Toxicology Letters 228 (2014) 241–247

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Effect of hexavalent chromium on histone biotinylation in human bronchial epithelial cells

Bo Xia[a,b,c], Xiao-hu Ren[a,b], Zhi-xiong Zhuang[b], Lin-qing Yang[b], Hai-yan Huang[b], Li Pang[c], De-sheng Wu[b], Jia Luo[a], You-li Tan[a], Jian-jun Liu[b,**], Fei Zou[a,*]

[a] Department of Occupational Health and Occupational Medicine, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, China
[b] Key Laboratory of Modern Toxicology of Shenzhen, Medical Key Laboratory of Health Toxicology, Laboratory of Modern Toxicology, Shenzhen Centre for Disease Control and Prevention, Shenzhen 518055, China
[c] College of Food Science and Technology, Hunan Agricultural University, East Renmin Road, Changsha 410128, Hunan, China

HIGHLIGHTS

- Cr(VI) (≥2.5 μM) induced histone deacetylation in 16HBE cells.
- Cr(VI) (≤0.6 μM) increased histone biotinylation in 16HBE cells.
- Cr(VI) (≥0.6 μM) affected the distribution of biotinidase in 16HBE cells.
- Cr(VI)-induced histone deacetylation takes part in adjusting histone biotinylation.

ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form 7 May 2014
Accepted 8 May 2014
Available online 21 May 2014

Keywords:
Chromium(VI)
Histone acetylation
Histone biotinylation

ABSTRACT

Chromium is a potent human mutagen and carcinogen. The capability of chromium to cause cancers has been known for more than a century, and numerous epidemiological studies have been performed to determine its carcinogenicity. In the post-genome era, cancer has been found to relate to epigenetic mutations. However, very few researches have focused on hexavalent chromium (Cr(VI))-induced epigenetic alterations. The present study was designed to investigate whether Cr(VI) would affect the level of a newfound epigenetic modification: histone biotinylation. Histone acetylation and histone biotinylation were studied in detail using human bronchial epithelial (16HBE) cells as an in vitro model after Cr(VI) treatment. Our study showed that Cr(VI) treatment decreased histone acetylation level in 16HBE cells. In addition, low doses of Cr(VI) (≤0.6 μM) elevated the level of histone biotinylation. Furthermore, immunoblot analysis of biotinidase (BTD), a major protein which maintains homeostasis of histone biotinylation, showed that the distribution of BTD became less even and more concentrated at the nuclear periphery in cells exposed to Cr(VI). Moreover, Cr(VI)-induced histone deacetylation may take part in the regulation of histone biotinylation. Together, our study provides new insight into the mechanisms of Cr(VI)-induced epigenetic regulation that may contribute to the chemoprevention of Cr(VI)-induced cancers and may have important implications for epigenetic therapy.

1. Introduction

Chromium, a commonly used industrial metal, is the most abundant heavy metal in the Earth's crust and is widely used by humans in various manufacturing industries, such as leather tanning and wood treatment, which caused environmental pollution and health concern worldwide (Vutukuru, 2005). For more than 100 years, numerous epidemiological studies have been performed on workers exposed to hexavalent chromium (Cr(VI)) to determine its carcinogenicity (Holmes et al., 2008). The epidemiology study on chromate production workers in 1948 showed that, compared to 1.4% in the reference population, 21.8% of the chromate workers deaths were due to respiratory cancer (Machle and Gregorius, 1948). However, the potential molecular mechanisms of carcinogenicity of Cr(VI) remain unclear.

* Corresponding author. Tel.: +86 20 61648301.
** Corresponding author. Tel.: +86 13501580129.
E-mail addresses: junii8@126.com (J.-j. Liu), public_xb@sina.cn (F. Zou).

http://dx.doi.org/10.1016/j.toxlet.2014.05.010
0378-4274/

As a well known potent oxidant, the formation of stable adducts caused by Cr(VI) is thought to contribute to its cytotoxic and genotoxic effects (Zhitkovich, 2005). Chemical and toxicological characteristics of chromium differ markedly on the basis of its valence state. Cr(VI) enters the cell through non-specific anion channels, and is subjected to a series of metabolic reductions to form the final stable metabolite Cr(III) (Stearns and Wetterhahn, 1994). These reactive intermediates and final products of Cr(VI) are able to induce the formation of stable DNA single or double-strand breaks, which may lead to a broad spectrum of DNA damage (Shi et al., 2004). The damage induced by Cr(VI) can lead to DNA replication inhibition, aberrant cell cycle checkpoints, dysregulated DNA repair mechanisms, which may all play an important role in Cr(VI) carcinogenesis (Nickens et al., 2010).

