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Short communication

Effects of soluble and particulate Cr(VI) on genome-wide DNA methylation in human B lymphoblastoid cells

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ABSTRACT

Several previous studies highlighted the potential epigenetic effects of Cr(VI), especially DNA methylation. However, few studies have compared the effects of Cr(VI) on DNA methylation profiles between soluble and particulate chromate in vitro. Accordingly, Illumina Infinium Human Methylation 450 K BeadChip array was used to analyze DNA methylation profiles of human B lymphoblastoid cells exposed to potassium dichromate or lead chromate, and the cell viability was also studied. Array based DNA methylation analysis showed that the impacts of Cr(VI) on DNA methylation were limited, only about 40 differentially methylated CpG sites, with an overlap of 15 CpG sites, were induced by both potassium dichromate and lead chromate. The results of mRNA expression showed that after Cr(VI) treatment, mRNA expression changes of four genes (TBL1Y, FZD5, IKZF2, and KIAA1949) were consistent with their DNA methylation alteration, but DNA methylation changes of other six genes did not correlate with mRNA expression. In conclusion, both of soluble and particulate Cr(VI) could induce a small amount of differentially methylated sites in human B lymphoblastoid cells, and the correlations between DNA methylation changes and mRNA expression varied between different genes.

1. Introduction

Cr(VI) is widely utilized in many industries such as chromate manufacturing, chrome plating, ferrochrome production and stainless steel welding, leading to occupational exposure and contamination of numerous drinking water supplies. It is estimated that tens of millions of people are exposed to chromium worldwide [1,2]. Human exposure to Cr(VI) may result in a variety of cytotoxic and genotoxic effects, eventually leading to cancer [3–6]. For example, epidemiological studies demonstrated a high incidence of lung cancer following occupational exposure to Cr(VI) [3]. However, the molecular mechanisms of chromium carcinogenesis are still an area of intense investigation. Epigenetic modification is one of the three well-accepted general carcinogenic paradigms of chromate-induced carcinogenesis, and the other two are multistage carcinogenesis and genomic instability [3]. Since the first introduction by Conrad Waddington in 1942, people are paying more and more attention to epigenetics, which plays a significant role in phenotypic expression [7]. Epigenetic changes are modifications of DNA, which occur without changing DNA sequences and can result in heritable changes in gene expression. DNA methylation is one of the most common and best understood epigenetic mechanisms, and global hypomethylation and site-specific hypermethylation are common features of human tumors [8].

Some previous studies have highlighted the potential epigenetic effects of Cr(VI). The first evidence came from a cell culture study, which demonstrated that potassium dichromate was able to induce DNA methylation in G12 cells and silence the expression of gpt transgene [9]. Population investigationsin chromate-induced lung cancer patients showed that increased DNA methylation was observed at the promoter regions of several tumor suppressor genes, such as p16, hMLH1, MGMT, and APC, and such changes can strongly affect the expression of these genes [10–12]. These studies suggested that chromate could alter epigenetic marks, which might contribute to its carcinogenic activity. However, no study investigated the effects of Cr(VI) on genome-wide DNA methylation patterns.

In the present study, a human B lymphoblastoid cell line, which has been successfully used to investigate the toxicity of various kinds of chemicals [13], bentonite particles [14,15], and cigarette

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smoke [16], was exposed to potassium dichromate or lead chromate for 24 h. Illumina Human Methylation450K Assay was used to analyze the DNA methylation profiles of cells, and the mRNA expression levels of the differentially methylated genes were further detected by real-time PCR.

2. Materials and methods

2.1. Cell culture and treatment

A human B lymphoblastoid cell line (Hmy2.CIR) was purchased from Cell Bank, Chinese Academy of Sciences. The cell line was established by Epstein–Barr virus transformation of peripheral blood mononuclear cells using phytohemagluttinin as a mitogen, and it grow in suspension culture with cells clumped in loose aggregates. The cells were grown in Iscove's Modified Dubecco's Medium (IMDM) cell culture medium (HyClone, USA) supplemented with 10% fetal bovine serum (HyClone, USA) in a 37 °C incubator with a humidified mixture of 5% CO_2 and 95% air. Cells were treated with 5 μM, 10 μM and 15 μM of potassium dichromate ($K_2Cr_2O_7$, Sigma, USA) or 1.25 μg/cm^2, 2.5 μg/cm^2, and 5 μg/cm^2 of lead chromate ($PbCrO_4$, Sigma, USA) for 24 h.

2.2. Intracellular chromium ion measurement

Intracellular chromium ion concentrations were detected with an inductively coupled plasma mass spectrometer (ICP-MS). After treatment with Cr(VI), cells were collected and treated with 0.075 M KCl, followed by 2% SDS. The lysate was sheered seven times through a needle and filtered to a vial. Chromium ion levels of the samples were then measured by standard ICP-MS methods.

