ROS

REVIEW ARTICLES

The Role of Epigenetics in the Toxic Effects Induced by Hexavalent Chromium

Huimin Feng, Jiaxing Liu, Guiping Hu, and Guang Jia

Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, China

Correspondence: jiaguangjia@bjmu.edu.cn (G.J.)

Feng H et al. Reactive Oxygen Species 5(14):107–117, 2018; ©2018 Cell Med Press
http://dx.doi.org/10.20455/ros.2018.821
(Received: October 22, 2017; Revised: November 28, 2017; Accepted: November 29, 2017)

ABSTRACT | The International Agency for Research on Cancer has classified compounds containing hexavalent chromium [Cr(VI)] as Group Ⅰ human carcinogens. Exposure to Cr(VI) via respiratory tract may affect the respiratory function and even lead to lung cancer. The reactive oxygen species (ROS) induced by long-term low-level chromium exposure could cause oxidative stress and DNA damage. There are three possible mechanisms of Cr(VI)-induced carcinogenesis, which are multistage carcinogenesis, genomic instability, and epigenetic modification. Epigenetic changes could be detected at the early stage of DNA damage, including changes of DNA methylation, histone modification, microRNA, circular RNA, long non-coding RNA, and other epigenetic modifications. This review will focus on the role of epigenetic modification in the toxic effects of Cr(VI) and the association between ROS and epigenetic modifications induced by Cr(VI).

KEYWORDS | Carcinogenesis; Circular RNA; Competitive endogenous RNA; DNA methylation; DNA repair; Epigenetics; Hexavalent chromium; Histone modification; Long non-coding RNA; MicroRNA; Reactive oxygen species

ABBREVIATIONS | ANRIL, antisense non-coding RNA in the INK4 locus; ceRNA, competitive endogenous RNA; circRNA, circular RNA; DDSR1, DNA damage-sensitive RNA1; DSB, double strand break; HR, homologous recombination; lncRNA, long non-coding RNA; MDA, malondialdehyde; miRNA, MicroRNA; NHEJ, non-homologous end-joining; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; ROS, reactive oxygen species

CONTENTS

1. INTRODUCTION

Chromium is one of the metallic elements present in rocks, animals, plants, soil, and volcanic ash and gases. There are two common chromium states that are Cr(III) and Cr(VI). Cr(III) is an important component of the glucose tolerance factor. Cr(VI) is widely used in industry and agriculture, mainly in chromium smelting, chromate production, chrome plating, stainless steel welding, and chrome pigment production. The yield of chromate in China is at the forefront in the world in line with the facts that a large number of people are exposed to it. Acute exposure to a high dose of Cr(VI) could cause irritation of the upper respiratory tract. However, chronic exposure to low doses of Cr(VI) could lead to multiple respiratory diseases, increasing the incidence of lung cancer.

As early as 1987, China included lung cancer induced by chromate manufacturing in the list of occupational tumors. The International Agency for Research on Cancer has classified compounds containing Cr(VI) as Group 1 human carcinogens since 1990 [1]. A low dose of Cr(VI) exposure could induce a mesenchymal epithelial transition and reinforce the invasion in the process of carcinogenesis of pulmonary epithelial cells [2]. Exposure to Cr(VI) could increase the activity of $CD4^+$ and $CD8^+$ T cells in the spleen of mice. Cell proliferation/activation was inhibited and cytokine release and cytolytic function were affected [3]. Long-term exposure to low-dose chromium could generate ROS that induced changes in oxidative stress and DNA damage. The corresponding epigenetic changes could be detected at the early stage of DNA damage, and there was little research on the role of epigenetics in the carcinogenesis of chromate.

There could be three possible mechanisms of chromate-induced carcinogenesis, which are multistage carcinogenesis, genomic instability, and epigenetic modifications. Of the three mechanisms, epigenetic modifications were most concerned by researchers. Epigenetic modifications refer to modifications unrelated to changes in the DNA sequence but related to changes in gene expression which are inheritable. In other words, the genotype has not changed but the phenotype has changed. In addition, the changes in gene expression are stable but reversible and inherited during development and cell proliferation. Nowadays, more and more epigenetic modifications have been explored, such as DNA methylation, histone modification, microRNA, circular RNA, and long non-coding RNA. Different epigenetic pathways could also influence each other to form competitive endogenous RNA network [4]. This review will illustrate the role of epigenetic modifications in Cr(VI)-induced toxicities and the association between ROS and Cr(VI)-mediated epigenetic modifications.

2. EPIGENETIC CHANGES INDUCED BY CHROMIUM

2.1. DNA Methylation

DNA methylation refers to the process of transferring methyl groups to a specific base with S-adenosylmethionine as a methyl donor under the action of methyltransferase. It regulates gene expression, leading to changes in chromatin structure, DNA conformation, DNA stability, and interaction between DNA and protein to control the expression of genes in a variety of diseases. Tumor development involves a variety of methylation changes, including hypomethylation of oncogenes and hypermethylation of tumor suppressor genes and repair genes, which ultimately lead to increased genomic instability and changes in the state of genes. Reactive oxygen species (ROS) can be produced in the reduction process of hexavalent chromium, including hydroxyl radicals and hydrogen peroxide. ROS can interfere with the ability of methyltransferase to bind with DNA, which may result in a change in the methylation sta-

