Minireview *Highlight Article*

Chromium exposure disrupts chromatin architecture upsetting the mechanisms that regulate transcription

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Impact statement

This mini-review highlights current evidence on the mechanisms through which hexavalent chromium (Cr(VI)) disrupts transcriptional regulation, an emerging area of interest and one of the central processes by which chromium induces carcinogenesis. Several studies have shown that Cr(VI) causes widespread DNA damage and disrupts epigenetic signatures, suggesting that chromatin may be a direct Cr(VI) target. The findings discussed here suggest that Cr(VI) disrupts transcriptional regulation by causing genomic architecture changes.

Abstract

Hexavalent chromium (Cr(VI)) is a highly mutagenic and carcinogenic chemical used in many industrial processes. Occupational exposure to chromium, occurring mostly by inhalation, constitutes a major lung cancer risk affecting chromium workers. Environmental exposure, on the other hand, mainly by ingestion of contaminated drinking water, is a widespread gastrointestinal cancer risk, affecting millions of people throughout the world. One of the major mechanisms through which Cr(VI) causes carcinogenic transformation is thought to be the disruption of transcriptional regulation. Indeed, Cr(VI)-directed DNA damage and crosslinking occurs preferentially at sites where active DNA replication and transcription processes take place. Accordingly, numerous studies have shown that Cr (VI) causes gene expression changes in a wide range of cell signaling pathways, resulting from Cr(VI)-induced direct macromolecular damage, alteration in transcription factor function, and disruption of epigenetic signatures. This brief review highlights past and current information on the impact of Cr(VI) on the various mechanisms of transcriptional regulation.

Keywords: Epigenetics, chromatin, genotoxicity, mechanisms, toxicology, transcription

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Introduction

Chromium (Cr) is an element abundantly available in the environment with unique chemical traits that make its various species valuable through both function and aesthetics. Of the several valence configurations that exist, the forms most commonly encountered in nature are trivalent, hexavalent, and elemental chromium (Cr(III), Cr (VI), and Cr(0), respectively). The toxicological profiles of elemental and trivalent chromium show little to no health risk; however, compounds containing hexavalent chromium possess notable toxicities and are major contributors to occupational exposure.

Although natural sources of hexavalent chromium exist, most of this compound is produced anthropogenically, with the intent to be used in a wide range of industrial applications, including stainless steel production and welding, chrome electroplating, pigments and dyes, and the manufacture of chromate compounds themselves.[1] It has been estimated that 558,000 US workers and 786,000 EU workers are exposed occupationally to Cr(VI), primarily through inhalation and dermal contact.[2] However, imperfect processing and disposal techniques expose a much larger proportion of local communities to chromium through airborne emissions and water contamination via industrial release and wastewater leach.[1,2] A recent study investigating the sources of groundwater Cr(VI) in California found that industrial contamination was largely responsible for acute concentrations, while monitoring wells in regions considered free of industrial pollution exceeded the 10 $\mu g\ L^{-1}$, possibly as a result of Cr(III) oxidation through anthropogenic and natural processes.[3,4] The contamination of Cr(VI) in ground waters, despite the complexity of its originating sources, indicates that much larger populations are chronically exposed to hexavalent

chromium through ingestion and highlights the need for studies assessing the potential adverse health outcomes associated with exposure.

Notably, Cr(VI) is a well-established respiratory carcinogen, though the mechanisms promoting carcinogenesis and progression remain unclear. Limited epidemiological data exist to suggest carcinogenic outcomes in humans following ingestion,[1,2] although numerous animal studies have suggested that at the molecular level, Cr(VI) has carcinogenic properties regardless of the route of exposure.[5,6] As a result, continued mechanistic studies of Cr(VI)'s role in genotoxicity and carcinogenesis may provide better foundations for risk assessment regarding population-level exposures. Past and current studies performed by several labs have identified that Cr(VI) may promote carcinogenesis through several different processes that include DNA damage and increased chromosome instability, protein–Cr–DNA adduct formation, silencing of tumor suppressors, and modification of epigenetic markers involved in the regulation of transcription and chromatin accessibility.

