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Molecular and Epigenetic Mechanisms of Cr(VI)-induced Carcinogenesis

Qiao Yi Chen[*,‡], **Anthony Murphy**[*,‡], **Hong Sun**[*], **Max Costa**[*,§]

[*]Department of Environmental Medicine, New York University School of Medicine, 341 East 25 Street, New York, NY 10016

Abstract

Chromium (Cr) is a naturally occurring metallic element found in the Earth's crust. While trivalent chromium ([Cr(III)] is considered non-carcinogenic, hexavalent chromium [Cr(VI)] has long been established as an IARC class I human carcinogen, known to induce cancers of the lung. Current literature suggests that Cr(VI) is capable of inducing carcinogenesis through both genetic and epigenetic mechanisms. Although much has been learned about the molecular etiology of Cr(VI)-induced lung carcinogenesis, more remains to be explored. In particular, the explicit epigenetic alterations induced by Cr(VI) in lung cancer including histone modifications and miRNAs, remain understudied. Through comprehensive review of available literature found between 1973–2019, this article provides a summary of updated understanding of the molecular mechanisms of Cr(VI)-carcinogenesis. In addition, this review identifies potential research gaps in the areas of histone modifications and miRNAs, which may prompt new niches for future research.

Keywords

Cr(VI); Carcinogenesis; Mechanism; Epigenetic

Introduction

Chromium (Cr) is a naturally occurring element found in soil, rocks and living organisms, and primarily exists in two stable valence states: trivalent chromium (Cr(III)) or hexavalent chromium (Cr(VI)) (Wilbur et al., 2012). Cr(III) is considered non-carcinogenic due to insufficient evidence in humans and animals. However, according to the International Agency for Research on Cancer (IARC) and U.S. Environmental Protection Agency (EPA), Cr(VI) compounds are classified as Group 1 and Group A human carcinogens, respectively(Stoss et al., 1983). Due to the potential adverse health effects resulting from exposure to Cr, the EPA established a maximum contaminant level of 0.1 mg/L total Cr in drinking water. However, Cr(VI) is often found in occupational settings as a result of

[§]Corresponding author: Professor Max Costa, Telephone: 845-731-3515, Fax: 845-351-2118, Max.Costa@nyumc.org.
[‡]Both authors contributed equally to this article

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industrial activities such as leather working, smelting, welding, and metal plating (Wilbur et al., 2012). Cr(VI) is also found in automobile exhaust and in tobacco products such as traditional and electronic cigarettes and hookahs (Williams et al., 2017). It has been estimated that 66% of current or former hazardous waste sites on the National Priorities List also contain Cr (Wilbur et al., 2012).

Cr(VI) is considered the biologically relevant form of Cr due to its ability to readily pass through the cell membrane via non-specific sulfate/phosphate anionic transporters. Cr(III) is capable of passing through cell membranes via diffusion or phagocytosis, albeit at much lower levels than Cr(VI) (Stout et al., 2009). Systemic toxicity attributable to Cr has been documented in the respiratory and pulmonary system, gastrointestinal system, dermis, and renal system (Wilbur et al., 2012). In addition, multiple mechanisms of carcinogenesis have been proposed involving oxidative stress, DNA damage and genomic instability, and epigenetic modulation. A comprehensive literature search was conducted through Pubmed using key words such as “Chromium”, “Cr(VI)”, “Carcinogenesis”, “Cancer”, “Epigenetic” and “Mechanisms”. In addition, the reference list of each article is also examined to prevent missing data. This review summarizes most current findings in the field of Cr(VI)-carcinogenesis, with a focus on epigenetic mechanisms. Furthermore, a comprehensive lists of genes, histone modifications, and miRNAs altered through Cr(VI) exposure is presented.

Chromate Exposure and Associated Health Risks

The primary non-occupational route of exposure to Cr is ingestion. However, for occupationally exposed individuals, exposure to Cr most often occurs via inhalation or dermal absorption (Wilbur et al., 2012). Adverse respiratory and pulmonary health effects due to Cr exposure include asthma (Bright et al., 1997), bronchitis and respiratory tract irritation (Khan et al., 2013), and nasal septum ulceration and perforation (Lindberg & Hedenstierna, 1983). Contact dermatitis is frequently documented following exposure to chromate and dichromate (Leijding et al., 2018), and more severe dermal reactions including skin burns, blisters, and skin ulcers have been reported (Wilbur et al., 2012). Gastrointestinal (GI) effects including chronic dyspepsia, gastric ulcers, and gastritis have all been documented following occupational exposure to Cr (Khan et al., 2013). Exposure to Cr(VI) can also cause acute tubular necrosis, which is localized to the proximal convoluted tubules and may result in rapid onset of renal failure (Wedeen et al., 1991).

As a prominent human carcinogen, Cr(VI) has long been reported to promote cancers of the lung and nasal and sinus cavities among occupationally-exposed workers. A study based on the Baltimore cohort which consisted of 2,357 participants, demonstrated a highly positive correlation between cumulative Cr(VI) exposure and lung cancer mortality rate (Gibb et al., 2000; Gibb et al., 2015). Another retrospective study based on 493 workers from a chromate production plant in Painesville further confirmed the correlation between lung cancer mortality and occupational Cr(VI) exposure (Luippold et al., 2003; Proctor et al., 2003). In addition, a meta-analysis of Cr(VI) exposure and GI cancers showed an increased risk of stomach cancer (relative risk = 1.27; 1.18 – 1.38) in Cr(VI)-exposed workers and an elevated stomach cancer mortality rate (rate ratio = 1.82; 1.11 – 2.91) in Cr contaminated regions (Beaumont et al., 2008; Welling et al., 2015; Zhang & Li, 1987). Despite the lack of

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conclusive evidence supporting a causal role of Cr(VI) in GI cancers in humans, associations between Cr(VI) ingestion and GI cancers have been documented in rodent models. In a two-year drinking water study conducted by the National Toxicology Program (NTP), oral exposure to sodium dichromate dihydrate in male and female rats increased the incidence of squamous cell neoplasms of the oral cavity. In mice there was an increase in incidence of neoplasms of the small intestine (duodenum, jejunum, or ileum) (NTP 2008). Together, these results suggest that Cr(VI) may act in a carcinogenic manner following ingestion, thereby raising concerns for non-occupationally exposed individuals. Molecular mechanisms involving oxidative stress and DNA damage are considered principal ways by which Cr(VI) exhibits its carcinogenic effects.