tus of the cytosine residues in the CpG site, an event closely related to the onset of lung cancer. Data from methylation gene chip showed that in the Cr(VI)-treated groups, the expression levels of p16 were lower but the methylation levels of CpG1, CpG31, and CpG32 of p16 were higher than those in the control group. The results revealed that there existed a negative correlation between p16 expression and its methylation levels, which pointed out the possibility of using p16 as a biomarker for Cr(VI)-induced epigenetic impairment [5]. A549 cells were treated with 5–15 μM dichromate and 1.25–5 μM lead chromate. After 24 h, whole genome hypomethylation occurred in these cells, followed by cell cycle arrest in G1 phase. Dichromate could significantly upregulate the expression levels of p16 mRNA while significantly downregulating expression levels of CDK4 and CDK6 mRNA. However, quantitative and qualitative results indicated that the methylation status of the p16 gene did not change after exposure to Cr(VI). Thus, the cell cycle arrest in G1 phase induced by Cr(VI) might be due to the hypomethylation of the whole genome, but the upregulated expression of p16 could be independent of DNA methylation [6]. Analysis of Illumina Infinium HumanMethylation450K BeadChip array showed that human B lymphoblastoid cells treated with potassium dichromate and lead chromate had approximately 40 differentially expressed methylated CpG sites and 15 overlapping CpG sites. Thus, both soluble and insoluble Cr(VI) could cause changes in DNA methylation sites in human B lymphoblastoid cells [7]. Sprague-Dawley rats were exposed to potassium dichromate at a concentration of 100 and 300 mg/L for 4 weeks, resulting in hypomethylation of the whole genome [8]. Chromium exposure in workers led to increased release of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) in the urine and hypomethylation of the whole genome [9].

2.2. Histone Modification

Post-translational modifications such as acetylation, phosphorylation, and ubiquitination of the histone N-terminal and C-terminal could alter the structure of chromatin and participate in the regulation of various biological functions such as DNA replication, DNA repair, and transcription. The study of histone modification by Cr(VI) has mainly focused on methylation and acetylation.

Xia et al. selected 16HBE cells as an in vitro model to determine the epigenetic modification induced by Cr(VI). First, they explored the changes of biotinidase and holocarboxylase synthetase that played an important role in the homeostasis of histone biotinylation. It was suggested that Cr(VI) could downregulate the level of biotinidase at the transcription level by histone acetylation [10]. Interestingly, they further found that when the concentration of Cr(VI) was ≤ 0.6 μM, the histone biotinidase was increased. When the concentration of Cr(VI) was ≥ 0.6 μM, the distribution of biotinidase was influenced. When the concentration of Cr(VI) was ≥ 2.5 μM, histone deacetylation occurred. In general, the acetylation of histones promoted the dissociation of DNA and histone octamers and the relaxation of nucleosomal structures, so that various transcription factors and synergistic transcription factors could bind specifically to DNA binding sites, activating the transcription of genes. On the other hand, the deacetylation of histones exerted the opposite effect. In cancer cells, acetylation of histones could restore the positive charge of histones, thus increasing the gravitational attraction between DNA and histones, so that the relaxation of nucleosomes became very tight, which was not beneficial for the expression of certain tumor suppressing genes. As a result, Cr(VI)-induced histone deacetylation might be correlated with Cr(VI)-induced cancers [11]. After exposure to Cr(VI), the global levels of histone H3K9 and H3K4 of dimerization and trimethylation were elevated in A549 cells, but global levels of histone H3K27 trimethylation and H3R2 dimerization were decreased. H3K9 could be enriched in the promoter region of MLH1 gene to affect the reduction of the expression level of MLH1 mRNA. For G9a, an H3K9 specific methyltransferase, its mRNA expression levels increased after exposure to Cr(VI), thereby increasing the global level of H3K9 dimerization. Interestingly, ascorbic acid could reverse the methylation level of H3K9 by reducing Cr(VI) and increasing the activity of histone demethylase [12].

2.3. MicroRNA

MicroRNAs (miRNAs) are small noncoding RNAs that affect plants, animals, and even human growth, and oxidative stress by epigenetic means. MiRNAs are negative regulatory factors of mRNA at the epigenetic level. Several studies suggested that miRNA

