducers were present, ascorbate dominated in Cr(VI) metabolism and led to the loss of the hypersensitivity to clonogenic killing by Cr(VI) in the presence of methoxyamine, which inhibits base excision repair of oxidative DNA damage in human lung H460 cells. Wong et al. [69] found that ascorbate-driven metabolism of Cr(VI) shifts its genotoxicity toward nonoxidative mechanisms.

In vitro experiments indicated that ascorbate reduced Cr(VI) at a higher rate than other reductants; for example, the rate for ascorbate was 10 times that of GSH [70, 71]. However, Martin et al. [66] suggested that GSH could reduce the oxidative damage of Cr(VI), while ascorbate could enhance the genetic toxicity of Cr(VI) by augmenting the activity of Cr(IV) intermediate products in the reduction process. On the other hand, Wong et al. [69] indicated that due to the lack of ascorbate, Cr(VI) reduction mainly depended on GSH leading to more production of ROS and Cr intermediates, suggesting that the reduction of Cr(VI) by ascorbate may produce less ROS and Cr intermediates. The exact mechanisms of Cr(VI) toxic defense are very complicated and need to be further elucidated.

Additionally, it was found that supplementation of antioxidant substances might help alleviate the toxicity of Cr(VI) in both in vivo and in vitro experiments. Singh and Chowdhuri [72] found that ascorbate supplementation could reverse the increase of ROS and oxidative stress which led to apoptotic death in the tested brain cells of Cr(VI)-exposed Drosophila. Moreover, the antioxidant *N*-acetylcysteine (NAC), a potent ROS scavenger, could increase GSH levels and prevent the damage produced by Cr(VI) exposure. Pretreatment of astrocytes with NAC attenuated the ROS production and mitochondrial membrane potential loss in Cr(VI)-treated astrocytes, and significantly increased the survival of astrocytes [73]. NAC pretreatment also led to a decrease in apoptosis and autophagy in HaCaT cells treated with Cr(VI) [74]. NAC inhibited Cr hypersensitivity in coadjutant Cr-sensitized albino guinea pigs by suppressing the effects of ROS. In addition, it was revealed that the natural dietary fla-