Molecular Mechanisms of Chromium Carcinogenesis

Oxidative Stress

Toxicity and carcinogenicity of Cr(VI) compounds is by virtue of their ability to readily enter the cells through isoelectric and isostructural anion transfer channels, which are used to transport HPO^{-}_{4} and SO^{2-}_{4} ions (Codd et al., 2001; Valko et al., 2005). Although Cr(VI) compounds do not bind directly to DNA, intermediates and byproducts of Cr(VI) metabolism can elicit a wide range of damages through DNA adducts and crosslinks (Kasprzak 1995). Notably, generation of reactive oxygen species (ROS) through detoxification is principally responsible for Cr(VI)-induced cellular damages such as DNA lesions, cytotoxicity, and tumor development (Chen et al., 2019; Shi et al., 1999; Wise et al., 2019; Zhitkovich 2005; Zhitkovich 2011). Cr species [(III), (IV), (V), and (VI)] are known to produce intracellular ROS. Specifically, ROS scavengers such as ascorbic acid and glutathione are able to detect and reduce Cr(VI) to Cr(III), thereby producing free radicals such as hydroxyl radicals and DNA-damaging intermediates such as Cr(V) and Cr(IV) (Arita & Costa, 2009; Chen et al., 2019; Jomova & Valko, 2011; Zhitkovich 2011).

During intracellular reduction of Cr(VI), hydroxyl radicals are generated through Fenton-like reactions in the presence of hydrogen peroxide (Sun et al., 2015). Endogenous superoxide anions and hydrogen peroxide produce hydroxyl radicals via Haber-Weiss-like reactions in the presence of Cr(VI) (Sun et al., 2015). Cr(VI) can also generate hydroxyl radicals through stimulated cells, which demonstrate upregulated activities of NADPH oxidase (Gao et al., 2002; Wang et al., 2000; Wang et al., 2004; Yao et al., 2008; Ye et al., 1995). Other reducing agents of Cr(VI) include flavoenzymes such as glutathione reductase and ferredoxin NADP+ oxidoreductase (Shi et al., 1999; Wise et al., 2019). In addition, Cr(VI) can disrupt the thioredoxin antioxidant system by irreversibly inhibiting thioredoxin reductase, which under normal conditions maintains thioredoxin in a reduced state (Myers & Myers, 2009). The thioredoxin antioxidant system promotes cell survival by defending against oxidative stress (Myers & Myers, 2009).

ROS including hydroxyl radicals, singlet oxygen, peroxides, and superoxides can serve as important secondary messengers and activators for various pathways including apoptosis, cell signaling, and homeostasis (Deyasagayam et al., 2004; Kwee 2014; Leonard et al., 2004; Valko et al., 2007; Wise et al., 2019). Specifically, Cr(VI) has been found to induce NF-kB, AP-1, and Nrf2 activation, each of which has important implications in cancer

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development (He et al., 2007; Klaunig et al., 2011; Valko et al., 2005; Ye et al., 1995). Hydroxyl radicals are able to react with guanine residues and generate radical adducts such as 8-hydroxy-deoxyguanosine (8-OH-dG), which is a prominent marker for oxidative damage in cancer (Valko et al., 2004). The accumulation of ROS can lead to oxidative stress and contribute to chronic inflammation, metabolic reprogramming, genetic instability, and development (Wang et al., 2016). Sprague-Dawley rats exposed to Cr(VI) for 5 days via intraperitoneal injection demonstrated enhanced antioxidant enzymes to combat oxidative stress in liver and kidneys as well as DNA damage in peripheral blood lymphocytes (Patlolla et al., 2009). In addition, adducts formed through Cr and ROS scavenger conjugation, including GSH-Cr-DNA, can generate bulky adducts and block proper DNA replication and repair (Chen et al., 2019; Quievryn et al., 2003; Zhitkovich 2005).

Cr(VI)-induced DNA Damage

It is widely accepted that Cr(VI) is capable of causing DNA damage following intracellular reduction in the form of apurinic/apyrimidinic sites (Casadevall & Kortenkamp, 1994) and by interacting with proteins (Wedrychowski et al., 1985), amino acids (Zhitkovich et al., 1995), or directly with DNA (DeLoughery et al., 2015), causing DNA single- (Christie et al., 1984) and double-strand breaks (DSBs) (DeLoughery et al., 2015). Upon intracellular reduction, Cr(VI) can form bulky Cr(III) binary adducts (i.e. Cr(III) – DNA) as well as ternary adducts (i.e. Cr(III)–ligand–DNA). Cr(VI) reduction in the presence of physiological concentrations of ascorbic acid demonstrated that ternary ascorbate-Cr(III)-DNA crosslinks were more mutagenic than their binary Cr(III)-DNA counterparts, with ternary complexes accounting for more than 90% of mutagenic damage (Quievryn et al., 2003). Cr(VI) is also capable of inducing 8-oxo-dG formation in proportion to Cr(VI) concentration *in vitro*, and 8-oxo-dG is often employed as a biomarker of exposure in chromate exposed individuals (Arakawa et al., 2012; Li et al., 2014). Using UvrABC and *Fpg* incision methods, Cr(VI) was found to induce bulky adducts and oxidative DNA damage at both dG's as well as dA's in the p53 gene (Arakawa et al., 2012).