2.3. CCK-8 assay

Cell viability was analyzed using a commercially available Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan), and it was performed according to the description by Zhang and Sun [17]. Briefly, 10 μl of CCK-8 solution was added to each well of a 96-well plate after treatment, and three wells were prepared for each concentration of $K_2Cr_2O_7$ or $PbCrO_4$. The cells were incubated at 37 °C for an additional 1 h, and then the OD value of each well was measured at the wavelength 450 nm on a microplate reader (Synergy2, BioTek, USA). The cell viability was calculated as our previous study [18].

2.4. Illumina Human Methylation 450K Assay

Genomic DNA extraction and bisulfate modification was performed according to our previous study [19]. DNA was extracted from human B lymphoblastoid cells using DNA extraction Kit (OMEGA BioTek, USA), and bisulphite conversion of genomic DNA was performed with the EZ DNA Methylation-Gold™ Kit (Zymo Research, Orange, USA) following the manufacturer's protocol.

According to Li et al's [20] description, bisulphite-converted genomic DNA was analyzed with Illumina Infinium Human Methylation 450K BeadChip kit (WG-314-1003). DNA samples extracted from cells treated with $K_2Cr_2O_7$ (5 μM) and $PbCrO_4$ (5 μg/cm^2) were selected for DNA methylation analysis, and three parallel samples were used in each treatment group. Bisulphite-converted DNA was denatured in sodium hydroxide, neutralized, and amplified with reagents provided by kit for 20–24 h at 37 °C. After precipitaion and resuspension, DNA samples were dispensed onto BeadChips, washed and placed into a fluid flow-through station for extension and staining. Finally, Illumina's iScan scanner was used to scan the BeadChip.

The data were extracted with BeadStudio v3.0 software. Methylation status of each CpG site was expressed as a beta (β) value ranged from 0 (completely unmethylated) to 1 (completely methylated). A Diff Score for a probe was computed, and for the CpG site, Diff Scores of corresponding probes were averaged. A nonparametric method was used for computation of detection *P*-values. Probe signals were ranked relative to signals of controls, and the Detection *P*-value was calculated as following: Detection *P*-value = 1-*R*/*N*, where *R* is the rank of the CpG site signal relative to controls and *N* is number of controls. Selection of the significantly differentially methylated CpG loci between samples was according to (1) a β-value difference of >0.10 and (2) a Diff Score of >10 or <−10.

2.4.1. mRNA quantification by real-time quantitative PCR

Total RNA was extracted from human B lymphoblastoid cells with TRIzol LS Reagent (Invitrogen, USA) according to manufacturer's protocols. Based on our previous study [21], mRNA quantification was performed with real-time quantitative PCR. The cDNA samples for mRNA quantification were prepared with reverse transcribing total RNA samples from human B lymphoblastoid cells treated with Cr(VI), and PrimeScript RT reagent Kit (Takara, Japan) was used for transcription. The qRT-PCR was performed in triplicate per sample for each gene using the SYBR Green Premix Ex Taq (Takara, Japan), and then analyzed by a 7500 fast Real-Time PCR System (Applied Biosystems, USA). The nucleotide sequences of primer pairs used to amplify each gene were listed in Table 1, including those for internal control gene-GAPDH. The thermal conditions for PCR were 40 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. The ΔCt was calculated as the $Ct_{target} - Ct_{reference}$, and fold changes were calculated as $2^{-\Delta\Delta Ct}$.

2.5. Statistical analysis

The difference of cell viabilityand mRNA expression of selected genes was analyzed using one-way ANOVA. The statistical analysis was performed with the program SPSS 11.0 for windows.

3. Results

3.1. Intracellular Chromium ion concentrations

Fig. 1 shows the results of intracellular chromium ion levels in human B lymphoblastoid cells exposed to potassium dichromate or lead chromate. Potassium dichromate concentrations of 5 μM, 10 μM, and 15 μM produced intracellular chromium ion levels of 1777.29 ± 41.79 μM, 2203.38 ± 242.08 μM, and 3442.23 ± 226.37 μM, respectively. Lead chromate concentrations of 1.25 μg/cm^2, 2.5 μg/cm^2, and 5 μg/cm^2 produced intracellular chromium ion levels of 463.02 ± 26.92 μM, 741.58 ± 21.93 μM, and 1085.08 ± 13.08 μM. The intracellular chromium ion concentrations of cells treated with these two chromium compounds were significantly higher than the controls ($p < 0.001$). Both potassium dichromate and lead chromate induced concentration-dependent increases ($r = 0.976$, $p < 0.001$; $r = 0.949$, $p < 0.001$) in intracellular chromium ion levels.

3.2. Cell viability

Our results (Fig. 2) indicated that the viabilities of cells treated with 5 μM, 10 μM and 15 μM of $K_2Cr_2O_7$ were all significantly ($P < 0.05$, $P < 0.01$) lower than that of controls, but only the highest concentration (5 μg/cm^2) of $PbCrO_4$ could significantly reduce cell viability. Moreover, the viability of cells treated with the highest concentration of $PbCrO_4$ was significantly ($P < 0.05$) higher than that of cells treated with the highest concentration of $K_2Cr_2O_7$,