could affect DNA damage induced by Cr(VI). Chandra et al. examined differentially expressed miRNAs in *Drosophila melanogaster* exposed to 5–20 μg/ml Cr(VI) for 24 and 48 h. The results showed that dme-miR-314-3p was the maximally upregulated miRNA which could downregulate mus309 accompanied by significantly increased DNA damage in its overexpression strain. It revealed that the Cr(VI)-induced carcinogenesis might be related to the increased cell cycle arrest and DNA damage caused by downregulated repair-deficient gene mus309, of which the miRNA dme-miR-314-3p was the epigenetic regulator [13]. Moreover, the expression of 28 miRNAs concerning with the major biological processes such as the redox process and the DNA damage and repair process, was significantly altered following Cr(VI) treatment. As to the DNA repair, downregulation of mus309 and mus312 and the upregulation of dme-miR-314-3p and dme-miR-79-3p occurred simultaneously [14]. Bollati et al. investigated workers from an electric furnace steel plant and analyzed the levels of metals and miRNA in the peripheral blood leukocytes. miR-146a was found to be negatively correlated with chromium levels [15]. An experiment was conducted with workers in a chromate production plant to explore the epigenetic changes of Cr(VI)-induced carcinogenesis. The results showed that there existed a significant association between the levels of miR-3940-5p and chromium in the blood. At the same time, the results suggested that the level of XRCC2 was negatively correlated with the frequency of micronucleus. These data revealed that high levels of exposure to Cr(VI) did not always exacerbate genetic damage, which might be due to the regulation of miR-3940-5p on DNA repair genes [16]. Further vitro experiments showed that Cr(VI) inhibited the expression of miR-3940-5p which led to suppressed formation of Rad51 foci and blocked double-strand break repair as well as downregulated XRCC2 protein. The results suggested that miR-3940-5p had a protective effect on Cr(VI)-induced DNA damage [17]. He et al. developed an in vitro model by exposing the BEAS-2B cells to Cr(VI) for a long period of time. Using this model, the study found that the expression levels of miR-143 were significantly reduced in cells transformed by Cr(VI). Inhibition of miR-143 led to Cr(VI)-induced cellular malignant transformation and angiogenesis by upregulating the expression of insulin-like growth factor-1 receptor and insulin receptor substrate-1 [18].

3. OTHER MECHANISMS OF EPIGENETIC MODIFICATIONS

3.1. Circular RNA

Circular RNAs (circRNAs) are special endogenous noncoding RNAs, which are another hotspot of the RNA family after miRNAs and long-non-coding RNAs. With the rapid development of high-throughput sequencing and bioinformatics, researchers have found that circRNAs existed in eukaryotes in a diverse, conservative, and stable manner. There have been no reports about changes of circRNAs concerning with chromate exposure, but circRNAs have been extensively studied as emerging biomarkers in the clinical field. Compared to normal tissue, there were at least 5,500 kinds of circRNA candidates and a series of circRNAs were differentially expressed in gastric cancer tissues. Among them, circPTV1 which was derived from the PVT1 gene, was often upregulated in gastric cancer tissues. CircPTV1 could be used as a sponge of the miR-125 family to promote cell proliferation. The researchers believed that circPVT1 could be used as a proliferative factor or prognostic marker in gastric cancer [19]. Studies have shown that ciRS-7 (also known as Cdr1as) is an inhibitor and sponge of miR-7 in embryonic brain and islet cells of zebrafish, but there is no significant difference in the expression level between hepatocellular carcinoma and normal matched tissues. CiRS-7 was negatively correlated with the expression of miR-7 in hepatocellular carcinoma complicated by hepatic microvascular invasion, and positively correlated with the expression of two target genes (PIK3CD and p70S6K) of miR-7. The above evidence suggested that ciRS-7 might be a biomarker and a therapeutic target for liver microvascular invasion of the liver carcinoma [20]. CircRNA could fulfill its biological function by interacting with other non-coding RNAs. There were studies concerning about circRNA on other heavy metals such as lead. Results of high-throughput RNA sequencing showed that both lncRpa and circRar1 could promote neuronal apoptosis. LncRpa and circRar1 could upregulate the expression levels of mRNA and protein of caspase8 and p38 by regulating its common target miR-671 [21]. CircRNA might also be associated with the drug resistance of breast cancer. The researchers analyzed differential expression profile between doxorubicin-resistant MCF-7

breast cancer cells and the control MCF-7 cells and verified by q-PCR. The results showed that the circ_0006528-miR-7-5p-Raf1 axis played a regulatory role in doxorubicin-resistant breast cancer [22].

3.2. Long Non-Coding RNA

Long non-coding RNAs (lncRNAs) are a class of transcripts whose length is greater than 200 nucleotides long and which do not encode proteins. LncRNAs are structurally similar to mRNAs. The lncRNAs between certain genes regulate the transcription process, the RNA production, and RNA silencing pathway [23]. LncRNAs have the characteristics of tissue specificity, spatiotemporal specificity, regulatory diversity, disease characteristics, and other significant features [24]. LncRNAs could regulate gene expression such as chromatin remodeling, transcriptional activation, transcriptional interference, intracranial transport in transcriptional or post-transcriptional levels by RNA interference, gene imprinting, and gene silencing [25]. Regulation of lncRNAs on genes was closely related to tumorigenesis, such as development of glioblastoma, breast cancer, colorectal cancer, liver cancer, and leukemia [26]. In general, abnormal regulation of lncRNAs had an effect on cellular functions such as cell proliferation, resistance to apoptosis, induction of angiogenesis, promotion of metastasis, and escape of tumor inhibitors [27]. Fang et al. suggested that lncRNAs were the basic regulators of transcription [28]. Several lncRNAs have been characterized and several action models have been proposed, such as acting as transcriptional active molecules, regulating RNA or protein conjugates, and directing ribonucleoprotein complexes to specific targets to regulate the transcription and expression of related genes [29].