More recently, DSBs caused by Cr-adducted DNA has been investigated in detail. Selective formation of Cr-induced DSBs occurred in euchromatin, despite the presence of Cr-DNA adducts in both euchromatin and heterochromatin (DeLoughery et al., 2015). It was further revealed that DSB repair signaling involving γH2AX, mono-, and di-ubiquitinated H2AX were dependent on ATR as opposed to the classical ATM mode of activation (DeLoughery et al., 2015). ATR is strongly activated by single-stranded tails as opposed to blunt-ended DSBs (Lukas et al., 2011). The authors further point out that mismatch repair operates by excision of one strand, producing a series of single-stranded DNA. This partly reveals that the nature of DNA damage following the formation of Cr-DNA adducts. Extended exposure (>48 h) to particulate Cr(VI), however, was shown to reduce Rad51 foci formation and protein expression, suggesting that the homologous recombination (HR) signaling pathway is repressed after longer exposures to Cr(VI) in particulate form (Qin et al., 2014). After 48 h, Rad51 was observed to aggregate in the cytoplasm, providing a possible explanation. It was later confirmed that prolonged exposure to particulate Cr(VI) does, in fact, inhibit HR repair (Browning et al., 2016). Rad51C is responsible for Rad51 nuclear import and stabilization of the Rad51 nucleofilament, and Rad51C foci formation was subsequently

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shown to be inhibited following prolonged exposure to particulate Cr(VI) (Browning et al., 2016).

Using formaldehyde-assisted isolation of regulatory elements and deep sequencing, Ovesen et al. reported, *in vitro*, that acute Cr(VI) versus chronic Cr(VI) treatment opened 3 times as many unique chromatin domains, and only eleven of these unique domains were shared between treatments (Ovesen et al, 2014). Chromatin domains surrounding both AP-1 and CTCF were found to become significantly more open after acute treatment whereas chromatin domains surrounding AP-1 and BAH2 were more open following chronic treatment. Furthermore, structural chromatin changes were not correlated with changes in global transcriptional response. Structural chromatin changes did, however, affect gene expression levels in target areas that vary with Cr(VI) concentration. Overall, the changes in chromatin structure in response to acute and chronic Cr(VI) suggests that the mechanisms governing Cr(VI)-induced transcriptional response are uniquely different depending on the dose, which may impact molecular events leading to carcinogenesis. Epigenetic Mechanisms of Chromium Carcinogenesis

Epigenetics refers to the reversible yet heritable changes in gene expression, independent of DNA sequence, caused by DNA hypo- or hypermethylation, histone tail post-translational modifications, and microRNAs (miRNAs). The 5 carbon of cytosine in DNA can be covalently methylated by DNA methyltransferases, or may be actively or passively demethylated. Histone post-translational modifications (PTMs) include, but are not limited to: acetylation, methylation, phosphorylation and citrullination, and can impact chromatin structure, thereby acting as gate-keepers for chromatin access (reviewed in Tessarz & Kouzarides, 2014). Histone PTMs also recruit or hinder epigenetic machinery or transcriptional regulators that direct gene expression. miRNAs, on the other hand, regulate gene expression by binding to complementary regions on target messenger RNAs (mRNAs) to dampen and fine-tune expression in the form of RNA degradation, and also reduce expression through induced decapping, induced deadenylation, altered cap protein binding, reduced ribosome occupancy, and sequestration of mRNA from translational machinery (reviewed in Mohr & Mott, 2015). Extensive studies have shown that Cr(VI) is capable altering gene expression and inducing cancer development through multiple epigenetic mechanisms. The following sections will examine these mechanisms in depth, and a comprehensive summary of genes altered by Cr(VI) exposure is listed in Table 1.

DNA Methylation

It was first reported that Cr(VI) could induce DNA methylation and silencing of the *gpt* transgene in G12 Chinese hamster lung cells (Klein et al., 2002). Since then, a number of studies have shown a wide range of epigenetic effects. Lou et al. found acute soluble Cr(VI) or particulate lead chromate induced global DNA hypomethylation, which maintained for 20 h (Lou et al., 2013). Aberrant DNA methylation, including global DNA hypomethylation as well as promoter specific DNA methylation, contributes to genomic instability and gene silencing, and has been identified in numerous cancer types and human diseases (reviewed in Pogribny & Beland, 2009). Hu et al. (2016) found that in human bronchial epithelial cells (16HBE), CpG sites on the p16 gene were significantly more methylated than controls

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following Cr(VI) exposure, and that p16 mRNA expression was negatively correlated with dose (Hu et al., 2016). The p16 tumor suppressor functions by inhibiting CDK4 and CDK6, which phosphorylate retinoblastoma protein and induces cell cycle arrest, and its inactivation via promoter methylation is common in lung cancers (Ohtani et al., 2004; Tam et al., 2013).