The intracellular fate of Cr(VI) and its toxicological outcomes

Key steps in the biotransformation and bioactivation of Cr (VI) are schematically presented in Figure 1. Following absorption, the majority of Cr(VI) is extracellularly reduced to Cr(III), which does not efficiently diffuse across cell membranes; the remaining Cr(VI) is rapidly transported across the cell membrane by the sulfate and phosphate anion transporters. Antioxidants such as glutathione (GSH), ascorbate, and cysteine reduce Cr(VI) stepwise one electron at a time to its trivalent state with ascorbate being the major reducer, forming reactive intermediates in between that include Cr(V), Cr(IV), and ultimately Cr(III). Reduction by ascorbate involves two simultaneous electrons, thus reducing the hexavalent form to Cr(IV) directly and skipping Cr(V), the first intermediate. The intermediates generated at each step are highly reactive and able to interact with DNA mainly at the phosphate backbone, generating both binary (Cr–DNA) and ternary adducts (X–Cr–DNA) with ascorbate, GSH, cysteine, and proteins. While DNA–Cr–DNA adducts have been demonstrated in vitro, they may be the result of experimental conditions in vitro that do not take place in vivo and may not be major contributors to the genotoxicity of chromium towards DNA.[7] Numerous studies have shown that chromium frequently crosslinks free amino acids, antioxidants such as GSH and ascorbate, and proteins to DNA, which then becomes a target of multiple DNA repair processes, most notably the mismatch repair pathway.[8–10] Of particular interest, Cr (VI)'s genotoxicity is exacerbated by the protective mechanisms used to detoxify it. Reynolds et al. have shown that the restoration of ascorbate, the main reducer of Cr(VI), in vitro prior to Cr(VI) increased chromosomal damage significantly and exhibited a 10-fold increase in mutagenicity.[11]

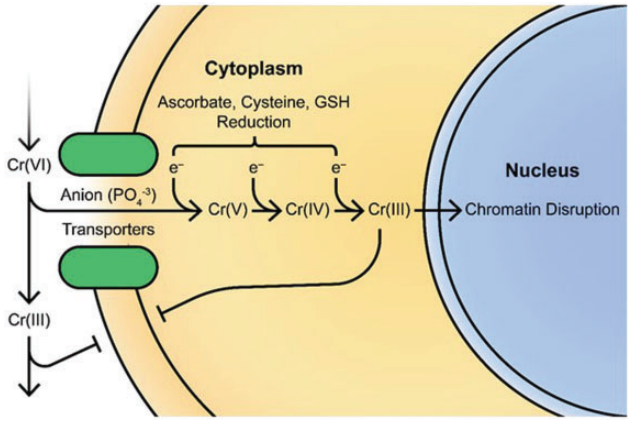

Figure 1. Cr(VI) uptake and biotransformation. Following absorption, Cr(VI) is largely reduced in extracellular fluids to Cr(III), hindering diffusion across cell membranes and promoting excretion from the body. Remaining Cr(VI) is rapidly shuttled across the lipid bilayer through non-specific anion transporters and reduced in a step-wise manner to Cr(III) by antioxidants, forming reactive intermediates capable of interacting with several intracellular substrates. Of note, chromium forms binary and ternary adducts with chromatin resulting in the disruption of regulatory mechanisms and double-strand break formation. (A color version of this figure is available in the online journal.)

Cr(VI) disrupts transcriptional regulation

Many studies have explored the transcriptomic changes that occur following exposure to Cr(VI), to identify the mechanisms that alter the transcriptional response. Intracellular Cr(VI) reduction generates a range of reactive oxygen species that damage key macromolecules, resulting in widespread changes in gene expression. A wide range of cell signaling pathways respond to Cr(VI) exposure including redox stress, calcium mobilization, energy metabolism, DNA repair, biosynthetic, cell proliferation, apoptosis, growth, differentiation, survival, and cell cycle regulation pathways.[12,13] As an example, Cr(VI) activates the MAPK family components JNK, p-38, and ERK1/2,[14] the Src family kinases,[15] NF-kB, AP-1, and Akt signaling[16]; and downregulates the expression of several tumor suppressor genes including p16,[17] hMLH1,[18,19] APC, MGMT,[19] PDCD4,[20] p15, p18, p19, p21, and p27[21] through diverse mechanisms. Direct DNA damage, bulky DNA–protein crosslinks, altered transcription factor function, epigenetic changes, and shifts in chromatin accessibility are some of the mechanisms through which Cr(VI) disrupts transcriptional programs. Figure 2 shows a summary of the sequence of molecular events leading to the disruption of transcription by chromium.

