Toxicology Letters 264 (2016) 71–78

Contents lists available at ScienceDirect

Toxicology Letters

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

Full length article

Gene expression profiling and bioinformatics analysis in 16HBE cells treated by chromium (VI)

Guiping Hu[a], Jiaxing Liu[a], Yongming Zhang[b], Pai Zheng[c], Lele Wang[a], Lin Zhao[a], Huadong Xu[a], Zhangjian Chen[a], Tiancheng Wang[d,*], Guang Jia[a,*]

[a] Department of Occupational and Environmental Health Science, School of Public Health, Peking University, Beijing 100191, China
[b] Department of Occupational and Environmental Health Science, School of Public Health, Baotou Medical College, Baotou, Inner Mongolia Autonomous Region 014030, China
[c] Editorial Department of Chinese Journal of Preventive Medicine, Chinese Medical Association, Beijing 100710, China
[d] Department of Clinical Laboratory, Third Hospital of Peking University, Beijing 100191, China

HIGHLIGHTS

- Gene expression profiling and bioinformatics analysis in 16HBE cells treated by chromium(VI)compound was performed.
- Cr(VI) toxicity effects may involve in multiple biological processes and signal pathways by some specific pathway.
- Some significantly differential expression genes can be used as potential biomarkers of Cr(VI) exposure.

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form 17 October 2016
Accepted 24 October 2016
Available online 26 October 2016

Keywords:
Hexavalent chromium Cr (VI)
Gene expression profile
Microarray analysis
Bioinformatics analysis

ABSTRACT

Hexavalent chromium [Cr(VI)] compounds are widely used in industry and agriculture and are also ubiquitous environmental contaminant which are recognized as one kind of carcinogen, mutagen and teratogen towards humans and animals. To determined the Cr(VI) toxicity effects, gene expression profile can be meaningful for discovering underlying mechanisms of toxicity, and identifying potential specific genetic markers of Cr(VI) exposure and effects. In the current study, gene expression profiling and bioinformatics analysis in 16HBE cells treated by chromium(VI) compound were performed. The MTT assay was done to determine the optimal Cr(VI) treated concentration and time. The mRNA expression profile was performed using Arraystar Microarray V3.0 at 10.00 μM Cr(VI). RT-qPCR was applied to verify some interested significantly altered genes at different treatment groups. Comprehensive analysis including biological processes, GO ontology network, pathway network analysis and gene-gene network analysis was conducted to identify the related biological processes, signal pathway and critical genes. It was found that Cr(VI) could induce reduced cells viability and alter gene expression profile of human bronchial epithelial cells. 2273 significantly differential expressed genes formed a complex network and some expressions changed in a Cr(VI) concentration dependent manner. In conclusion, Cr(VI) toxicity effects may involve in oxidative stress, inflammation, energy metabolism, protein synthesis endocytosis, ion binding, DNA binding and metabolism, cell morphogenesis, cell cycle regulation, autophagy, apoptosis, cell death, and carcinogenesis by some specific pathway. Meanwhile, some significantly differential expression genes can be used as potential biomarkers of Cr(VI) exposure.

1. Introduction

Hexavalent chromium [Cr(VI)] and its compounds have been recognized to be carcino genic (IARC, 1990) based on numerous observations in occupational epidemiology studies (Rosenman and Stanbury, 1996). Since the widespread use of chromium in industries and agriculture, amounts of chromium slag and

* Corresponding authors.
E-mail addresses: tcwang@bjmu.edu.cn (T. Wang), jiaguangjia@bjmu.edu.cn (G. Jia).

http://dx.doi.org/10.1016/j.toxlet.2016.10.015
0378-4274/

chromium waste water were discharged into environment which induced increasing concern about the environmental influence and health effects of Cr(VI) (Gao and Xia, 2011; Rosenman and Stanbury, 1996). Except a higher lung cancer risk, workers in chromate production, plating, pigments, welding and leather tanning industries historically exposed to Cr(VI) also suffered from an increased risk of immune disorders (Beaver et al., 2009), stomach (Welling et al., 2015) and intestinal (Thompson et al., 2014), liver (Patlolla et al., 2009) and kidney damage (Wang et al., 2011). However, the mechanisms of multi-system disorders and genetic damage of Cr(VI) exposure still need further investigation.

Chromate Cr(VI) closely resembles sulfate (SO_4^{2-}) and thus could enter cells via an anion carrier (Buttner and Beyersmann, 1985). As a strong oxidant, Cr(VI) can produce a series of reactive oxygen species (ROS) and undergo a series of metabolic reductions to form reactive Cr(V) and Cr(IV) intermediates as well as the final stable metabolite Cr(III), which could induce oxidative stress or genetic damage (Myers, 2012). It is extensively accepted that genetic lesions induced by Cr(VI) are associated with ROS, Cr-DNA adducts, oxidized bases, DNA-protein crosslinks, energy metabolism and DNA strand breaks which dominate the underlying mechanisms of apoptosis and carcinogenesis (Myers, 2012; Nickens et al., 2010). Our previous studies (Li et al., 2014) have found that 8-OHdG and micronucleus (MN) in blood have a positive correlation with blood Cr(VI) and might be used as potential early genetic damage biomarkers. Many studies revealed that the intricate genetic damage repair pathways were activated upon DNA damage and played a critical role in the repair of Cr(VI)-induced DNA strand breaks (Halasova et al., 2012; Reynolds et al., 2004; Zhitkovich et al., 2005). What's more, the cell apoptosis related cell cycle regulation pathways are involved in the DNA damage induced by Cr(VI) exposure (Hu et al., 2016). However, more evidences on toxicity effects of Cr(VI) exposure and bioinformatics analysis method are still required.