In lung tumors obtained from chromate workers in Japan, methylation of both DNA repair genes, hMLH1 (28%) and MGMT (20%), and tumor suppressor APC (86%), were detected using nested methylation-specific PCR (Ali et al., 2011). In non-chromate lung cancer tumors, methylation of hMLH1 was not detected, and methylation of APC was detected in only 44% of tumors. In a cross-sectional study examining CpG methylation of DNA repair genes, HOGG1, MGMT, XRCC1, ERCC3, and RAD53, methylation of HOGG1, MGMT, and RAD53 were significantly higher in chromate-exposed workers as well as in 16HBE cells treated with Cr(VI) (Hu et al., 2018). CpG sites within HOGG1, MGMT, and RAD51 were similarly modified in both lymphocytes from chromate-exposed workers and in Cr(VI)-treated 16HBE cells, revealing a distinct methylation pattern.

Recently, Cr was found to inhibit the levels of 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-foC), and 5-carboxylcytosine (5-caC) in mouse embryonic stem cells - all 10–11 translocation (TET) protein-induced derivatives of 5-methylcytosine (5-mC) (Xiong et al., 2017). While TET1–3 mRNA expression was found to be unchanged, TET protein activity decreased by 62.1% and 2-hydroxyglutarate (2-HG; endogenous TET inhibitor) was found dramatically increased. 2-HG can act as a competitive inhibitor of TET 5-methylcytocine hydroxylases due to structural similarities with α-KG, a necessary co-substrate for conversion of 5mC to 5-hmC (Xu et al., 2011). TET proteins and their 5-mC modifying activities are crucial for epigenetic reprogramming during both development and active DNA demethylation (reviewed in Kohli & Zhang, 2013).

Histone Posttranslational Modifications

The roles that histone modifications play in chromatin homeostasis are dynamic and dependent on factors such as the degree of modification (e.g. mono-, di-, and tri-methylation), location, and on histone cross-talk, which is context specific. Histone modification is tightly controlled by enzymatic activities of histone methyltransferases, demethylases, histone acetyltransferases (HATs), and deacetylases (HDACs), among others. For instance, HATs catalyze the transfer of acetyl groups from acetyl CoA to histone lysine residues, HDACs remove acetyl groups from histones. Acute high dose Cr(VI) exposure has been found to induce various histone modifications including increase in global histone 3 lysine 9 (H3K9) and histone 3 lysine 4 (H3K4) di- and trimethylation, and decrease global histone 3 lysine 27 (H3K27) trimethylation and histone 3 arginine 2 (H3R2) dimethylation, *in vitro* (Sun et al., 2009), see Table 2. Furthermore, H3K9 dimethylation was found enriched in the MLH1 promoter, and MLH1 promoter methylation was correlated with a decrease in MLH1 mRNA expression. H3K9 dimethylation and subsequent MLH1 silencing may be attributable to histone methyltransferase G9a, which specifically methylates H3K9, and was also found to increase at both the transcriptional and protein level. However, histone modifying enzymes including, but not limited to, SUV39H1, which methylates H3K9 (me2

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and me3), and EZH2, which methylates H3K27, can also contribute to aberrant histone methylation following Cr(VI) exposure (Wang et al., 2018). Cr(VI) has also been shown to inhibit JHDM2A, a H3K9 demethylase, by reducing ascorbic acid availability (Sun et al., 2009). Specifically, the ascorbic acid reserve needed for JHDM2A demethylase activity is depleted upon intracellular reduction of Cr(III), which can subsequently lead to elevated H3K9me2 levels.

Cr(VI) not only interferes with histone methyltransferases and demethylases, but has also been shown to crosslink HDAC1-DNMT1 to the *Cyp1A1* promotor (Schenekenburger et al., 2007). Total HDAC1 activity following Cr(VI) was shown to be unaffected by Cr(VI), yet local deacetylase activity was shown to be sufficient to impact histone acetylation levels in *Cyp1A1* (Schenekenburger et al., 2007). In addition to HDAC1, both HDAC2 and HDAC3 protein expression levels in 16HBE cells were found to increase following 24 h exposure to Cr(VI) (Xia et al., 2014). Corresponding to this increase in HDAC2 and HDAC3, global levels of H3 and H4 acetylation were found to decrease (Xia et al., 2014). Xia et al. 2014 further showed that Cr(VI) significantly decreased biotinidase (BTD) at both the protein and mRNA levels, and this decrease was dependent on histone acetylation. Accordingly, histone biotinylation was shown to be inversely related to Cr(VI) at low (0.6 μM) doses (Xia et al., 2014). Lastly, Cr(VI) was found to significantly reduce histone 4 lysine 16 (H4K16) monoacetylation – a hallmark of cancers (Chen et al., 2016; Fraga et al., 2005).

Among many acetylated histone lysine residues, H4K16 acetylation is the most prevalent modification for controlling the formation of higher-order chromatin structure and modulating the functional interaction between non-histone proteins and chromatin fibers (Zhang et al., 2017). Located in the basic patch of the H4 N-terminal tail, acetylation of H4K16 can hinder the interaction between core histones such as H4 and H2A/H2B, and dynamically regulate chromatin related processes including chromatin condensation, DNA replication, transcription, repa ir, damage responses, as well as overall genome stability (Dorigo et al., 2003; Shogren-Knaak et al., 2006; Shogren-Knaak & Peterson, 2006). Due to considerable influence of this particular histone modification, it is not surprising that the loss of H4K16 acetylation has been reported as an important hallmark for many human cancers [35].

MOF, Males absent on the first, a member of the MYST (MOZ, Ybf2 (Sas3), Sas2, and Tip60) family of histone acetyltransferase (Chen et al., 2015; Li et al., 2010; Taipale et al., 2005), is an H4K16-specific HAT. Interestingly, although H4K16 can be acetylated by many different histone acetyltransferases, studies suggest that MOF may be the only HAT capable of acetylating H4K16 in intact cells. As shown in HeLa and HepG2 cell lines, knockdown of MOF led to reduction in H4K16 acetylation while other histone acetylation marks remained unchanged (Taipale et al., 2005; Smith et al., 2005). Loss of MOF and subsequent H4K16ac have been linked to greater genome instability and tumor development (Fraga et al., 2005).

