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DNA Methylation Modifications Induced by Hexavalent Chromium

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Abstract

Human exposure to hexavalent chromium [Cr(VI)] increases the risk for certain chronic diseases and cancers. While the genotoxic and carcinogenic effects of hexavalent chromium exposure are better-characterized, various mechanistic processes involved in Cr(VI)-induced carcinogenesis are still a matter of debate. In recent years, studies have shown that epigenetic modifications, especially DNA methylation, may play a significant role in Cr(VI)-induced carcinogenesis. The aim of this review is to summarize the effects of Cr(VI) on global and gene-specific DNA methylation that are involved in the toxic and carcinogenic effects of Cr(VI) exposure.

Keywords

hexavalent chromium; DNA methylation; Hypermethylation; Hypomethylation

Introduction

Chromium is a common heavy metal that exists in several oxidation states. The most stable and common forms in the environment are the trivalent Cr(III) and the hexavalent Cr(VI) species, which display quite different chemical properties^[1]. Cr(III), as hydroxide, sulfate or phosphate precipitates, is not easily transported across cellular membranes, but nevertheless serves as an essential trace element with a role in maintaining proper glucose, lipid and carbohydrate metabolism in humans^[2,3]. Cr(III), such as [Cr3O(O2CCH2CH3)6(H2O)3]·NO3, which is a kind of Cr(III) supplementation, is available in many daily foods such as some vegetable and animal fats and Cr(III)-containing nutritional supplements can decrease the concentration of glucose in the blood, reduce the level of cholesterol and low density lipoprotein, and help muscle development^[4,5]. Deficiency of Cr(III) may cause cardiovascular disease, diabetes and other metabolic diseases^[6]. However, recent reports on CrCl₃ as an additive show that it can produce

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Cr-DNA adducts and cause DNA damage^[2]. However, Cr(VI) is the most toxic form of chromium. It is usually associated with oxygen as CrO_4^{2-} or $\text{Cr}_2\text{O}_7^{2-}$ ions, which are freely soluble in water and easily transported across the cellular membrane nonspecific anion channels^[1]. Once inside the cells, Cr(VI), as the form of $\text{Cr}_2\text{O}_7^{2-}$, is rapidly reduced to Cr(III), generating excessively high concentrations of intracellular Cr(III) which in turn result in the production of reactive oxygen species (ROS) and Cr-DNA adducts. ROS are a group of oxygen-containing molecules which have high chemical reactivity due to an unpaired electron^[7]. Such molecules may harm cellular function and have long been linked to pathological outcomes^[8].

According to estimates by The International Agency for Research on Cancer (IARC), there are millions of workers occupationally exposed to Cr(VI) in a variety of industries, including stainless steel welding, chrome plating, electroplating, leather, printing and dyeing manufacturing process^[9]. Previous studies have indeed indicated that Cr(VI) has significant effects on the health of occupationally exposed populations^[9–15]. The incidence of skin mucosal stimulation, dermatitis, asthma, nasal septum perforation in people exposed to Cr(VI) through swallowing, inhaling, or contacting is significantly higher than in people who have never been exposed to Cr(VI)^[10]. Long term exposure to Cr(VI) can lead to infertility as well as tumors of the larynx, nose, lungs, liver, and kidney^[16]. Chronic environmental exposure to Cr(VI) compounds has been shown to induce cancers and other diseases in exposed individuals^[9]. Epidemiological investigations showed that the risk of lung cancer were doubled in workers occupationally exposed to Cr(VI), and in some populations increased by as much as 80 fold^[11]. Therefore, IARC has identified Cr(VI) as a confirmed human carcinogen^[11,12].