Recently, many data are interesting and shed light on the role of Cr(VI) in epigenetic modifications, which are changes in cellular functions that occur without altering the genetic material resulting in tumorigenesis. The earliest report showed that Cr(VI) was able to induce DNA methylation and silence the expression of the gpt transgene in a cell line expressing the bacterial gpt reporter gene (Klein et al., 2002). Furthermore, in chromate induced human lung cancers, DNA methylation was found to be increased in the promoter region of the DNA mismatch repair (hMLH1) gene (Labra et al., 2004) and the tumor suppressor gene p16 (Kondo et al., 2006). Interestingly, Cr(VI) could cross link the histone deacetylase 1–DNA methyltransferase 1 complexes to the chromatin of the Cyp1a1 promoter and inhibit histone marks induced by AHR-mediated gene transactivation, including trimethylation of H3 Lys-4, phosphorylation of histone H3 Ser-10 and various acetylation marks in histones H3 and H4 (Schnekenburger et al., 2007). These data suggested that, other than genotoxic effects, epigenetics may contribute to the carcinogenicity of Cr(VI).

Biotinylation of lysine (K) residues in histones is a novel, enigmatic histone modification. The discovery that biotinidase (BTD) has biotinyl-transferase activity, in addition to biotinidase hydrolase activity, presents possibility that biotin may participate directly in epigenetic changes, and the specific transfer of biotin to histones by BTD provides a possible explanation for why biotin is found in the nucleus of eukaryotic cells and the nature of its role in the regulation of protein transcription (Hymes and Wolf, 1999). Although biotin content in the nucleus is relatively small, it has been shown to regulate the transcription of glucokinase synthesis in early studies (Chauhan and Dakshinamurti, 1991). The fact that biotinylation increased for all classes of histones in G1, S, G2 and M phase of the cell cycle compared to G0 phase suggests that biotinylation of histones is a part of a general mechanism in nucleus (Stanley et al., 2001).

The aim of the current study was to investigate whether Cr(VI) would affect the level of histone biotinylation, if it is so then what is the underlying mechanism? In our previous study, we found that Cr(VI) caused down regulation of BTD in human bronchial epithelial (16HBE) cells by modifications of histone acetylation (Xia et al., 2011). In the current study, we further investigated the effect of Cr(VI) on histone biotinylation. In addition, we also performed analyses of the distribution of the two histone biotinylation-related proteins, BTD and holocarboxylase synthetase (HCS), in Cr(VI)-induced cells. Furthermore, we examined the effect of Cr(VI) on histone acetylation and tested the hypothesis that Cr(VI)-induced modifications of histone acetylation contribute to the regulation of histone biotinylation. Our study demonstrated that treatment of 16HBE cells with low doses of Cr(VI) ($\leq$0.6 μM) increased the level of histone biotinylation. In addition, Cr(VI)-induced histone deacetylation caused down regulation of BTD, and that at least partially resulted in the level of histone biotinylation revert to normal. These results suggest that Cr(VI) can modulate the level of histone biotinylation processes involving histone acetylation.

2. Materials and methods

2.1. Chemicals, materials and cell lines

Potassium chromate (K_2CrO_4) was purchased from Sigma–Aldrich. It was dissolved in sterile deionized water to 50 mM as the stock solution and stored at 4 °C. The stock solution was diluted with minimum essential medium (MEM) (without FBS) to experimental concentration before the chemical treatment. MEM, heat-inactivated fetal bovine serum (FBS), antibiotics (streptomycin and penicillin) and trypsin-EDTA solution were all purchased from Gibco BRL-Life Technologies (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO) was purchased from BIO BASIC Inc. The human bronchial epithelial (16HBE) cell line was a kind gift from Prof. Gruenert D.C. (California University, CA, USA). Trichostatin A (TSA) was from Sigma and stored at −20 °C.

