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Cr(VI)-induced methylation and down-regulation of DNA repair genes and its association with markers of genetic damage in workers and 16HBE cells☆

Guiping Hu [a, 1], Ping Li [b, 1], Xiaoxing Cui [c], Yang Li [a, d], Ji Zhang [e], Xinxiao Zhai [f], Shanfa Yu [g], Shichuan Tang [d], Zuchang Zhao [h], Jing Wang [f], Guang Jia [a, *]

[a] Department of Occupational and Environmental Health Sciences, School of Public Health, Peking University, Beijing, 100191, China
[b] Laboratory of Nutrition, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, 100045, China
[c] Nicholas School of the Environment, Duke University, Box 90328, Durham, NC, USA
[d] Beijing Key Laboratory of Occupational Safety and Health, Beijing, 100054, China
[e] Jinan Center for Disease Control and Prevention, Jinan, Shandong Province, 250021, China
[f] Yima Center for Disease Control and Prevention, Sanmenxia City, Henan Province, 472300, China
[g] Institute of Occupational Disease Prevention, Zhengzhou City, Henan Province, 450052, China
[h] Sanmenxia Municipal Center for Disease Control and Prevention, Sanmenxia, Henan Province, 472000, China

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ABSTRACT

To examine the mechanism of hexavalent chromium [Cr(VI)]-induced carcinogenesis, a cross-sectional study in workers with or without exposure to Cr(VI) as well as *in vitro* administration of Cr(VI) in 16HBE cells was conducted. We explored the associations between Cr(VI) exposure, methylation modification of DNA repair genes and their expression levels, and genetic damage. Results showed that hypermethylation of CpG sites were observed in both occupationally exposed workers and 16HBE cells administrated Cr(VI). DNA damage markers including 8-hydroxydeoxyguanosine (8-OHdG) and micronucleus frequency in Cr(VI)-exposed workers were significantly higher than the control group. Among workers, blood Cr concentration was positively correlaed with the methylation level of CpG sites in DNA repair genes including CpG6,7, CpG8, CpG9,10,11 of *MGMT*, CpG11 of *HOGG1*; CpG15,16,17, CpG19 of *RAD51*, and genetic damage markers including 8-OHdG and micronucleus frequency. Significant negative association between methylation levels of CpG sites in DNA repair genes and corresponding mRNA was also observed in 16HBE cells. This indicated that Cr(VI) exposure can down-regulate DNA repair gene expression by hypermethylation, which leads to enhanced genetic damage. The methylation level of these CpG sites of DNA repair genes can be potential epigenetic markers for Cr(VI)-induced DNA damage.

1. Introduction

Hexavalent chromium [Cr(VI)] and its compounds are carcinogens present in the environment and at the workplace (Hausladen and Fendorf, 2017; Turner, 2017; Wilbur et al., 2012). Cr(VI) compounds are basic chemical raw materials that have been widely used in industrial and agricultural production, resulting in potential emission into water, soil, air, and food. This poses considerable concerns for the exposure of occupational workers and the general population (Gao and Xia, 2011; Hausladen and Fendorf, 2017).

There are three possible routes of exposure to Cr(VI) and its compounds, viz. inhalation, dermal contact, and ingestion. After entry into the cell facilitated by anion carriers, Cr(VI) can introduce a series of chromium intermediates and reactive oxygen species (ROS) (Hu et al., 2017; Myers, 2012; Valko et al., 2006). This can lead to cross-linked products with biological macro-molecules, resulting in the formation of Cr-DNA, Cr-Protein, DNA-DNA, DNA-Protein, which promotes the breakage of DNA strands and chromosomes

Abbreviations: Cr, Chromium; 16HBE cells, Human Bronchial Epithelial cells; MALDI-TOF-MS, Matrix Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry; 8-OHdG, 8-hydroxydeoxyguanosine.

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* Corresponding author.
E-mail address: jiaguangjia@bjmu.edu.cn (G. Jia).

[1] Hu G and Li P contributed equally to this manuscript and are co-first authors.

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(Liu and Dixon, 1996; Nickens et al., 2010; Persson et al., 1986; Snow and Xu, 1991; Zhitkovich, 2005). If not properly repaired, genetic damage may contribute to the process of carcinogenesis (Peterson-Roth et al., 2005; Wise et al., 2008).

DNA damage and defects in DNA repair mechanisms are the first steps in the development of many diseases (Cavalcante et al., 2017). A close healing network including direct repair, excision repair, mismatch repair and recombination repair pathways that exist to ensure the stability of chromosome with high fidelity amplification of DNA (Browning et al., 2016; Hu et al., 2016b; O'Brien et al., 2003). It was demonstrated that successful repair of genetic damages depended on the degree of DNA damages and the sound function of the DNA repair system (O'Brien et al., 2003). Previous studies have reported that several DNA repair genes are actively involved in response to Cr(VI)-induced genetic damages. RAD51 was observed to participate in the homologous recombination, and function alteration of DNA after Cr(VI)-induced DNA double-strand break (Browning et al., 2016; Li et al., 2016b). MGMT is a key enzyme responsible for direct repair that removes O^6-guanine adducts from DNA. The level of methylation of *MGMT* had been observed to be significantly associated with lung cancer (Yang and Li, 2016). *HOGG1* encodes the enzyme responsible for the excision of 8-oxoguanine. XRCC1 is involved in the efficient repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents (O'Brien et al., 2003).