Cr(VI) alters transcription factor and epigenetic modifier functions

Cr(VI)-induced direct DNA damage accounts for major transcriptional disruption due to RNA polymerase arrest and elongation stasis. The presence of DNA double-strand breaks (DSBs), DNA–protein crosslinks, DNA–chromium, and X–DNA–chromium adducts combine to suppress mRNA transcript synthesis.[22,23] The fact that chromium-induced DNA lesions and crosslinks form preferentially in the nuclear matrix DNA,[24] a nuclear sub-compartment where transcription and replication occur, suggests that Cr

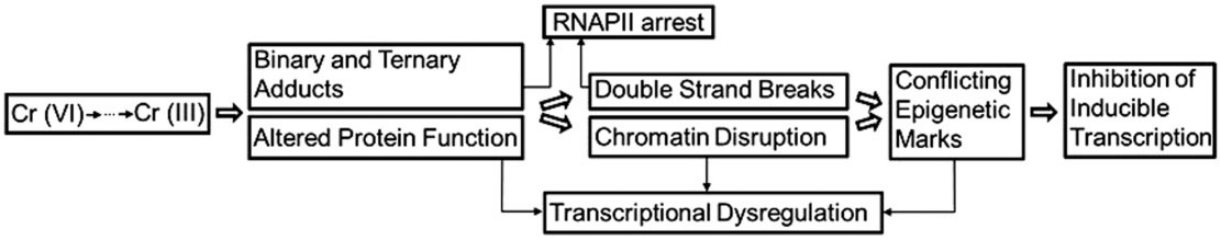

Figure 2. Cr(VI) disrupts transcriptional regulatory mechanisms. The reduction of intracellular Cr(VI) yields reactive intermediates that damage several key macromolecules and form bulky DNA lesions, such as Cr–DNA adducts and protein–Cr–DNA crosslinks, resulting in widespread DNA double-strand breaks, arrest of RNA polymerase-II (RNAPII) transcription elongation and global chromatin disruption. Direct effects of Cr(VI) on proteins such as transcription factors lead to transcriptional disruption in pathways regulated by these proteins. The altered chromatin configuration arising from Cr(IV) bulky lesions gives way to conflicting epigenetic marks (altered DNA methylation, histone modifications, and non-coding RNA profiles) that not only inhibit transcription of inducible genes but also destabilize the global transcription machinery.

(VI) targets transcriptional regulatory mechanisms and directly impacts transcription factor function. Accordingly, Cr(VI) has been shown to impair the expression of highly inducible genes that bear active promoters, without blocking the transcription of constitutively expressed genes. Bulky DNA–protein lesions arising from Cr(VI)-mediated crosslinks are thought to block the expression of several inducible genes.[25–28] Evidence from our laboratory indicates that Cr(VI) crosslinks the repressive DNMT1-HDAC1/2 complex to the *Cyp1a1* promoter, preventing RNAPII recruitment and induction by the aryl hydrocarbon receptor ligand, benzo[*a*]pyrene.[25] It is thus plausible that global changes in the transcriptome arise partly from Cr(VI)-induced DNA–protein crosslinks involving epigenetic writer/reader molecules that cause extensive epigenomic alterations and shifts in chromatin accessibility. Context-dependent changes in transcription can be anticipated, depending on the local chromatin configuration, the specific epigenetic factor that is crosslinked, and the affected genomic locus.

Cr(VI) selectively hinders heterodimerization of coactivator molecules with transcription factors, therefore curtailing the expression of target genes. Pretreatment with Cr(VI) prevented dimerization between the p65 subunit of NF-κB and the transcriptional coactivator CBP, inhibiting IL-8 induction.[29] In addition, Cr(VI) inhibited the transactivation domains of the MTF1 transcription factor,[30] preventing heterodimerization with the histone acetylase complex p300 and RNAPII recruitment to the metallothionein-1 gene promoter.[26] Cr(VI)-generated ROS and alterations in kinase activity are thought to alter the transactivation activity of both p300 and CBP, and to limit their interaction with transcriptional factors.

While these and other studies[31] have shown that Cr(VI) does not affect the binding of transcription factors to DNA, Cr(III) complexes were shown to alter Sp1 and TFIID binding to cognate DNA sequences, thereby blocking active transcription of reporter genes.[32] Furthermore, work using a thiolate complex modeling a 2-cysteine, 2-histidine zinc finger (similar to the zinc fingers found in CCCTC Binding Factor (CTCF)) indicates that Cr(V/IV) derivatives cause release of Zn(II) from the tetrahedral zinc finger.[33] Zinc finger proteins are a large and widely diverse family of DNA, RNA, and protein-binding proteins involved in transcription, epigenetic, and chromatin regulation. This raises the important question whether Cr(VI) destabilizes the DNA binding ability of zinc finger proteins and if this mechanism drives transcriptional and epigenetic disruption after Cr(VI) exposure.