Activation of DNA damage in cells could trigger complex signal cascades which lead to cell cycle arrest, allowing cells to repair the damage. If the damage is irreparable, the cells are compensated by apoptosis or cell senescence. Previous studies have shown that Cr(VI) could cause double strand breaks (DSBs) in DNA and increase genome instability to induce cancer. DSBs could be repaired precisely by homologous recombination repair mechanisms. However, recent studies have suggested that Cr(VI) could inhibit homologous recombination repair by mismatching RAD51 [30]. MiRNAs and lncRNAs played an important role in cell cycle regulation and apoptosis and might be key participants in DNA-damage response. With further exploration of lncRNAs, it was found that DNA repair was tightly linked to lncRNAs. DNA repair consists mainly of base excision repair, nucleotide excision repair, non-homologous end-joining (NHEJ) repair, and homologous recombination (HR) repair. DSBs are the most difficult to repair among various types of DNA damage. When DSB occurred, there was no complementary strand to provide genetic information to repair the fracture. Then the homologous recombination repair should be started. The proteins of the phosphatidylinositol 3-kinase-like protein kinase family mediated the NHEJ pathway or homologous recombinant HR pathway to repair the DSB. ATM, ATR and DNA-PKcs played an important role in the process of DNA repair [31]. The choice of the repair pathway was affected by the stage of cell cycle. During G1 phase, DSBs were primarily repaired by the NHEJ pathway, which involved direct reconnection of DNA ends. While in the S/G2 phase, the HR pathway was dominant using homologous DNA template sequences for error-free repair [32]. In the process of DNA repair based on the HR signaling pathway, DSBs were detected by the MRE11-RAD50-NBS1 complex, and then ATM was activated by autophosphorylation [33]. Activation of ATM led to the phosphorylation of various DNA repair factors such as core histone variants H2AX, CtIP, and BRCA1, and exonuclease EXO1 [34]. BRCA1 activation promoted terminal resection and homologous recombination [35]. The extensive recombination and modification of chromatin rupture sites could provide repair factors for DSB repair [36]. So far, there have been little research on the function of lncRNAs in DNA repair after the Cr(VI)-induced DNA damage. Therefore, this section will focus on the function of lncRNAs in DNA repair and discuss the mechanism currently reported to provide a theoretical basis for the subsequent studies.

3.2.1. LncRNAs Involved in DNA Repair Based on the HR Signaling Pathway

DNA damage-sensitive RNA1 (DDSR1) has been clearly demonstrated in the study of lncRNAs associated with DNA repair. DDSR1 is an lncRNA produced in response to DNA damage through the ATM NF-κB pathway. Studies showed that DDSR1 was activated during the recruitment of DNA repair fac-

tors. DDSR1 modified the recruitment of HR repair factors such as BRCA1 and RAP80 to DSB by influencing the HR repair pathway. The absence of DDSR1 resulted in a sharp decrease in the efficiency of HR repair, indicating that the basal level of DDSR1 was significantly associated with DNA repair levels. These facts indicated that DDSR1 affected DNA repair by separating the BRCA1-RAP80 complex and preventing its binding to mixed DNA [37].

The deletion of antisense non-coding RNA in the INK4 locus (ANRIL) in the INK4 locus also inhibited the HR repair [38], but the specific molecular mechanism of ANRIL-mediated HR repair remained unknown. Similar to DDSR1, ANRIL regulated gene expression and DNA repair by HR [39]. It was shown that prostate-specific lincRNA PCAT1 was also involved in the regulation of HR. The expression of PCAT1 did not respond to DNA damage but inhibited homologous recombination of the DNA in prostate tissue by causing post-transcriptional blockade of BRCA2 [40].

3.2.2. LncRNAs Involved in DNA Repair Based on the NHEJ Signaling Pathway

Nonhomologous end joining pathway 1 (LINP1) was found to be a long-non-coding RNA closely related to breast cancer which could enhance NHEJ activity by providing a support for Ku80 and DNA-PKcs. Once the DSB occurred, the Ku80-Ku70 heterodimer recruited LINP1 to the damaged DNA, and then LINP1 increased the activity of NHEJ-mediated DNA repair by stabilizing Ku80 and DNA-PKcs complexes [41]. Cells without LINP1 expression could still repair DNA through the NHEJ pathway, indicating that LINP1 did not appear to be a prerequisite for the NHEJ process. However, forced expression of LINP1 in non-LINP1-expressing cells enhanced the activity of NHEJ-mediated DNA repair. In addition, the NHEJ pathway might also be a key source of genome rearrangement and instability.

Changes in lncRNA expression after DNA damage were also shown to be regulated by the p53 transcription factor. The presence of multiple p53-binding sites in the lincRNA-p21 promoter region could bind with hnRNP-K to stop the transcription of p53 and promote apoptosis [42]. After DNA damage, p53 activated transcription of lncRNAs including CDKN1A, PANDA, and lincRNA-p21. PANDA bound to NF-YA transcription factor to inhibit apoptosis after its transcription. PANDA, lincRNA-p21, and CDNK1A cooperated to regulate apoptosis [43]. P53 could also mediate gene silencing of PINT by interacting with polycomb repressive complex 2 [44].

3.3. Competitive Endogenous RNA

Poliseno et al. proposed a hypothesis of competitive endogenous RNAs (ceRNAs) that mRNAs, pseudogenes, lncRNAs, and circRNAs, as well as other endogenous RNA molecules contained certain miRNA binding sites. As illustrated in **Figure 1**, the ceRNAs could compete for the binding sites of the same miRNA to decrease the inhibition of target mRNA regulated by certain miRNAs. As a result, the expression level of the target gene could be increased and a series of biological behaviors, including tumorigenesis, could be regulated [4, 45].