Stressor protein NUPR1 promotes tumorigenesis through mechanisms relating to cell cycle, apoptosis, and more recently uncovered, chromatin remodeling. Consistent with previous studies demonstrating the ability of Cr(VI) to reduce global H3 and H4 acetylation, Chen et al. showed that Cr(VI) induced NUPR1, see Figure 1 (Chen et al., 2016). NUPR1 induction

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resulted in the reduction of MOF and H4K16 acetylation, and subsequently led to cell transformation, as indicated by acquired ability for anchorage-independent growth in BEAS-2B cells (Chen et al., 2016). NUPR1-induced loss of H4K16ac and downregulation of MOF supports the assertion that the chromatin remodeling ability of NUPR1 is a mechanism driving Cr(VI)-induced lung carcinogenesis. However, NUPR1 and its relationship with other chromatin influencers, post-translational modifications governing protein expression and activity, and transcriptional regulation remain to be unveiled.

MicroRNAs

In comparison to DNA methylation and histone post-translational modifications, less is known about the influence of Cr(VI) on microRNAs (Table 3), yet they represent critical gene regulatory mechanisms and are often dysregulated in cancers. For instance, miR-143 was found to be downregulated in Cr(VI)-transformed cells, and was also found to be repressed in human lung cancer cells, see Figure 2 (He et al., 2013). Repression of miR-143 was capable of inducing cell transformation and angiogenesis via upregulation of insulin-like growth factor-1 receptor (IGF-IR) and insulin receptor substrate-1 (IRS1). IGF-IR/IRS1 was found to upregulate interleukin-8 (IL-8) as well as activate downstream ERK/HIF-1a/NF-kB signaling pathway to induce transformation and tumor angiogenesis. miRNA profile analysis of *Drosophila melanogaster* larva exposed to varying concentrations of Cr(VI) for 24–48 h showed 28 significantly dysregulated miRNAs targeting major biological processes (Chandra et al., 2015). Concurrent downregulation of miRNA gene targets, *mus309* and *mus312*, *acon*, and *pyd*, which function in DNA repair, oxidation-reduction processes, and stress activated MAPK cascade, respectively, were also reported. A significant dose-dependent increase in miR-21 and dose-dependent decrease in mRNA and protein expression of its target gene, PDCD4, has also been observed (Pratheeshkumar et al., 2016). PDCD4 acts as a tumor suppressor, in part, via regulating E-cadherin. E-cadherin was subsequently found to be downregulated, and upregulation of active b-catenin and TCF4 was evident. Oncogenic c-MYC and uPAR, targets of b-catenin/TCF4-dependent transcription, were increased in a dose-dependent manner, and chromatin immunoprecipitation analysis showed their association with both uPAR and c-MYC promoters. uPAR expression has been shown to enhance tumor growth and metastasis, and has been associated with cancer stem-cell like property in small cell lung cancer (Gutova et al., 2007; Xing & Rabbani, 1996). In addition, the study revealed that Cr(VI) was able to induce phosphorylation and activation of the signal transducer and activator of transcription-3 (STAT3) as well as increased IL-6 secretion. Previous studies have shown that STAT3 can bind directly to the miR-21 promoter upon IL-6 activation (O'hara et al., 2007; Pratheeshkumar et al., 2016), thus Cr(VI) may promote miR-21 expression via the IL-6/STAT3 pathway.

Conclusion

Extensive research has solidified Cr(VI) as a potent human carcinogen, especially in the context of lung cancer. Although epigenetic alteration has been proposed as an important mechanism underlying Cr(VI) carcinogenicity, researchers are still trying to elucidate the specific changes as well as the epigenetic machineries responsible for mediating these alterations. This review provides an updated summary of current findings on mechanisms of

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Cr(VI) carcinogenesis, with a focus on epigenetic changes. In addition, this review also revealed that while much focus has been placed on the role of oxidative stress in Cr(VI) carcinogenesis, less focus has been given to the role of histone modifications and miRNAs. The current research gap in this area may provide a new niche for future research, which can generate new studies in the effort to provide a more comprehensive understanding of the mechanisms of Cr(VI) carcinogenesis.

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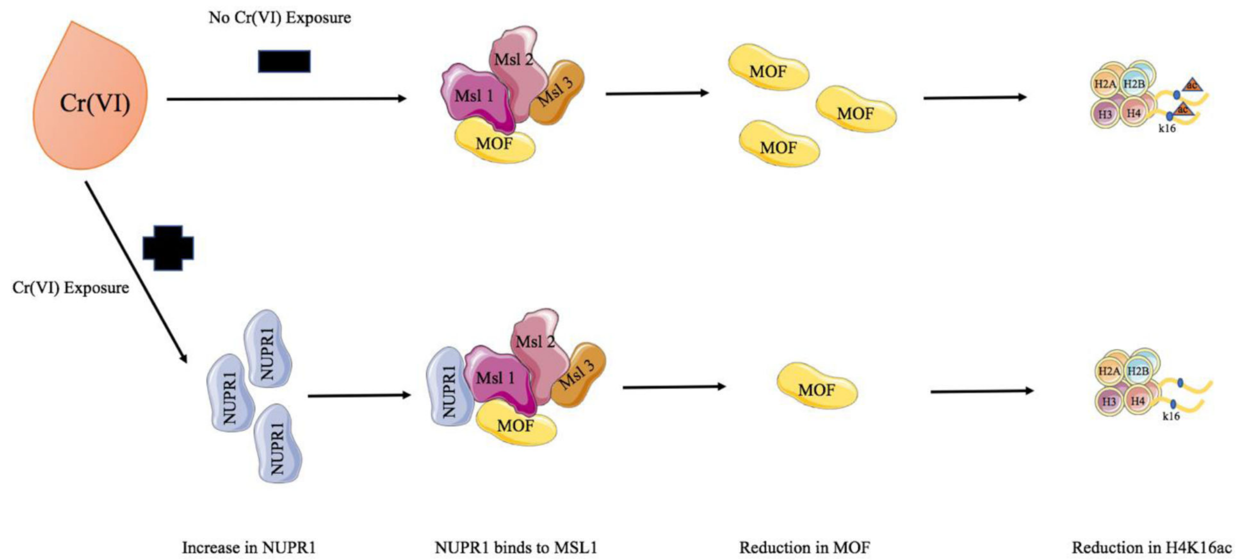