However, the mechanisms underlying the carcinogenesis of Cr(VI) are not yet fully elucidated. Chromium induced epigenetic modifications are among those attracting current interest^[17]. Epigenetic modifications, including chemical modifications to DNA or histones, are heritable changes that alter the structure of chromatin without changing DNA nucleotide sequences^[18]. Among the various kinds of epigenetic modifications, DNA methylation is the most intensely studied in the context of Cr(VI) exposure. Many studies have investigated the features of DNA methylation induced by chromium (Table 1). In addition, changes in DNA methylation have been related to the progress of tumor in lung cancer patients with chromate exposure^[19–23].

Overview of DNA methylation

DNA methylation, catalyzed by DNA methyl transferases (DNMTs), is the covalent addition of a methyl group to the carbon-5 position of cytosine (C) to form 5-methylcytosine (5mC) in cytosine-guanine (CpG) dinucleotides^[24]. The regions of the genome that are rich of CpG sequence are called CpG islands, the length of which has to exceed 200 bp with greater than 50% GC content^[25]. CpGs affecting gene expression are often located in the promoter region and in the first exon of genes. CpG sites outside the CpG islands are usually methylated, while those inside the CpG islands are non-methylated. Abnormal methylation of CpG sites of certain genes can cause diseases and cancers^[26–28]. The

evidence from previous studies showed that DNA hypermethylation of specific gene and genome hypomethylation were both related to carcinogenesis^[29–35].

Influence of Cr(VI) on mechanisms of DNA methylation

Intact chromium particles have no effect on the cells when they are phagocytosed into the cell. However, particulate Cr(VI) can produce a chromate anion and a cation when it is partially dissolved outside the cell. The cation has no adverse effect on the cell after entering the cell through nonspecific channel protein (Fig. 1). However, when Cr(VI) entering the cell through anion transport, Cr(VI) is metabolically reduced by reductive agents, such as ascorbate, glutathione (GSH), cysteine, lipoic acid, hydrogen peroxide, NAD(P)H, fructose and ribose non-enzymatically to Cr(V), Cr(IV), Cr(III), and generates ROS^[36]. The Cr species produced during the reduction of Cr(VI) can form ternary Cr-DNA adducts with DNA. These adducts may be partly responsible for DNA damage induced by Cr(VI)^[37]. ROS are chemically reactive molecules containing oxygen, and it includes peroxides, superoxide, hydroxyl radical, and single oxygen, which possibly contribute to the pathophysiology and oncology of Cr(VI)-induced diseases^[38–40]. Excessive quantity of ROS can cause injury to cellular proteins, lipids, and DNA, leading to a state of oxidative stress^[41–43]. A previous study has demonstrated that ROS can induce DNA methylation modifications through oxidative stress and then further contribute to the multiple steps of carcinogenesis^[39].

DNA methyl transferases (DNMTs) catalyze DNA methylation reactions and are typically up-regulated by oxidative stress. Mammals have five DNMTs: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. Among these enzymes, DNMT1 and DNMT3B will form the DNMT-Containing Complexes with Sirtuin1 (SIRT1) and polycomb repressive complex 4 (PRC4) after hydrogen peroxide (H₂O₂) treatment. SIRT1 can interact with DNMT1 and other proteins during the process of DNA damage^[44,45]. Additionally, PRC4 is another important protein of the complexes, which have been proven to interact with DNMTs in response to H₂O₂-induced DNA damage, probably increasing DNA methylation. Maybe that is the reason for DNMT-Containing Complexes localization at sites of H₂O₂-induced DNA damage. However, it is still unclear whether the expression of DNMTs is related to the formation of DNMT-Containing Complexes. As reported, dynamic regulation of DNA methylation is achieved through a cyclic enzymatic cascade mainly comprised of DNMTs and TET dioxygenases, which converts cytosine (C) to 5mC and 5mC to 5-hydroxymethylcytosine (5-hmC), respectively^[46]. Furthermore, 5-hmC can be converted back to cytosine (C) by unknown mechanisms. Thus, the increased levels of DNMTs and DNMT-Containing Complexes may contribute to the changes of DNA methylation status by promoting the conversion of cytosine (C) to 5mC. Previous studies demonstrated that under the condition of oxidative stress, deoxyriboseguanosine monophosphate (dGMP) can develop into 8-hydroxydeoxyguanosine (8-OHdG), which is a marker of DNA oxidative damage. Meanwhile, 8-OHdG can also negatively affect DNA methylation of nearby cytosines^[47]. On the other hand, 8-OHdG is related to demethylation coupled with oxidative damage repair followed by global hypomethylation^[39]. Furthermore, the level of DNA methylation can be decreased by the conversion of 5mC to 5hmC, which may take place either