The expression profiles of mRNAs, lncRNAs, and circRNAs in germline stem cells from male and female mice were measured and predicted with a biological information method. The results of the network prediction showed that lncRNA Meg3 and cirRNA Igf1r could compete with miRNA-15a-5p to increase the expression of target genes including Inha, Acsl3, Kif21b, and Igfbp2 [46]. Another study found that lncRNA FOXD3-AS1 could promote lung epithelial cell death induced by oxidative stress. There was an interaction between miR-150 and FOXD3-AS1 in human lung epithelial cells. In addition, miR-150 mimics inhibited the expression of FOXD3-AS1. The antisense oligonucleotides of FOXD3-AS1 significantly increased the intracellular levels of miR-150. MiR-150 protected lung epithelial cells from oxidative stress, whereas FOXD3-AS1 could trigger apoptosis. The results demonstrated that FOXD3-AS1 acted as a sponge or a competitive endogenous non-coding RNA for miR-150, thereby limiting its ability to promote cell growth and exacerbate hyperoxia-induced pulmonary epithelial cell apoptosis [47].

4. THE ROLE OF ROS IN EPIGENETIC CHANGES INDUCED BY CR(VI)

Cr(VI) could alter levels of oxidative stress by inducing ROS [48]. Sprague-Dawley rats showed a decrease in plasma levels of malondialdehyde (MDA)

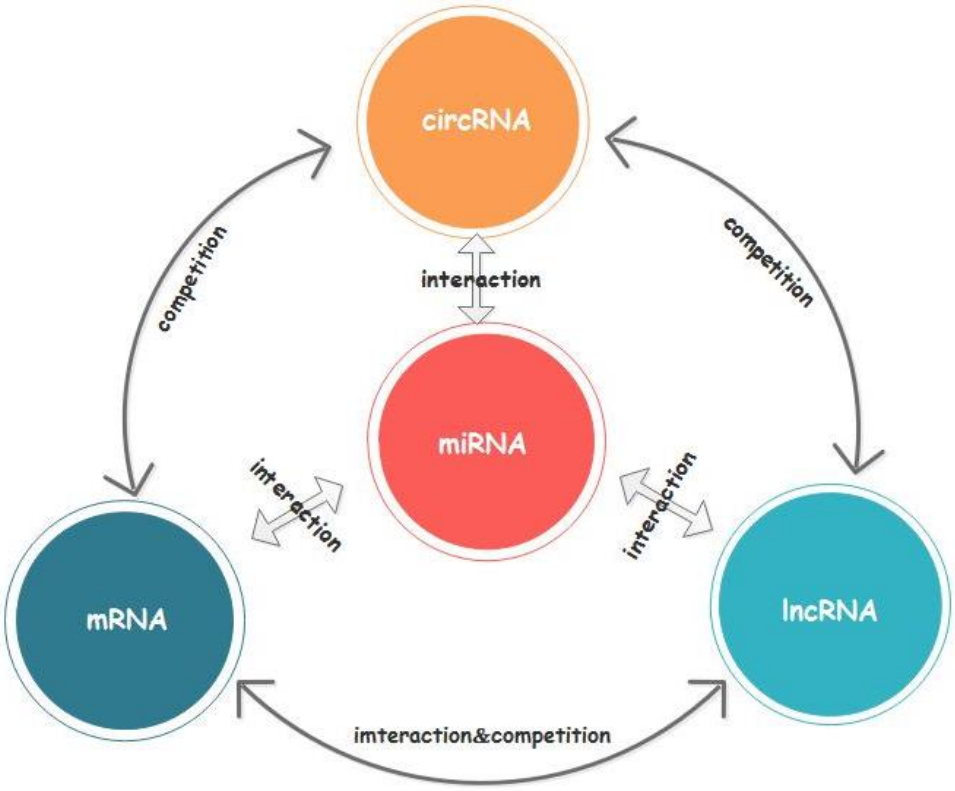

FIGURE 1. The ceRNA network. See detailed description in text (Section 3.3).

and a decrease in the methylation level of whole genome DNA after four weeks of exposure to potassium dichromate at a concentration of 100 and 300 mg/L. Moreover, there was a good negative correlation between the MDA levels and the whole genome DNA methylation levels [8]. Groundwater in Cr(VI)-contaminated areas could increase serum activities of aspartate aminotransferase and alanine aminotransferase and hepatic MDA levels and decrease the levels of hepatic superoxide dismutase and reduced form of glutathione, and serum glutamic acid, resulting in liver injury in mice. Vitamin C had an antagonistic effect on liver damage caused by potassium dichromate [49]. Estrogen metabolites could also play a protective role in ROS-mediated oxidative stress caused by Cr(VI) [50]. Another study suggested that when the L-02 hepatocytes were treated with 4 μM Cr(VI), the severe impairment of cells could be mitigated by the reduction of VDAC1 and Sam50 through BAPTA regulation. In addition, when the L-02 hepatocytes were treated with Cr(VI) at a concentration below 4 μM, cell damage could be improved by knocking down VDAC1. Furthermore, restraining the overload of intracellular Ca^{2+} could be an effective means of protecting the L-02 hepatocytes form the toxicity of Cr(VI) [51]. 8-OH-dG, generated from ROS-mediated DNA oxidation, could act as a signaling agent for gene activation. When the 8-OH-dG was formed in the potential G-quadruplex-forming sequence, the enhanced gene expression occurred as well as the expression of 8-oxo guanine DNA glycosylase, which induced base excision repair, resulting in the formation of abasic sites. The results suggested that 8-OH-dG was related to epigenetic modification and the G-quadruplex formation sequence and could act as a sensor for oxidative stress induced by ROS [52]. MiRNA was shown to be one of the major regulators in the epigenetics that was involved in the redox homeostasis of cells by affecting the expression of various target genes as redox activators or scaven-

gers. The intracellular level of ROS, in turn, could regulate the expression of ROS-reactive miRNAs, known as ROSmir, through modulating its target genes [53].