Figure 1.

Model illustrating Cr(VI)-induced reduction in H4K16ac through NUPR1 induction

The model depicts Cr(VI)-induced up-regulation of NUPR1, which can potentially bind to MSL complex (MSL1) and thereby hinder MOF transcription and subsequent histone H4K16 acetylation (Chen et al., 2015; Chen et al., 2016; Gironella et al., 2009).

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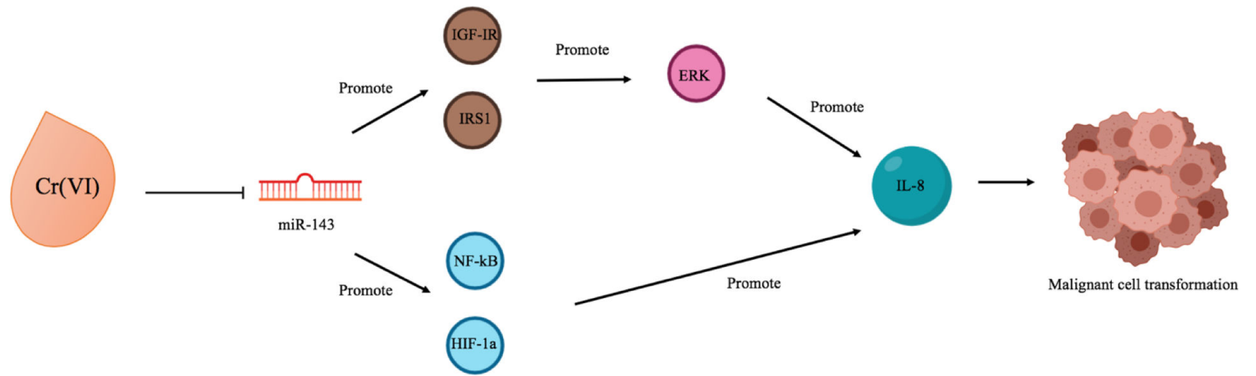

Figure 2.
Model illustrating Cr(VI)-induced miR-143 reduction and subsequent cell signaling response

The model portrays Cr(VI)-induced reduction in miR-143, which has been shown to promote IL-8 and malignant cell transformation through two signaling pathways: IGF-IR/IRS1/ERK and HIF-1a/NF-kB (He et al., 2013).

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Table 1.

Summary of genes altered by Cr(VI)

Gene Name	Direction of change	Chromium compound	Dose	Length of exposure	Tissue type	Reference
Angjogenin	Up	Na2Cr2O7	0.25 uM	6 mo	BEAS-2B	Kim et al., 2016
AP1	Up	K2Cr2O7	5 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2014
ATM	Down	K2Cr2O7	L uM	48 hr	BEAS-2B	Abreu et al., 2018
ATR	Up	K2Cr2O7	1 uM	48 hr	BEAS-2B	Abreu et al., 2018
Bcl-xL	Up	Na2Cr2O7	100 nM	3 mo	BEAS-2B	Dai et al., 2017
Bcl2	Up	Na2Cr2O7	100 nM	3 mo	BEAS-2B	Dai et al., 2017
Bcl2	Up	Cr(VI)	0.3 uM	30 wk	BEAS-2B, keratinocytes	Ganapathy et al., 2017
Bcl2	Up	K2Cr2O7	0.5 uM	4 wk	BEAS-2B	Huang et al., 2017
Bcl2	Up	Na2Cr2O7·2H2O	5 uM	24 wk	BEAS-2B	Medan et al., 2012
Bcl2	Up	Na2Cr2O7·2H2O	10 uM	24 hr	BEAS-2B	Son et al., 2017
BTD	Down	K2CrO4	6.25–12.5 uM	24 hr	16HBE	Xia et al., 2011
Capspase-9	Up	K2Cr2O7	0.2 uM	0–6 hr	A549	Ge et al., 2019
Caspase 3	Up	K2Cr2O7	0.2 uM	0–12 hr	A549	Ge et al, 2019
Caspase-8	Up	K2Cr2O7	0.2 uM	0–6 hr	A549	Ge et al., 2019
CDK4	Down	K2Cr2O7; PbCrO4	5–15 uM; 1.25–5 uM	2–24 hr	A549, human B lymphoblastoid	Lou et al., 2013
CDK6	Down	K2Cr2O7; PbCrO4	5–15 uM; 1.25–5 uM	2–24 hr	A549, human B lymphoblastoid	Lou et al., 2013
Cox-2	Up	K2Cr2O7	5 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2014
Cox-2	Up	Na2CrO4	20 uM	6, 12 hr	MEFs	Zuo et al., 2012
Cox2	Up	K2Cr2O7	5 uM	24 hr	BEAS-2B	Roy et al., 2016
E-cadherin	Down	K2Cr2O7	1.5–15 uM	10 hr-1 wk	BEAS-2B	Ding et al., 2013
ERCC3	Down	K2Cr2O7	0.6 uM	24 hr	16HBE	Hu et al., 2018
ERK	Up	Na2Cr2O7	50–100 uM	1–2 hr	A549	Wang & Shi 2001
EZH2	Up	K2Cr2O7	.25 uM	20,40 wk	BEAS-2B, 16HBE	Wang et al., 2018
FBP1	Down	Na2Cr2O7	100 nM	3 mo	BEAS-2B	Dai et al., 2017
G9a	Up	K2CrO4	5–10 uM	24 hr	A549	Sun et al., 2009
G9a	Up	K2Cr2O7	.25 uM	20,40 wk	BEAS-2B, 16HBE	Wang et al., 2018
Gene 33	Down	Na2CrO4	1–5 uM	48–72 hr	BEAS-2B	Park et al., 2016
Gli2	Up	K2Cr2O7	0.5 uM	4 wk	BEAS-2B	Huang et al., 2017