The alternative to toxic effects involved in Cr(VI) exposure can be associated with oxidative stress, DNA damage, DNA repair, apoptosis elements acting and so on via a variety of genes (Nigam et al., 2014). A marked gene expression profile or signal regulation pathway induced by Cr(VI) could contribute to a comprehensive insight into toxicity effects of Cr(VI) exposure. The Cr(VI)-altered cellular gene expression profile could be treated as the possible molecular based navigation of the onset or progress of various effects (Nigam et al., 2014). Previous studies have shown altered genes expression that a number of genes were changed in response to acute or high Cr(VI) exposure to human cells (Andrew et al., 2003; Ye and Shi, 2001). However, the Cr(VI) biological activities are complicated and remained to be fully investigated. To better understand the characteristic alerted genes and mechanisms of Cr (VI) toxic effect, microarray analysis was used to identify the mRNA expression profile for Cr(VI) treated 16 HBE cells. Besides, the real-time quantitative polymerase chain reaction (RT-qPCR) was used to verify some interested significantly altered genes identified by gene microarray. Bioinformatics analysis including go ontology net analysis, pathway analysis and gene-gene-net analysis were also performed to analyze the possible biological processes, signal pathway and critical genes and mechanisms of Cr(VI) exposure effects.

2. Materials and methods

2.1. Cell culture and treatment condition and time

Human bronchial epithelial cell lines (16HBE cells) were purchased from the tumor cell library of Chinese Academy of Cell Resource Center (Shanghai, China). 16HBE cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin, and maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

Depending on the experiment design, 1×10^4 16 HBE cells were treated in 96-well plates with dichromate ($Cr_2O_7^{2-}$) (Sigma, USA) stoke solution diluted in cell culture medium at various concentrations: 0.00, 0.63, 1.23, 2.50, 5.00, 10.00, 20.00, 50.00 and 100.00 μM for 12, 24 and 48 h. Then, the other treatment does were considered by the following experiment. All controls were exposed to medium with the same volume as Cr(VI) stoke solution but replaced by ddH_2O (Sigma, USA) and underwent the same condition as experimental group.

2.2. RNA isolation and mRNA gene expression profile analysis

According to the experiment design, 1.2×10^6 16 HBE cells were treated in 6-well plates with various Cr(VI) concentrations. Then, the total RNA was extracted using TRIzol reagent (InvitrogenTM, USA) according to the manufacturer' s protocol. The NanoDrop 2000c spectrophotometers (Thermo, USA) were used to measure the absorbance at 260 nm (A260) and at 280 nm (A280) and evaluate the RNA integrity and concentration. RNA integrity was assessed by standard denaturing agarose gel electrophoresis. RNA was amplified and transcribed into cDNA utilizing a random priming method, and sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) with minor modifications by Arraystar Human gene Microarray V3.0 (8×60 K, Arraystar Inc., Rockville, MD, USA) which can detect 26,109 coding transcripts.

2.3. Cell proliferation and cell viability rate

MTT assay was used to analyze the cell viability and cell proliferation (Mosmann, 1983). Approximately 5×10^3 cells were plated in each well of 96-well plate to substrate for 24 h. The cells were then exposed to 0.00, 0.63, 1.23, 2.50, 5.00, 10.00, 50.00 and 100.00 μM Cr(VI) for 12, 24 or 48 h. Finally, the absorbance was measured at 492 nm on Microplate Reader (Thermo Fisher, USA). Each sample was repeated three times in triplicate parallel on separate cell cultures ($n \geq 3$ for cytotoxicity). The reduction in cell viability was expressed as a percent normalized to non-treated control cells. As described in our previous work (Hu et al., 2016), to detect the cell proliferation and cell viability rate, all the groups were analyzed by SPSS software to calculate the IC50 for Cr(VI) treatment concentration and time.

2.4. Differential expressed gene analysis

To analyses the differential expressed gene, six samples were send to do Microarray including three control samples and three treated samples. Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. Quantile normalization and subsequent data processing were performed with using the GeneSpring GX v12.1 software package (Agilent Technologies). After quantile normalization of the raw data, mRNAs that at least 3 out of 6 samples have flags in Present or Marginal ("All Targets Value") were chosen for further data analysis. The criterias of up-regulation and down-regulation of differential expressed genes were fold change >2 and fold change <0.5 respectively. Differential expressed mRNAs were identified through Fold Change filtering and the statistical significance of differential expressed mRNAs between the two groups were identified through random variance model Hierarchical Clustering and combined analysis were performed using homemade scripts. These p values were considered statistically significant if they were <0.05.