5. CONCLUSION

As discussed above, Cr(VI) could change the gene expression at the epigenetic level. Furthermore, Cr(VI) could induce DNA damage by producing ROS, and ROS themselves also caused changes in DNA methylation and miRNA expression levels. Cr(VI) could trigger epigenetic changes by DNA methylation, histone modifications, and miRNAs. These epigenetic modifications might alter chromatin remodeling, transcriptional activation, transcriptional interference, intracranial transport at transcriptional or post-transcriptional levels through RNA interference, gene imprinting, and gene silencing mechanisms. However, further studies are needed to determine whether these epigenetic regulations have long-term effects on Cr(VI)-induced carcinogenesis and whether the function of epigenetics is sufficient for Cr(VI)-induced cancer development. So far, there has been little reporting on the role of circRNAs and lncRNAs in the mechanism of carcinogenesis induced by Cr(VI), which indicates a research direction. Although Poliseno et al. proposed the ceRNA hypothesis, the authenticity of the hypothesis still requires much research to verify [4]. In addition, epigenetic changes are reversible. In this regard, vitamin C had a certain antagonistic effect on liver damage caused by potassium dichromate [49], and vitamin C could also reverse the methylation level of H3K9 by reducing Cr(VI) and increasing the activity of histone demethylase [12]. These studies suggested that we might be able to delay the development of diseases or even treat diseases by pharmacological inhibition of methylation. It is of great significance to further study the role of epigenetics in the carcinogenesis induced by Cr(VI).

ACKNOWLEDGMENTS

This work was supported by projects grants from the National Natural Science Foundation of China (81573118 and 81673118). The authors declare no conflicts of interest.

REFERENCES

1. IARC. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Chromium, Nickel and Welding*. International Agency for Research on Cancer, Lyon. France.1990, pp. 1–648.
2. Ding SZ, Yang YX, Li XL, Michelli-Rivera A, Han SY, Wang L, et al. Epithelial-mesenchymal transition during oncogenic transformation induced by hexavalent chromium involves reactive oxygen species-dependent mechanism in lung epithelial cells. *Toxicol Appl Pharmacol* 2013; 269(1):61–71. doi: 10.1016/j.taap.2013.03.006.
3. Dai L, Xu W, Li H, Frank JA, He C, Zhang Z, et al. Effects of hexavalent chromium on mouse splenic T lymphocytes. *Toxicol In Vitro* 2017; 45(Pt 1):166–71. doi: 10.1016/j.tiv.2017.09.006.
4. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* 2011; 146(3):353–8. doi: 10.1016/j.cell.2011.07.014.
5. Hu G, Li P, Li Y, Wang T, Gao X, Zhang W, et al. Methylation levels of P16 and TP53 that are involved in DNA strand breakage of 16HBE cells treated by hexavalent chromium. *Toxicol Lett* 2016; 249:15–21. doi: 10.1016/j.toxlet.2016.03.003.
6. Lou J, Wang Y, Yao C, Jin L, Wang X, Xiao Y, et al. Role of DNA methylation in cell cycle arrest induced by Cr (VI) in two cell lines. *PLoS One* 2013; 8(8):e71031. doi: 10.1371/journal.pone.0071031.
7. Lou J, Wang Y, Chen J, Ju L, Yu M, Jiang Z, et al. Effects of soluble and particulate Cr(VI) on genome-wide DNA methylation in human B lymphoblastoid cells. *Mutat Res Genet Toxicol Environ Mutagen* 2015; 792:12–8. doi: 10.1016/j.mrgentox.2015.08.004.
8. Wang Y, Wu W, Yao C, Lou J, Chen R, Jin L, et al. Elevated tissue Cr levels, increased plasma oxidative markers, and global hypomethylation of blood DNA in male Sprague-Dawley rats exposed to potassium dichromate in drinking water. *Environ Toxicol* 2016; 31(9):1080–90. doi: 10.1002/tox.22117.
9. Wang TC, Song YS, Wang H, Zhang J, Yu SF, Gu YE, et al. Oxidative DNA damage and global DNA hypomethylation are related to folate