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Gene Name	Direction of change	Chromium compound	Dose	Length of exposure	Tissue type	Reference
GLP	Up	K2Cr2O7	.25 uM	20,40 wk	BEAS-2B, 16HBE	Wang et al., 2018
GRO-a	Up	ZnCrO44Zn(OH)2	50 uL	0–24 hr	BAL fluid	Beaver et al., 2008
GRP78	Up	K2Cr2O7	0.2 uM	12 hr	A549	Ge et al., 2019
HCS	Up	K2CrO4	6.25–12.5 uM	24 hr	16HBE	Xia et al., 2011
HDAC2	Up	K2CrO4	2.5–5 uM	24 hr	16HBE	Xia et al., 2014
HDAC3	Up	K2CrO4	2.5–5 uM	24 hr	16HBE	Xia et al., 2014
HIF-la	Up	K2Cr2O7	2.5–10 uM	0–24 hr	DU145	Gao et al., 2002
HIF-la	Up	K2Cr2O7	5 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2014
HIF-a	Up	Na2Cr2O7	0.25 uM	6 mo	BEAS-2B	Kim et al., 2016
HO-1	Up	K2Cr2O7	5 uM	24hr	Mycoplasma-free human dermal fbroblast	Joseph et al., 2008
HO-1	Up	Na2Cr2O7·2H2O	1 uM acute, .125–5 uM chronic	24 hr	BEAS-2B	Son et al., 2017
HOGG1	Down	K2Cr2O7	0.6 uM, 1.2 uM, 2.5 uM, 5.0 uM, 10.0 uM and 20.0 uM	24 hr	16HBE	Hu et al., 2018
Hsp90a	Down	K2Cr2O7	1 uM	48 hr	BEAS-2B	Abreu et al., 2018
HSPA1A	Down	K2Cr2O7	1 uM	48 hr	BEAS-2B	Abreu et al., 2018
IL-6	Up	ZnCrO44Zn(OH)2	50 uL	0–24 hr	BAL fluid	Beaver et al., 2008
IL-6	Up	Na2Cr2O7	0.25 uM	6 mo	BEAS-2B	Kim et al., 2016
IL-6	Up	K2Cr2O7	0–2 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2014
IL-8	Up	K2Cr2O7	0–2 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2014
iNOS	Up	K2Cr2O7	5 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2014
JNK	Up	K2Cr2O7	10–80 uM	l-12 hr	CL3	Chuang et al., 2000
MGMT	Down	K2Cr2O7	20 uM	24 hr	16HBE	Hu et al., 2018
MMP-1	Up	Na2Cr2O7	0.25 uM	6 mo	BEAS-2B	Kim et al., 2016
NQO1	Up	Na2Cr2O7	100 nM	3 mo	BEAS-2B	Dai et al., 2017
Nrf2	Up	K2Cr2O7	1–5 uM	24 hr	BEAS-2B	Roy et al., 2016
Nr£2	Up	Na2Cr2O7·2H2O	1 uM acute, .125–5 uM chronic	24 hr	BEAS-2B	Son et al., 2017
NUPR1	Up	K2CrO4	5–10 uM	24 hr	BEAS-2B	Chen et al., 2016

