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miR-3940-5p associated with genetic damage in workers exposed to hexavalent chromium

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HIGHLIGHTS

- Plasma miR-3940-5p is significantly decreased by hexavalent chromium exposure.
- Hexavalent chromium exposure can increase micronuclei frequency in peripheral lymphocytes, but micronuclei frequency does not continue to increase at a higher hexavalent chromium exposure.
- miR-3940-5p modulates hexavalent chromium-induced genetic damage by regulating XRCC2 gene.

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ABSTRACT

To understand the regulation of genetic damage by epigenetics at the early stage of carcinogenesis after hexavalent chromium (Cr(VI)) and assessed genetic damage to explore their association with DNA repair genes mediated by differently expressed miRNA. Genetic damages were evaluated using cytokinesis-block micronucleus assay (CBMN) and serum 8-hydroxyguanine (8-OHdG) ELISA assay. Blood Cr level showed significant association with plasma miR-3940-5p level ($r = -0.33$, $P = 0.001$) and non-linear relationship with micronuclei frequency in CBMN and serum 8-OHdG level ($\beta_{std} = 0.29$, $P = 0.039$; $\beta_{std} = 0.35$, $P = 0.001$), with micronuclei frequency not increasing apparently under high Cr exposure. In contrast, no significant association was found between plasma miR-3940-5p level and the two genetic indicators. However, plasma miR-3940-5p level was linked to micronuclei frequency under high blood Cr level ($\beta_{std} = 0.18$, $P = 0.015$). To explore the effect of miR-3940-5p on genetic damage under high Cr exposure, the protein expression levels of miR-3940-5p-mediated DNA repair genes in leukocytes were quantified using enzyme-linked immunosorbent assay for subjects with high blood Cr level. The results showed that XRCC2 and BRCC3 protein levels were statistically associated with miR-3940-5p level respectively ($\beta_{std} = -0.31$, $P = 0.010$; $\beta_{std} = -0.24$, $P = 0.037$). Meanwhile, a weak but statistically negative association between XRCC2 level and micronuclei frequency was found ($\beta_{std} = -0.15$, $P = 0.027$). These data suggests that high Cr(VI) does not always aggravate genetic damage after reaching a high Cr(VI) exposure in real situation, which may be due to the regulation of miRNA on DNA repair genes responsive to high Cr(VI) exposure.

1. Introduction

Chronic hexavalent chromium (Cr(VI)) exposure has long been known to increase cancer incidence and is classified as type I carcinogen (Straif et al., 2009). Cr(VI) and its compounds are widely used and persistent in the environment. Recent epidemiological studies have found that carcinogenic risks associated with Cr(VI) are substantially higher than previously thought (Salnikow and Zhitkovich, 2008). The increased environmental and

Abbreviations: Cr(VI), hexavalent chromium; CBMN, cytokinesis-block micronucleus assay; 8-OHdG, 8-hydroxyguanine; IQR, interquartile range; $Na_2Cr_2O_7$, sodium dichromate; ICP-MS, inductively coupled plasma mass spectrometry; ELISA, enzyme-linked immunosorbent assay; NSCLC, non-small cell lung carcinoma; BMI, body mass index; KHSRP, KH-type splicing regulatory protein.

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occupational Cr(VI) exposure poses a huge concern about its effect on general population and occupational workers. *In vitro* test showed that cells exposed to Cr(VI) are subjected to several types of DNA damage, including base modification, single-strand breaks, double-strand breaks, Cr-DNA adducts, DNA-Cr-DNA adducts, and protein-Cr-DNA adducts, during Cr(VI) reduction in cytoplasm (Kuo et al., 2003; Wise et al., 2008). These damages without accurate repairment can result in genetic damage and ultimate carcinogenesis (Wise et al., 2008).

An uptake-reduction model and DNA damage mechanism are widely accepted to explain how genetic damages are induced by Cr(VI) and then initiate carcinogenesis (Zhitkovich, 2011). Therefore, genetic damage indicators are often chosen as markers of Cr(VI) exposure at the early stage of carcinogenesis. An epidemiological study showed that Cr(VI) exposure can induce high increase of urinary 8-hydroxydeoxyguanosine (8-OHdG) (Kuo et al., 2003), and our previous investigation found that Cr (VI) exposure can increase binucleated lymphocytes with micronuclei frequencies (BNMN) in occupational Cr(VI) exposed workers (Liu et al., 2012).

Although Cr(VI) is an established human carcinogen and it is widely accepted that genetic damages dominate the underlying mechanisms of carcinogenesis, it is impossible to reconcile cancer development after Cr(VI) exposure with any simple mechanism involving DNA lesions. Recently, epigenetic mechanisms have been involved in the regulation of oncogenes, tumor suppress genes and DNA repair genes (Ziech et al., 2010). Both genetic and epigenetic changes are major causes of disordered cellular programming in cancer. Latest studies suggest that epigenetic factors, including histone modification, DNA methylation and non-coding small RNAs, exert important roles in metal-induced carcinogenesis (Baccarelli and Bollati, 2009).