passively or actively. Under these circumstances, one can envision that both regional DNA hypermethylation and global DNA methylation may occur through oxidative stress after chromium exposure (Fig. 2).

Global DNA methylation

Global DNA methylation modification induced by hexavalent chromium plays an important role in many chemical carcinogenesis including *in vitro*, *in vivo* and population studies^[48–51]. Labra *et al.*^[29] evaluated genetic damage and epigenetic alteration induced by potassium dichromate in *Brassica napus L.* They found increased global DNA methylation in the CCGG-DNA sequence after treatment of 10–200 mg/l potassium dichromate in *Brassica napus L.*, and the methylation levels were positively correlated to potassium dichromate concentration. Cozza *et al.*^[52] detected Cr(VI)-dependent differences in DNA methylation between Cr-resistant and wild type strains of a green algae. Global DNA hypermethylation of plants might be associated with plant self-protection mechanisms, which induced cell cycle arrest with the consequent reduction of plant growth and development^[29]. In humans, it was suggested that chronic occupational chromate exposure could induce folate depletion in conjunction with its action as a direct mutagen^[30]. Exposure resulted in further reduced thymidylate synthesis and nucleotide imbalance and DNA disintegrity, and finally accelerated double-strand DNA breaks, genetic instability, and chromosome fragility^[30–32]. In our previous study, a human B lymphoblastoid cell line (Hmy2.CIR) and a human lung cell line (A549) were exposed to 5–15 μ M potassium dichromate and 1.25–5 μ g/cm² lead chromate for 2–24 hours. Our results showed that both Cr(VI) compounds resulted in global DNA hypomethylation followed by cell cycle arrest at G1 phase in both cell lines^[53]. We suggested that the decreased methylation level of global DNA might be linked to the cell cycle arrest induced by Cr(VI)^[53]. We also studied the effect of hexavalent chromium on DNA methylation in Sprague-Dawley rats after exposure to potassium dichromate through drinking water for 4 weeks. The results showed that oral ingestion of hexavalent chromium through drinking water caused global DNA hypomethylation in genomic DNA extracted from whole blood of male rats, and a good correlation was found between increased malondialdehyde (MDA) levels and reduced global DNA methylation^[54]. MDA is a marker for secondary products of lipid peroxidation^[55]. MDA concentration has been measured to examine oxidative stress and as an indicator of oxidative imbalance during the onset of many diseases^[56]. Flohr *et al.*^[57] studied the effects of textile, metal–mechanic and pulp and paper industrial waste containing Cr(VI) on DNA methylation in Nile tilapia, with potassium dichromate as a positive control. The results showed that the soluble fractions of these industrial wastes are capable of inducing oxidative damage and increase global DNA methylation of *Oreochromis niloticus*. However, Steinberg *et al.*^[58] evaluated blood samples from patients receiving metal-on-metal hip resurfacing (n=30), which increases Cr and Co levels in blood, and patients receiving conventional non-metal-on-metal hip replacements (n=30) and found no metal-dependent changes in DNA methylation. In summary, we conclude that Cr(VI) could contribute to diseases and cancer via induced changes in global DNA hypomethylation, which can be mediated via oxidative stress or other mechanisms.