deficiency in chromate manufacturing workers. *J Hazard Mater* 2012; 213–214:440–6. doi: 10.1016/j.jhazmat.2012.02.024.
10. Xia B, Yang LQ, Huang HY, Pang L, Hu GH, Liu QC, et al. Chromium(VI) causes down regulation of biotinidase in human bronchial epithelial cells by modifications of histone acetylation. *Toxicol Lett* 2011; 205(2):140–5. doi: 10.1016/j.toxlet.2011.05.1032.
11. Xia B, Ren XH, Zhuang ZX, Yang LQ, Huang HY, Pang L, et al. Effect of hexavalent chromium on histone biotinylation in human bronchial epithelial cells. *Toxicol Lett* 2014; 228(3):241–7. doi: 10.1016/j.toxlet.2014.05.010.
12. Sun H, Zhou X, Chen H, Li Q, Costa M. Modulation of histone methylation and MLH1 gene silencing by hexavalent chromium. *Toxicol Appl Pharmacol* 2009; 237(3):258–66. doi: 10.1016/j.taap.2009.04.008.
13. Chandra S, Khatoon R, Pandey A, Saini S, Vimal D, Singh P, et al. Dme-miR-314-3p modulation in Cr(VI) exposed *Drosophila* affects DNA damage repair by targeting mus309. *J Hazard Mater* 2016; 304:360–9. doi: 10.1016/j.jhazmat.2015.10.075.
14. Chandra S, Pandey A, Chowdhuri DK. MiRNA profiling provides insights on adverse effects of Cr(VI) in the midgut tissues of *Drosophila melanogaster*. *J Hazard Mater* 2015; 283:558–67. doi: 10.1016/j.jhazmat.2014.09.054.
15. Bollati V, Marinelli B, Apostoli P, Bonzini M, Nordio F, Hoxha M, et al. Exposure to metal-rich particulate matter modifies the expression of candidate microRNAs in peripheral blood leukocytes. *Environ Health Perspect* 2010; 118(6):763–8. doi: 10.1289/ehp.0901300.
16. Li Y, Li P, Yu S, Zhang J, Wang T, Jia G. miR-3940-5p associated with genetic damage in workers exposed to hexavalent chromium. *Toxicol Lett* 2014; 229(1):319–26. doi: 10.1016/j.toxlet.2014.06.033.
17. Li Y, Hu G, Li P, Tang S, Zhang J, Jia G. miR-3940-5p enhances homologous recombination after DSB in Cr(VI) exposed 16HBE cell. *Toxicology* 2016; 344–346:1–6. doi: 10.1016/j.tox.2016.02.003.
18. He J, Qian X, Carpenter R, Xu Q, Wang L, Qi Y, et al. Repression of miR-143 mediates Cr (VI)-induced tumor angiogenesis via IGF-IR/IRS1/ERK/IL-8 pathway. *Toxicol Sci* 2013; 134(1):26–38. doi: 10.1093/toxsci/kft101.
19. Chen J, Li Y, Zheng Q, Bao C, He J, Chen B, et al. Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric cancer. *Cancer Lett* 2017; 388:208–19. doi: 10.1016/j.canlet.2016.12.006.
20. Xu L, Zhang M, Zheng X, Yi P, Lan C, Xu M. The circular RNA ciRS-7 (Cdr1as) acts as a risk factor of hepatic microvascular invasion in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2017; 143(1):17–27. doi: 10.1007/s00432-016-2256-7.
21. Nan A, Chen L, Zhang N, Liu Z, Yang T, Wang Z, et al. A novel regulatory network among LncRpa, CircRar1, MiR-671 and apoptotic genes promotes lead-induced neuronal cell apoptosis. *Arch Toxicol* 2017; 91(4):1671–84. doi: 10.1007/s00204-016-1837-1.
22. Gao D, Zhang X, Liu B, Meng D, Fang K, Guo Z, et al. Screening circular RNA related to chemotherapeutic resistance in breast cancer. *Epigenomics* 2017; 9(9):1175–88. doi: 10.2217/epi-2017-0055.
23. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012; 489(7414):57–74. doi: 10.1038/nature11247.
24. Vella S, Gallo A, Lo Nigro A, Galvagno D, Raffa GM, Pilato M, et al. PIWI-interacting RNA (piRNA) signatures in human cardiac progenitor cells. *Int J Biochem Cell Biol* 2016; 76:1–11. doi: 10.1016/j.biocel.2016.04.012.
25. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol* 2012; 9(6):703–19. doi: 10.4161/rna.20481.
26. Ellis BC, Molloy PL, Graham LD. CRNDE: A long non-coding RNA involved in cancer, neurobiology, and development. *Front Genet* 2012; 3:270. doi: 10.3389/fgene.2012.00270.
27. Wang P, Ren Z, Sun P. Overexpression of the long non-coding RNA MEG3 impairs in vitro glioma cell proliferation. *J Cell Biochem* 2012; 113(6):1868–74. doi: 10.1002/jcb.24055.
28. Fang Y, Fullwood MJ. Roles, functions, and mechanisms of long non-coding RNAs in cancer. *Genomics Proteomics Bioinformatics* 2016; 14(1):42–54. doi: 10.1016/j.gpb.2015.09.006.
29. Zhang C, Peng G. Non-coding RNAs: an emerging player in DNA damage response.