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Gene Name	Direction of change	Chromium compound	Dose	Length of exposure	Tissue type	Reference
OGG1	Down	Na2Cr2O7	0–100 uM	16 hr	A549	Hodges & Chipman, 2002
p-16	Up	K2Cr2O7; PbCrO4	5–15 uM; 1.25–5 uM	2–24 hr	A549, human B lymphoblastoid	Lou et al., 2013
p-PERK	Up	K2Cr2O7	0.2 uM	12 hr	A549	Ge et al., 2019
p38	Up	K2Cr2O7	10–80 uM	l-12 hr	CL3	Chuang et al., 2000
p38	Up	Na2Cr2O7	50–100 uM	3 hr	A549	Wang & Shi, 2001
p53	Up	Na2Cr2O7	20–100 uM	l-2 hr	A549	Wang & Shi, 2001
PDCD4	Down	K2Cr2O7	5 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2016
RAD51	Down	K2Cr2O7	0.6 uM, 1.2 uM, 2.5 uM, 5.0 uM, 10.0 uM and 20.0 uM	24 hr	16HBE	Hu et al., 2018
Snail	Down	K2Cr2O7	1.5–15 uM	10 hr-1 wk	BEAS-2B	Ding et al., 2013
SOD1	Up	Na2Cr2O7·2H2O	1 uM acute, . 125–5 uM chronic	24 hr	BEAS-2B	Son et al., 2017
SOD2	Up	Na2Cr2O7	100 nM	3 mo	BEAS-2B	Dai et al., 2017
SUV39H1	Up	K2Cr2O7	.25 uM	20,40 wk	BEAS-2B, 16HBE	Wang et al., 2018
TNF-a	Up	K2Cr2O7	5 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2014
TNF-a	Up	K2Cr2O7	1–5 uM	24 hr	BEAS-2B	Roy et al., 2016
Twist	Down	K2Cr2O7	1.5–15 uM	10 hr-1 wk	BEAS-2B	Ding et al., 2013
VEGF	Up	K2Cr2O7	2.5 uM	0–24 hr	DU145	Gao et al., 2002
VEGF	Up	Na2Cr2O7	0.25 uM	6 mo	BEAS-2B	Kim et al., 2016
Vimentin	Up	K2Cr2O7	1.5–15 uM	10 hr-1 wk	BEAS-2B	Ding et al., 2013
XRCC1	Down	K2Cr2O7	2.5 uM, 10.0 uM and 20.0 uM	24 hr	16HBE	Hu et al., 2018
yH2AX	Up	Na2CrO4	5 uM	6 & 24 hr	BEAS-2B	Park et al., 2016

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Table 2.

Summary of histone modifications altered by Cr(VI)

Histone Mark	Direction of change	Chromium compound	Dose	Length of exposure	Tissue type	Reference
H4K16ac	Down	K2CrO4	10 uM	24hr	BEAS-2B	Chen et al., 2016
H3K27me3	Down	K2CrO4	5–10 uM	1 hr	A549	Sun et al., 2009
H3K4me2	Up	K2CrO4	10 uM	1 hr	A549	Sun et al., 2009
H3K4me3	Up	K2CrO4	10 uM	1 hr	A549	Sun et al., 2009
H3K9me2	Up	K2CrO4	5–10 uM	1 hr	A549	Sun et al., 2009
H3K9me3	Up	K2CrO4	5–10 uM	1 hr	A549	Sun et al., 2009
H3R2me2	Down	K2CrO4	5–10 uM	1 hr	A549	Sun et al., 2009
H3K27me3	Up	K2Cr2O7	.25 uM	20,40 wk	BEAS-2B, 16HBE	Wang et al., 2018
H3K9me2	Up	K2Cr2O7	.25 uM	20,40 wk	BEAS-2B, 16HBE	Wang et al., 2018
H3ac	Down	K2CrO4	2.5–5 uM	24 hr	16HBE	Xia et al., 2014
H4ac	Down	K2CrO4	2.5–5 uM	24 hr	16HBE	Xia et al., 2014
H3K4me3	Up	K2CrO4	0.5–10 uM	24 hr	A549	Zhou et al., 2009

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Table 3.

Summary of miRNAs altered by Cr(VI)

Gene Name	Direction of change	Chromium compound	Dose	Length of exposure	Tissue type	Reference
miR-1-3p	Up	K2Cr2O38	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-10-3p	Up	K2Cr2O29	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
mR-10-5p	Up	K2Cr2O28	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-1002-5p	Up	K2Cr2O16	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-12-5p	Up	K2Cr2O10	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-13b-3p	Up	K2Cr2O12	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-143	Down	Na2Cr2O7·2H2O	1 uM	6 mo	BEAS-2B	He et al., 2013
miR-184-3p	Up	K2Cr2O36	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-21	Up	K2Cr2O7	2.5–5 uM	24 hr	BEAS-2B	Pratheeshkumar et al., 2016
miR-2493-5p	Up	K2Cr2O15	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-2494-5p	Up	K2Cr2O14	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-252-3p	Up	K2Cr2O40	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-276a-3p	Up	K2Cr2O25	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-277-3p	Up	K2Cr2O17	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-279-3p	Up	K2Cr2O11	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-281-2-5p	Up	K2Cr2O35	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-289-5p	Up	K2Cr2O19	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-2a-3p	Up	K2Cr2O30	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-2b-3p	Up	K2Cr2O31	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-306-5p	Up	K2Cr2O32	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-314-3p	Up	K2Cr2O8	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-31a-5p	Up	K2Cr2O27	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-34-5p	Up	K2Cr2O24	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-375-3p	Up	K2Cr2O26	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015

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Gene Name	Direction of change	Chromium compound	Dose	Length of exposure	Tissue type	Reference
miR-389-3p	Up	K2Cr2O39	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-3940-5p	Down	Cr(VI)	5 IQR	3–10 yr	Human blood	Li et al., 2014
miR-3940-5p	Down	Na2CrO4	5–10 uM	0, 12 hr	16HBE	Li et al., 2016
miR-7-5p	Up	K2Cr2O33	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-79-3p	Up	K2Cr2O9	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-8-3p	Up	K2Cr2O23	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-954-5p	Down	K2Cr2O7	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-956-3p	Up	K2Cr2O18	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-959-3p	Up	K2Cr2O41	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-964-5p	Up	K2Cr2O42	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-970-3p	Up	K2Cr2O37	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al., 2015
miR-986-5p	Up	K2Cr2O13	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-998-3p	Up	K2Cr2O34	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-9a-3p	Down	K2Cr2O20	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015
miR-9a-5p	Up	K2Cr2O21	5–20 ug/mL	24, 48 hr	Drosophila mid-gut tissue	Chandra et al, 2015
miR-9c-5p	Up	K2Cr2O22	5–20 ug/mL	24, 48 hr	Drosophik mid-gut tissue	Chandra et al., 2015