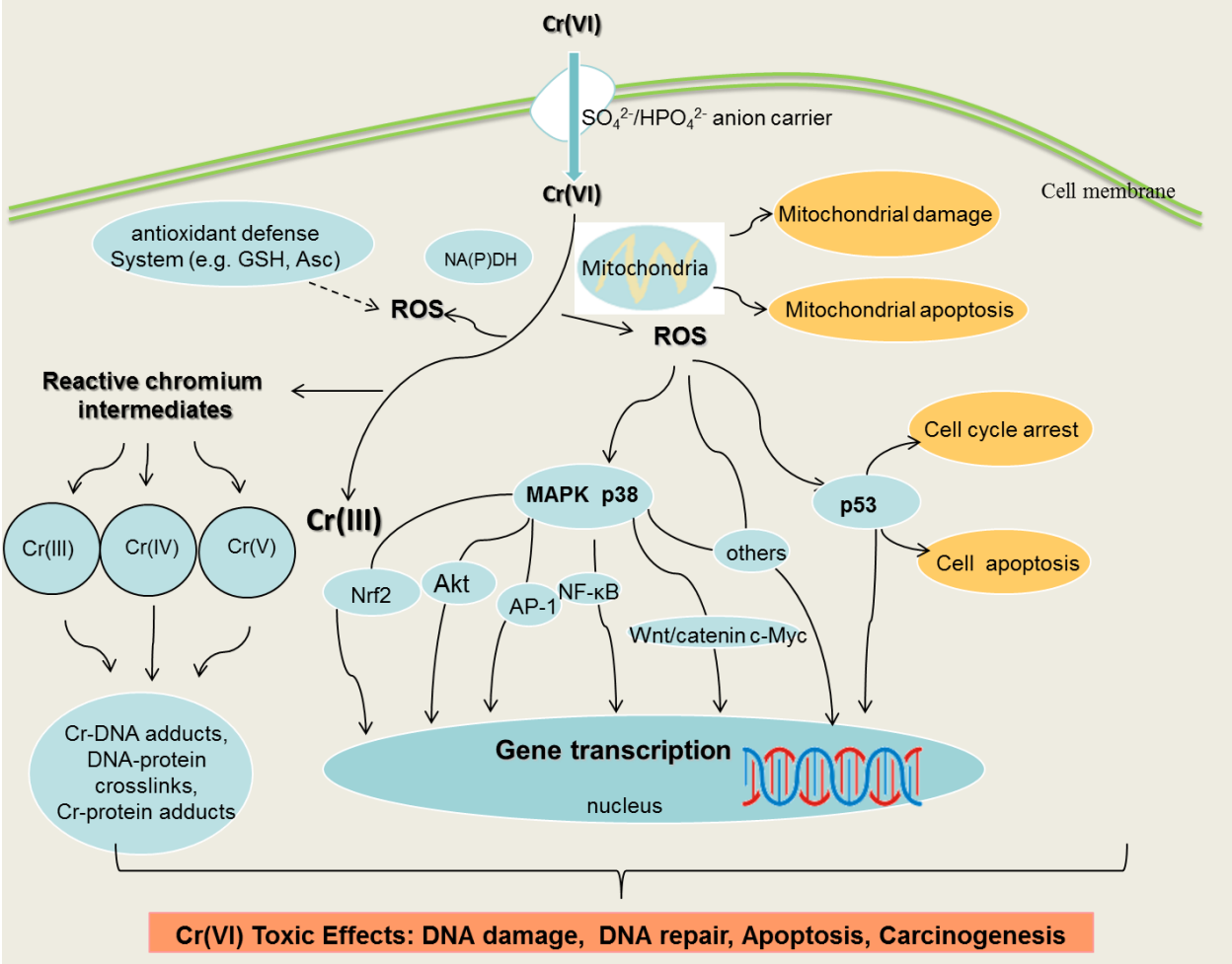

FIGURE 2. ROS and Cr(VI)-induced signaling pathways. See text (Section 4) for detailed description. Asc denotes ascorbate.

vonoid luteolin, which possesses potent antioxidant and anti-inflammatory properties, could prevent BEAS-2B cells from Cr(VI)-induced carcinogenesis by scavenging ROS, and therefore serves as a potential chemopreventive agent against Cr(VI)-induced malignant transformation [73].

6. CONCLUSION

Collectively, evidence suggests that ROS may be a key mediator of Cr(VI)-induced dysregulation of cell growth, differentiation, apoptosis, cell cycle, and DNA repair, leading to carcinogenesis. ROS are increased in a time- and dose-dependent manner in the reduction of Cr(VI) by various biological systems, in particular, microsomes, mitochondria, and ascorbate. Cr(VI) can also increase mitochondrial electron leak from the respiratory chain to reinforce the genetic damage and apoptosis. The toxic effects of Cr(VI) are tightly linked with the activation of redox-sensitive factors such as Akt, AP-1, NF-κB, Nrf2, MAPK, and p53 via the recycling of electrons through the antioxidant network. As summarized in **Figure 2**, the Cr(VI)-related signaling cascades lead to the activation of several redox-regulated factors

(AP-1, p53, NF-κB, Nrf2, Akt, MAPK). To defend Cr(VI) toxic effects, both enzymatic and non-enzymatic antioxidants are activated to control the elevated ROS. However, the exact processes involved in Cr(VI) toxic effects are much more intricate than the reduction of Cr(VI) via redox reactions. In addition, the antioxidant defenses are also a double edge sword, because they can either prevent or reduce Cr(VI) damage or promote Cr(VI) toxicity by producing intermediate molecules. Thus, it is still needed to further explore the potential effects of oxidative and reductive species involved in Cr(VI)-induced toxicity.

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