2.2. Cell culture and chemical treatment

Cells were cultured in MEM medium supplemented with 10% (v/v) heat-inactivated FBS and antibiotic supplement (penicillin 100 U/ml and streptomycin 100 μg/ml) at 37 °C in a humidified incubator with 5% CO_2. When the cultured cells had grown to about 80% confluence, they were treated with different concentrations of Cr(VI) generally ranged from 0.3 to 5 μM for 24 h, and 0.1% sterile deionized water was used as the solvent control. In previous publications on the toxicology of chromate, concentrations of chromate ranged from 0.2 to 800 μM, and incubation times ranged from 1 min to several days (Holmes et al., 2008). Therefore, we chose 24 h as the incubation time in our study, and 5 μM (<1/2 LC_{50}) was chosen as the highest concentration in this study. TSA was added to the medium after treatment with Cr(VI) to the termination of the experiments. Detailed treatment procedures are outlined in the following sections or described in the figure legends.

2.3. Confocal microscopy analysis

Fluorescence staining experiments were conducted as described before (Narang et al., 2004). After treatment with Cr(VI) for 24 h, 16HBE cells, grown on coverslips, were fixed in freshly prepared 4% paraformaldehyde in PBS for 5 min followed by washing 3 times with PBS prior to permeabilization by 0.3% Triton-X in PBS for 5 min. Fixed cells were then incubated with primary antibodies diluted 1:1000 in 1% BSA-PBST for about 16 h at 4 °C, washed with PBS and incubated with fluorescein-labeled secondary antibody diluted 1:400 in PBST at 37 °C for 30 min in the dark and counterstained with DAPI in PBS for 5 min. All the immunofluorescence staining were observed using Confocal Laser Scanning Microscopy (Leica Tcs sp5, Leica Microsystems) under dark field and the images were analyzed by Image-Pro Plus software (IPP). The following antibodies were used in this study: BTD (K-17) Antibody (sc-48432, santa cruz), HCS (N-19) Antibody (sc-23732, santa cruz), IgG-FITC Secondary Antibodies (santa cruz).

2.4. Histone extraction

Histone extraction was performed as described previously (Shechter et al., 2007). Briefly, cells were collected by trypsinization and centrifugation, and washed twice with PBS, then the cell pellet was resuspended in hypotonic lysis buffer (10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 1 mM KCl, 1 mM dithiothreitol, 20 mM N-ethylmaleimide, 10 mM Na-butyrate, phosphatase inhibitors and protease inhibitor cocktail) and incubated for 30 min at 4 °C. The cells were then centrifuged and resuspended in 0.4 M HCl and incubated overnight at 4 °C. Soluble chromatinized histones were precipitated by trichloroacetic acid (TCA) and the histone pellet was then dissolved in 100 μl ddH_2O. Because there were no housekeeping proteins left after histone extraction, we quantified the content of histones in each sample using Micro BCA Protein Assay Kit (23235, Pierce), which was used for normalization of the levels of histone modifications. The histone solution was separated on 15% SDS/PAGE gel. The following antibodies were used in this study: anti-acetyl-histone H3 Antibody (06-599, Upstate), anti-acetyl-histone H4 Antibody (06-598, Upstate), Streptavidin HRP Conjugate (21130, Pierce).

2.5. Western blot analysis

After the indicated treatments, total protein of 16HBE cells was extracted with lysis buffer (7 M Urea, 2 M Thiourea, 4%CHAPS, 10 mM Tris). The lysates were then centrifuged and the insoluble debris was discarded. After boiling at 99 °C for 5 min, proteins were then separated on 10% polyacrylamide gels, and transferred to polyvinylidene fluoride membranes. Membranes were blocked in Tris-buffered saline (TBS) containing 0.1% (vol/vol) Tween 20 and 5% fat-free milk for 1 h at room temperature, and then incubated with primary antibodies overnight at 4 °C and with secondary antibodies for 1 h at room temperature. GAPDH was used for normalization of protein levels. Antibody signals were detected using Image J quantification software. The following antibodies were used in this study: HDAC2 (C-8) Antibody (sc-9959, santa cruz), HDAC3 (N-19) Antibody (sc-8138, santa cruz), BTD (K-17) Antibody (sc-48432, santa cruz), HCS (N-19) Antibody (sc-23732, santa cruz).