The epigenome as a target of chromium

DNA methylation. DNA methylation is a well-studied epigenetic mark that responds dynamically to diverse environmental factors and is important to modulate gene expression and maintain genomic stability.[34] DNA methylation state directs genomic binding of several classes of transcription factors and regulatory proteins.[35] For example, CTCF, a protein that controls genome architecture and transcription, has been shown to have varying genomic occupancy that is tightly linked with the DNA methylation status; regions with high methylation having low CTCF binding.[36] With Cr(VI)-induced widespread methylome changes, it can be anticipated that there is global disruption in transcriptional regulation arising from diverse changes in transcription factor and co-regulator protein binding.

Cr(VI) upregulates the stress protein NUPR1 via promoter demethylation[37] and Cr(III) treatment hypomethylates the *45S* ribosomal RNA gene in mouse sperm.[38] Studies in human lung cancer samples indicate that chromium specifically targets and silences the tumor suppressor genes $p16^{INK4a}$ and Human MUTL Homolog 1 (hMLH1) through promoter hypermethylation, though the question remains whether this is a targeted response or the selection of cell populations with deficient repair systems.[17,19] Global DNA hypomethylation induced by chromium has been shown to contribute to genomic instability and cell cycle arrest[39] in vitro, in vivo, and in exposed chromate manufacturing workers.[39–41] Cr(VI) also causes a general decline in the DNA demethylation intermediates 5-hydroxylmethylcytosine, 5-formylcytosine, and 5-carboxycytosine, and inhibits the TET demethylation proteins,[42] likely through depletion of ascorbate which is a cofactor of the TET proteins alongside α-ketoglutarate and iron. Furthermore, the resultant massive DNA damage after Cr (VI) exposure may recruit most of the repair proteins that are also required for base excision repair that occurs during active DNA demethylation. Overall, genome-wide studies to characterize the methylome response to Cr(VI) have yet

to be carried out. These will detail the genomic sites of methylation changes, and whether these correlate with Cr (IV) changes in other epigenetic marks and alterations in gene transcription.

Post-translational histone modifications. Post-translational histone modifications play a crucial role in regulating chromatin accessibility and transcription. While histone acetylation is generally associated with open and transcriptionally active chromatin, the role of histone methylation in regulating gene expression is context-dependent. For example, whereas tri-methylated lysine-4 and lysine-6 in histone H3 (H3K4me3 and H3K6me3) occur in actively transcribing genes, tri-methylated lysine-9 and lysine-27 in the same histone (H3K9me3 and H3K27me3) are associated with gene silencing.[43]

Cr(VI) treatment reduces the global H3 and H4K16 acetylation levels, an effect attributed to the direct downregulation of the histone acetyltransferase, MOF, by NUPR1 which are induced upon Cr(VI) exposure.[37] Chromium was also found to increase the levels of H3K4me3, and the repressive H3K9me2 and H3K9me3 marks by upregulating expression of the histone methyltransferases G9a, GLP, and SUV39H1, but decreased the global presence of H3K27me3 and H3R2me2.[18,44,45] Wang et al., however, reported an increase in H3K27me3 that was attributed to Cr(VI) upregulation of EZH1.[45] Cr(VI) depletes cellular ascorbate required as a cofactor of the H3K9me2 demethylase JHDM2A, and supplementation of Cr(VI)-treated cells with ascorbate led to H3K9me2 demethylation.[37] Thus, the increase in H3K9me2 corresponds to depletion of cellular ascorbate stores. At specific gene targets, chromium upregulates the expression of NUPR1 by increasing promoter H3K9 and H3K14 acetylation,[37] while silencing hMLH1 by increasing the promoter presence of H3K9me2.[18]

A hallmark of cancer, loss of global H4K16 acetylation co-occurs with loss of H4K20me3 and DNA hypomethylation of repetitive sequences.[46] Additionally, a global increase in H3K9me3 is associated with tumor progression in multiple cancers.[47] These chromium-induced alterations in local and global histone marks indicate widespread changes in chromatin accessibility and transcription, and a collapse of epigenetic homeostasis. Furthermore, the altered chromatin environment may impact the processing and repair of DSBs induced by Cr(VI), since for DSB repair to proceed, it is necessary for the local chromatin to undergo nucleosomal displacement and relaxation by histone acetylation and chromatin remodeling.[48] Altogether, whether the DNA methylation and histone modification changes arising from chromium exposure may directly influence chromatin reorganization remains a question to be investigated. Alternatively, these epigenetic changes may arise from direct effects of Cr(VI) on the chromatin landscape.