Mutat Res Rev Mutat Res 2015; 763:202–11. doi: 10.1016/j.mrrev.2014.11.003.
30. Browning CL, Wise JP, Sr. Prolonged exposure to particulate chromate inhibits RAD51 nuclear import mediator proteins. *Toxicol Appl Pharmacol* 2017; 331:101–7. doi: 10.1016/j.taap.2017.05.030.
31. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature* 2009; 461(7267):1071–8. doi: 10.1038/nature08467.
32. Bunting SF, Callen E, Wong N, Chen HT, Polato F, Gunn A, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 2010; 141(2):243–54. doi: 10.1016/j.cell.2010.03.012.
33. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, Tkac J, et al. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell* 2013; 49(5):872–83. doi: 10.1016/j.molcel.2013.01.001.
34. Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. *Science* 2013; 339(6120):700–4. doi: 10.1126/science.1231573.
35. Price BD, D'Andrea AD. Chromatin remodeling at DNA double-strand breaks. *Cell* 2013; 152(6):1344–54. doi: 10.1016/j.cell.2013.02.011.
36. Lukas J, Altmeyer M. A lncRNA to repair DNA. *EMBO Rep* 2015; 16(11):1413–4. doi: 10.15252/embr.201541309.
37. Sharma V, Khurana S, Kubben N, Abdelmohsen K, Oberdoerffer P, Gorospe M, et al. A BRCA1-interacting lncRNA regulates homologous recombination. *EMBO Rep* 2015; 16(11):1520–34. doi: 10.15252/embr.201540437.
38. Wan G, Mathur R, Hu X, Liu Y, Zhang X, Peng G, et al. Long non-coding RNA ANRIL (CDKN2B-AS) is induced by the ATM-E2F1 signaling pathway. *Cell Signal* 2013; 25(5):1086–95. doi: 10.1016/j.cellsig.2013.02.006.
39. Prensner JR, Chen W, Iyer MK, Cao Q, Ma T, Han S, et al. PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer. *Cancer Res* 2014; 74(6):1651–60. doi: 10.1158/0008-5472.CAN-13-3159.
40. Congrains A, Kamide K, Ohishi M, Rakugi H. ANRIL: molecular mechanisms and implications in human health. *Int J Mol Sci* 2013; 14(1):1278–92. doi: 10.3390/ijms14011278.
41. Zhang Y, He Q, Hu Z, Feng Y, Fan L, Tang Z, et al. Long noncoding RNA LINP1 regulates repair of DNA double-strand breaks in triple-negative breast cancer. *Nat Struct Mol Biol* 2016; 23(6):522–30. doi: 10.1038/nsmb.3211.
42. Hoa NN, Kobayashi J, Omura M, Hirakawa M, Yang SH, Komatsu K, et al. BRCA1 and CtIP are both required to recruit Dna2 at double-strand breaks in homologous recombination. *PLoS One* 2015; 10(4):e0124495. doi: 10.1371/journal.pone.0124495.
43. Marin-Bejar O, Marchese FP, Athie A, Sanchez Y, Gonzalez J, Segura V, et al. Pint lincRNA connects the p53 pathway with epigenetic silencing by the Polycomb repressive complex 2. *Genome Biol* 2013; 14(9):R104. doi: 10.1186/gb-2013-14-9-r104.
44. Freeman JA, Espinosa JM. The impact of post-transcriptional regulation in the p53 network. *Brief Funct Genomics* 2013; 12(1):46–57. doi: 10.1093/bfgp/els058.
45. Poliseno L, Pandolfi PP. PTEN ceRNA networks in human cancer. *Methods* 2015; 77–78:41–50. doi: 10.1016/j.ymeth.2015.01.013.
46. Li X, Ao J, Wu J. Systematic identification and comparison of expressed profiles of lncRNAs and circRNAs with associated co-expression and ceRNA networks in mouse germline stem cells. *Oncotarget* 2017; 8(16):26573–90. doi: 10.18632/oncotarget.15719.
47. Zhang D, Lee H, Haspel JA, Jin Y. Long noncoding RNA FOXD3-AS1 regulates oxidative stress-induced apoptosis via sponging microRNA-150. *FASEB J* 2017; 31(10):4472–81. doi: 10.1096/fj.201700091R.
48. Balasaraswathi K, Jayaveni S, Sridevi J, Sujatha D, Phebe Aaron K, Rose C. Cr-induced cellular injury and necrosis in *Glycine max L.*: biochemical mechanism of oxidative damage in chloroplast. *Plant Physiol Biochem* 2017; 118:653–66. doi: 10.1016/j.plaphy.2017.08.001.
49. Effects of hexavalent chromium in groundwater and potassium dichromate exposure on liver and blood glucose in mice and the effect of vitamin

C intervention. *J Environ Health* 2012; 29(08):714–7.
50. Sawicka E, Dlugosz A. The role of 17beta-estradiol metabolites in chromium-induced oxidative stress. *Adv Clin Exp Med* 2017; 26(2):215–21. doi: 10.17219/acem/62217.
51. Yi X, Xiao F, Zhong X, Duan Y, Liu K, Zhong C. A Ca^{2+} chelator ameliorates chromium (VI)-induced hepatocyte L-02 injury via down-regulation of voltage-dependent anion channel 1 (VDAC1) expression. *Environ Toxicol Pharmacol* 2017; 49:27–33. doi: 10.1016/j.etap.2016.11.007.
52. Fleming AM, Ding Y, Burrows CJ. Oxidative DNA damage is epigenetic by regulating gene transcription via base excision repair. *Proc Natl Acad Sci USA* 2017; 114(10):2604–9. doi: 10.1073/pnas.1619809114.
53. Bhat AV, Hora S, Pal A, Jha S, Taneja R. Stressing the (epi)genome: dealing with reactive oxygen species in cancer. *Antioxid Redox Signal* 2017. doi: 10.1089/ars.2017.7158.