MicroRNAs (miRNAs) are short, endogenous non-coding small RNAs with the ability to silence or degrade target mRNAs by base pair, repressing the expression of genes at the post-transcriptional level (Bartel, 2004). miRNAs not only play critical roles in various biological processes, but also mediate specific mechanisms of toxicity involved in a wide spectrum of environmental chemicals (Baccarelli and Bollati, 2009). miREnvironment, an online database focusing on miRNA-environment factors, has indexed more than 800 miRNAs which are associated with 260 chemicals including many heavy metals: arsenic, cadmium, lead and others (Yang et al., 2011). Therefore, it is important to identify and validate miRNAs that can be induced by Cr(VI) exposure, which may bring further mechanistic understanding and harbor profiles potentially useful to identify at-risk individuals.

Circulating miRNAs have been shown to be stabilized in blood without RNase degradation by inclusion in various protein complexes or membranous particles (Arroyo et al., 2011). The profile of miRNAs in serum and plasma has been shown to reflect disease states such as cancer (Bryant et al., 2012) as well as organ damage and injury (Dorn, 2011). Considering that accumulation of DNA damage in DNA repair genes, oncogenes and tumor suppressor genes are associated with tumor-prone phenotypes, epigenetic regulation of these genes may promote or inhibit carcinogenesis (Lahtz and Pfeifer, 2011). It is not surprising to assume that miRNA-mediated epigenetic processes are involved in Cr(VI)-induced genetic damage and carcinogenesis. Exploring the roles of miRNA in genetic damage indicators will promote our understanding of the underlying mechanisms at an early stage of Cr(VI)-induced carcinogenesis.

In present study, we investigate the effects of Cr(VI) exposure on plasma miRNAs profiles in chromate production workers, and validate miRNAs which are related to Cr(VI) exposure. To further explore the association of miRNAs with genetic damage, their target DNA repair genes are quantified on transcriptional level. Finally, BNMN and serum 8-OHdG are detected to evaluate genetic damage and explore the association of miRNA-mediated DNA repair with them.

2. Materials and methods

2.1. Study design

We recruited 117 subjects (age: 36 ± 8 years; male/female: 82/35) in a chromate production (sodium dichromate, $Na_2Cr_2O_7$) plant in Henan, China. 87 subjects were blue-collar workers exposed to chromate from different work sections (exposed group, subjects exposed to chromate by inhalation for ~5.0 (IQR: 3.0–10.0) year), and 30 subjects were employees that had no direct contact with any chromium products (including managers, officers, support crew, and so on) as controls (control group). No one had changed job positions from exposed group to control group, and vice versa. We only recruited subjects from these workers who have worked more than one year to make their potential epigenetic and genetic changes detectable. These subjects were free of cancer, cardiovascular diseases, kidney diseases and pulmonary diseases. All participants were required to answer questions regarding age, gender, smoking status, alcohol drinking status, current and history of diseases, and received physical examinations. Ethical approval for this study was granted by the Committee of the Health Science Center, Peking University and local government authorities. A written informed consent was obtained from all participants prior to being enrolled.

2.2. Blood collection and assessment of Cr(VI) biological exposure

Post-shift blood samples on fasting state were collected. A total of 15 mL venous blood was also collected in three sets of vacuum blood collection tubes (INSEPACK, Beijing, China). The heparin anticoagulant blood was sampled for whole blood element concentration analysis. The EDTA2K anticoagulant blood samples were collected for miRNAs analysis. Plasma were obtained by centrifugation at 200 g for 10 min to precipitate the cellular components of blood specimen for miRNA quantification. The third set of tube was used to separate buffy-coat within 30 min blood draw for leukocytes protein extraction, and serum samples were collected for serum 8-OHdG detection. Subsequently, all the samples were stored at $-80\,^{\circ}C$ until analysis. Blood Cr level in heparin-anticoagulated blood samples was measured by inductively coupled plasma mass spectrometry (ICP-MS) (Model Elan DRC II, PerkinElmer, Waltham, MA, USA) as internal exposure to reflect Cr(VI) exposure as described previously (Wang et al., 2012).

2.3. Microarray miRNA expression profile

Plasma miRNAs seems better biomarker than cellular miRNAs in sample preparation, extraction and stability. Twenty EDTA2K anticoagulant plasma samples were randomizedly selected from exposed group and control group, respectively. Each of the five samples in the same group were pooled together to minimize the variety caused by small sample size and reduce microarray cost. Finally, eight pooled plasma samples were obtained: four pools represented controls and the other four pools stood for exposed individuals. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE53135 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53135). Total RNA was extracted from plasma using a commercial column-based system following the manufacturer's instructions with the following modifications (Qiagen miRNeasy Mini Kit Qiagen, USA).