MicroRNAs. MicroRNAs are a large family of short RNA oligonucleotides that silence target gene expression by binding mRNA and leading to its degradation. Since miRNA regulate broad transcriptional networks, they are involved in several important biological processes.[49] In steel and chromate production workers, chromium exposure was found to increase the expression of miR-222[50] and decrease the expression of miR-3940-5p, which was associated with DNA damage.[51] These results were replicated in vitro, where Cr(VI) downregulated the expression of miR-3940-5p allowing for DNA repair to proceed.[52] In a recent study, He et al. showed that miR-143 was significantly suppressed in Cr(VI)-transformed cells, which exhibited a malignant phenotype attributed to miR-143 downregulation.[53] Chromium-induced upregulation of miR-21 and the resultant downregulation of the tumor suppressor gene PDCD4 have been implicated in the malignant transformation of cells by Cr(VI).[20] It is thus plausible that Cr(VI) disrupts multiple transcription pathways within the transcriptome through the direct dysregulation of miRNA expression profiles.

Chromatin structure and function

Transcriptional regulation hinges on the coordinated binding of transcription factors to canonical DNA motifs, a configuration that arises from local nucleosomal displacement and chromatin remodeling events. On a higher level, genomic regions with similar transcriptional activity and chromatin configuration are organized into chromatin loops and insulated topologically associating domains by CTCF and the cohesin complex. Previous work in our laboratory indicates that Cr(VI) alters chromatin accessibility by causing local and genome-wide nucleosomal position shifts and occupancy changes.[54] These changes disrupt the accessibility of transcription factor binding motifs of CTCF, AP1, BORIS, and BACH2 proteins.[54,55] Cr(VI) opens the chromatin surrounding AP1 sites, while the BACH2 and CTCF binding sites are opened in a manner dependent on Cr (VI) concentration.[55] This chromatin changes may not only impact CTCF and AP1 occupancy of their cogent DNA motifs, but it may also predispose these sites to direct damage by Cr(VI). Furthermore, direct transcriptional changes of genes within the AP1 and BACH2 signaling pathways are affected, and it is likely that Cr(VI)-induced alterations in CTCF function may give rise to global changes in three dimensional (3D) chromatin organization and widespread disruption in the transcriptome.

A hallmark of Cr(VI) exposure as it pertains to DNA damage is the formation of phosphorylated γ-H2AX foci, a well-established marker for DSBs. Interestingly, DeLoughery et al. have found that Cr–DNA adducts form non-preferentially across the genome; however, formation of DSBs was localized to euchromatin, or active regions of the genome, and were likely initiated as single-stranded as a result of ATR activation.[56] Recent advances have demonstrated that DSBs occurring within a transcribed gene can recruit factors that suppress expression until the damage is resolved. However, as noted by DeLoughery, genes that are initially activated may become suppressed over time due to localized DSB formation. Chronic, low-dose exposure to Cr (VI) in Hepa-1c1c7 cells resulted in the gradual accumulation of y-H2AX foci of which many failed to resolve following removal of the challenge,[57] suggesting that DNA repair

might be increasingly deficient over time. It is plausible that regions involved in the early response to Cr(VI) exposure are highly susceptible to damage and suppression with time. Notwithstanding, the broad expansion of γ-H2AX domains require further study to determine the positioning of the break relative to any affected gene.

Current initiatives and future directions

Numerous studies investigating the mechanisms of Cr(VI) toxicity suggest that the hexavalent species functions as a carcinogen at the molecular level, though the mechanisms are not fully understood. Promising studies have highlighted the dual-role that antioxidants play, both by helping to detoxify Cr(VI) as well as by enhancing its genotoxicity through ternary adduct formation with DNA. The capacity to directly interact with DNA and tether protein complexes highlights the role that Cr(VI) may play in reshaping the chromatin landscape. To this end, there is a need for deep genome-wide studies to outline the dynamics of epigenetic changes that arise from Cr(VI) exposure. Current work in our laboratory is geared towards addressing these questions and to further understand the impact of Cr(VI) on 3D chromatin architecture, focusing on CTCF as a direct target of chromium. Ultimately, there is need to integrate epigenomic, transcriptomic and chromatin architecture data arising from Cr(VI) studies in order to build a comprehensive model and an understanding of the impact of chromium on transcriptional regulatory mechanisms.

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DECLARATION OF CONFLICTING INTERESTS

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