Gene-specific DNA methylation

Hexavalent chromium not only causes global DNA methylation abnormalities but also alters methylation patterns of certain genes, some of which may be related to cell transformation and malignancy. Several studies have shown altered DNA methylation in specific genes of chromium exposed populations^[31,33,34,54,59–62]. For instance, a population-based study found that the promoter of the tumor suppressor gene *p16* was hypermethylated in workers occupationally exposed to chromate for more than 15 years. The methylation status of *p16* gene was not changed in workers with exposure time less than 15 years, suggesting that the effect of hexavalent chromium on the methylation status at the promoter of *p16* gene was associated with exposure time^[33]. Hypermethylation of the *p16* promoter can reduce its protein expression, leading to up regulation of the *CDK4* gene, and cell cycle dysfunction. The disordered cell cycle is closely related to the occurrence of many tumors^[34]. Ali *et al.* have attempted to obtain insights into the methylation of CpG islands in 4 genes [*adenomatosis polyposis coli (APC)*, *O6-methylguanine-DNA methyltransferase (MGMT)*, *human mut-L homologue (hMLH1)*, and *p16*] in 36 chromate lung cancers and 25 nonchromate lung cancers. *MGMT* and *hMLH1* are involved in DNA repair, and the inactivation of these genes could result in increased mutagenicity, whereas, *APC* and *p16* take part in the control of the cell cycle and are frequently inactivated in human cancers. Methylation in the promoter of these genes was significantly higher in chromate associated lung cancers than in nonchromate associated lung cancers. The results also showed a significant inverse correlation between DNA methylation levels at the promoter of *p16* and *hMLH1* genes and the magnitude of the decrease or loss of the corresponding gene products. Aberrant DNA methylation of *hMLH1* gene led to the reduced protein expression of *hMLH1* and dysfunction of DNA mismatch repair (MMR), resulting in microsatellite instability (MSI) and increased mutation of cancer-related genes. In addition, Ali *et al.* also documented a reduction of *APC* protein induced by hypermethylation of *APC* gene, which was possibly involved in altering the Wnt signaling pathway and chromosomal instability^[34]. The results suggested that lung cancers from chromate-exposed workers may be linked with genetic instability that are initiated by DNA methylation alteration of some tumor suppressors^[34]. In agreement with those observations, Takahashi *et al.*^[35] found that the promoter region of DNA mismatch repair gene *hMLH1* was highly methylated in chromate lung cancer, which was associated with the inhibition of *hMLH1* protein expression.

To examine the relationship between chromium exposure and variation in mitochondrial (mt) DNA methylation, Yang *et al.*^[63] enrolled 29 pairs of control-exposure chromate workers. They found that the methylation levels of *mitochondrially encoded tRNA phenylalanine (MT-TF)* and *mitochondrially encoded 12S RNA (MT-RNR1)* genes were significantly lower in Cr-exposed workers than those in control group a few years later. The results suggested that methylation level of mtDNA had the possibility of acting as an alternative effect biomarker for chromium exposure^[63]. For instance, Xiao *et al.*^[64] revealed that Cr(VI) decreased the activity of mitochondrial respiratory chain complex I and II, thus leading to ROS accumulation. According to the results of Yang and Xiao, we speculated Cr(VI) may inhibit energy metabolism through decreasing the methylation of mitochondrial DNA. Alternatively, Cr(VI) could impact mtDNA copy number, although

this hypothesis has not yet been tested. Finally, another study demonstrated *Wnt inhibitory factor 1* (*WIF1*), *APC*, and *MLH1* genes exhibited elevated promoter DNA methylation in chromate workers compared to the workers who were not exposed to chromate^[60]. The increased *WIF1* methylation may influence Wnt signaling pathway and ultimately participate in carcinogenesis.