Existing studies are suggestive that Cr(VI) exposure may downregulate the expression of DNA repair genes via methylation modification, which may lead to increased levels of genetic damage (Pierron et al., 2014; Wang et al., 2016). However, this mechanism has not been elucidated yet. In this study, we hypothesize the Cr(VI) can modify DNA repair genes including *MGMT*, *HOGG1*, *XRCC1*, *ERCC3* and *RAD51* via methylation, and thus impact on DNA stability and subsequent genetic damage. We conducted a cross-sectional study in workers with and without occupational Cr(VI) exposure to examine the association between methylation modification of DNA repair genes, their expression level and genetic damage markers. As methylation modification of CpG sites showed tissue and time specificity (Wan et al., 2015), SV40-immortalized human bronchial epithelial cells (16HBE cells) were administrated Cr(VI) *in vitro* to further validate the molecular mechanism. To our knowledge, this study is the first one that combines findings from occupationally exposed individuals and *in vitro* toxicological experiments in 16HBE cells in investigating the mechanism of Cr(VI)-induced DNA damage.

2. Materials and methods

2.1. Study design

A cross-sectional study was designed to understand methylation of DNA repair genes and their association with markers of genetic damage in workers with and without occupational exposure to Cr(VI). Cr(VI) was administrated to human bronchial epithelial cell line (16HBE cells) *in vitro* to further explore the molecular mechanism of Cr(VI)-induced genetic damage.

2.2. Study subjects with and without occupational exposure to Cr(VI)

The study protocol was approved by Medical Ethics Committee of Peking University Health Science Center, Beijing, China. 117 subjects were recruited from the same factory, consisting of 87 blue-collar workers with occupational exposure to chromate from different work sections (the exposure group) and 30 employees who worked in administrative offices in the same factory without Cr(VI) exposure (the control group). Individuals with skin infections, cancer, cardiovascular diseases, kidney diseases, pulmonary diseases, and medical history of allergy, asthma and allergic rhinitis were excluded from the study. All subjects had been working for at least three months at the same work section and for at least one year in this factory. All subjects signed informed consent, completed a questionnaire and undertook a physical examination. The questionnaire was used to collect self-reported information from the study subjects, including weight, height, smoking status, alcohol intake, occupational history, personal medical history, medication use, hair dye use, house decoration, radiation exposure, and the use of personal protective equipment.

2.3. Air sample collection and Cr assessment

Air sampling was conducted in accordance with the sampling method for the monitoring of hazardous substances in the air outlined by the national standard in China (GBZ159-2004). Air samples were collected using mixed cellulose ester MCE filters (Φ37 mm, Pall, USA) at a flow rate of 1 L/min (Sp730, TSI Corporation, USA). For all six work sections, 10 sampling points were collected at each work section. Each sampling session lasting 8 h and was conducted during regular working hours. The mass of Cr captured on the MCE filter were measured by atomic absorption spectrometry (Thermo Fisher, USA). The mass of Cr on the filter was divided with the total volume of air that was pulled through the filter during the sampling period to calculated Cr concentration in air samples.

2.4. Blood collection and analysis of Cr, micronucleus frequency, and 8-OHdG

10 mL peripheral venous blood was drawn from each subject after a work shift into two tubes (Greiner, Kremsmuenster, Austria). One tube was coated with anticoagulant for plasma separation and subsequent measurement of Cr concentration, micronucleus frequency, and methylation levels. The other tube was coated with pro-coagulant for serum separation and 8-OHdG quantification in serum. The concentration of blood Cr, an indicator for Cr(VI) internal exposure, was measured by inductively coupled plasma mass spectrometry (ICP-MS) (Model Elan DRC II, PerkinElmer, Waltham, MA, USA) following a previously described protocol (Song et al., 2012).

Within 4 h after the heparinized blood collection, 0.4 mL peripheral venous blood was incubated with 5 mL RPMI-1640 culture medium supplemented with 30 μg/mL phytohemagglutinin (PHA), double-stranded anti-DNA antibody, 10% calf serum, and 0.002 mol/L glutamine at 37 °C for 72 h. After 44 hours of incubation, 200 μL (6 μg/mL) cytochalasin-B (Sigma, St. Louis, MO, USA) was added to the cultures (a final concentration of 6 mg/mL). In each individual, the presence of micronucleus was evaluated in 1000 binuclear lymphocytes according to Fenech's protocol (Fenech, 2007). Serum 8-OHdG concentration was measured using a highly sensitivity 8-OHdG ELISA kit (JaICA, Shizuoka, Japan) following the manufacturer's instructions. As the performance of 8-OHdG ELISA kits may be affected by high-molecular-weight substances such as proteins, serum samples were filtered using a Microcon YM-10 ultrafilter (Millipore, Billerica, MA, USA) with a cut-off of molecular weight at 10 kDa. The filtrates were used to conduct ELISA kit instead of serum.

2.5. Human bronchial epithelial 16HBE cells and chromium treatment

The 16HBE cell line was purchased from the tumor cell library of