In addition to the population studies mentioned above, a series of *in vitro* studies have also shown that Cr(VI) induced aberrant methylation in specific genes. For example, Hu *et al*^[61] have examined DNA methylation modifications after treating human bronchial epithelial cells with 0–20 µM potassium dichromate, which showed both hypermethylation of *p16* and reduced expression of *p16*. No change of DNA methylation was found in the *TP53* gene, although *TP53* gene expression increased after treatment of hexavalent chromium at the concentration higher than 5µM. Interestingly, the results showed that CpG1 methylation levels of *p16* gene were directly proportional to cell damage, suggesting that CpG methylation of *p16* gene could be used as a marker of chromium exposure. Our previous study examined the effects of soluble and particulate chromate on genome-wide DNA methylation in human B lymphoblastic cells using the Illumina Infinium Human Methylation 450K BeadChip array. We identified two sets of about 40 differentially methylated CpG sites induced by potassium dichromate and lead chromate, with 15 CpG sites shared by both treatments. The results of mRNA expression showed that after Cr(VI) treatment, mRNA expression changes of four genes (*TBL1Y*, *FZD5*, *IKZF2*, and *KIAA1949*) were consistent with their DNA methylation alteration. We found that both soluble and insoluble chromate could decrease the methylation levels of *TBL1Y*, *FZD5*, and *IKZF2* genes followed by reduced mRNA expression of these genes^[62]. *TBL1Y* is a Y-linked gene, which is involved in male fertility^[65]. *FZD5* is an important member of the Wnt family, which is involved in the development of prostate, brain and other tissues^[66]. *IKZF2* gene is involved in lymphocyte regulation and plays an important role in the early growth of the hematopoietic system^[67]. *KIAA1949* is originally cloned from a fetal brain cDNA library, which can be expressed in multiple normal human tissues including spleen, lung, liver, ovary, and other adult and fetal tissues^[68]. However, the function of *KIAA1949* gene is still unclear. These genes may play important role in adverse effects of hexavalent chromium exposure on physiological function. Another study investigated the effects of soluble and insoluble chromate on *gpt* gene *in vitro* [V79-derived, mammalian cell line (G12)] system. They found that insoluble barium chromate induced significant level of mutations in these cells but did not cause DNA methylation changes, but potassium chromate salts could induce aberrant DNA methylation followed by *gpt* gene silencing in this system, and all epigenetically silenced variants examined to date could be reactivated by 5-azacytidine to re-express the transgene^[69].

Conclusions

The evidence described above indicates that exposure to Cr(VI) can perturb DNA methylation states and subsequently induce cancer and other diseases. However, the exact mechanism of DNA methylation dysregulation caused by chromium is still unclear. We suggest that Chromate-induced oxidative stress is a good paradigm to account for chromate-

induced DNA methylation dysregulation. In Fig.1, Fig. 2, and Fig. 3, we propose a potential mechanism of Cr(VI)-induced carcinogenesis. Oxidative stress may be the first step in the process of carcinogenesis induced by hexavalent chromium. ROS generated in the process of oxidative stress can take part in the expression regulation of tumor genes and tumor suppressor genes through DNA methylation modification. The accumulating evidence discussed above demonstrates that hexavalent chromium exposure can change global and gene-specific DNA methylation, which is associated with dysregulation of gene expression. The review of currently available information indicates that chromium induces epigenetic alterations in plants, cell lines, animals, and human populations. Notably, population studies of DNA methylation profiles of chromium exposure workers are lacking. The role of DNA methylation in the carcinogenic effects of hexavalent chromium may become clearer after addition of genome-wide DNA methylation data in human population.

Overall, it is easy to envision that chromium significantly affects a large number of genes by modifying the status of DNA methylation, which play an important role in carcinogenesis. For instance, *p16* gene was frequently investigated by researchers, and all of the results showed increased methylation levels of *p16* gene after chromium exposure. As mentioned above, *p16* gene is a tumor suppressor gene whose protein abundance is affected by the transcription rate and methylation status of the gene. Thus, changed methylation state of *p16* gene may be partially responsible for chromium induced cancers. Thus, it is urgent to clearly explore the association between *p16* gene methylation state and chromium induced tumorigenesis but also further document the hypermethylation of *APC*, *MGMT* and *hMLH1* in tumors potentially caused by chromium exposure. Nevertheless, the mechanism underlines the hypermethylation of these genes is still unclear. ROS and ternary Cr-DNA adducts may influence methylation changes in these genes. The carcinogenic effects of chromium seem to be associated largely with epigenetic other than genetic changes.

Cohort studies of DNA methylation changes induced by hexavalent chromium could also be enhanced. For instance, it will be helpful to explore the entire process of the alteration of global and gene-specific DNA methylations from early-life to adult using state of the art approaches that yield genome-wide data with nucleotide resolution. Furthermore, the addition of DNA methylation profile, will illuminate the potential role of various carcinogenic mechanisms. These would enhance studies that already demonstrated consistent results regarding the methylation status of candidate genes.

In summary, we review DNA methylation changes associated with tumorigenesis after hexavalent chromium exposure. Evidently, chromium-associated DNA methylation remains an understudied area. To make the mechanism of DNA methylation more generalizable, additional studies in across ethnic groups and large cohorts are warranted. It will also be interesting to consider novel prevention strategies and treatments that are targeted to hexavalent chromium induced cancers by modifying DNA methylation levels of related genes. In addition to DNA methylation, histone modification, non-coding RNA regulation and other epigenetic mechanism should also continue to be studied after exposure of heavy metals, such as hexavalent chromium.

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Figure 1.
Membrane transportation of chromium and the generation of ROS.

Figure 2. Oxidative stress induced by chromium and aberrant DNA methylation in global DNA and specific gene.

Figure 3.

The effects of global DNA hypomethylation and gene-specific DNA hypermethylation induced by chromium on biological processes including cell cycle, genome instability, and Wnt signaling.

Table 1.

Summary of Cr (VI) Effects on Specific genes DNA methylation DNA Methylation

Species	Tissue/Cell line	Cr (VI) concentration	Time	Methylation(%)	Expression of gene/protein	Reference
Human	30 chromate lung cancers and 38 non-chromate lung cancers	-	>15 years	Hypermethylated <i>p16</i> promoter region	Decreased expression of <i>p16</i> gene	25
Human	36 chromate lung cancers and 25 non-chromate lung cancers	-	<15 year	No change of <i>p16</i> gene methylation	-	26
Human	Human bronchial epithelial cells(16HBE)	0-20 μ M potassium dichromate	>15 years	Hypermethylated <i>APC</i> , <i>MGMT</i> , <i>hMLH1</i> , <i>p16</i> genes	Decrease or loss of <i>hMLH1</i> , <i>p16</i> gene products and decreased expression of <i>APC</i> , <i>MGMT</i> genes; reduced function of DNA MMR; MSI; increased mutations of cancer-related genes; active Wnt signal pathway	46
Human	Human B lymphocytes	soluble and insoluble chromate	-	Hypermethylated CpG1, CpG31 and CpG32 of <i>p16</i> gene, no change in <i>TP53</i> gene	Decreased expression of <i>p16</i> gene; increased <i>TP53</i> gene expression after treatment of hexavalent chromium higher than 5 μ M; CpG1 methylation level of <i>p16</i> gene was directly proportional to cell damage	47
Human	29 pairs of control-exposure chromate workers	-	-	Hypomethylated <i>TBL1Y</i> , <i>FZD5</i> , <i>IKZF2</i> genes	Decreased expression of <i>TBL1Y</i> , <i>FZD5</i> , <i>IKZF2</i> genes	48
Rat	G12 cells	soluble and insoluble chromate	-	Hypomethylated <i>MT-TF</i> and <i>MT-RNR1</i>	Potassium chromate salts can induce aberrant DNA methylation followed by <i>gpt</i> gene silencing	49