Biochemical and Biophysical Research Communications 446 (2014) 590–595

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

# Analysis of trichloroethylene-induced global DNA hypomethylation in hepatic L-02 cells by liquid chromatography–electrospray ionization tandem mass spectrometry

Hang Zhang [a,b,1], Wen-Xu Hong [a,1], Jinbo Ye [a,1], Xifei Yang [a], Xiaohu Ren [a], Aibo Huang [a], Linqing Yang [a], Li Zhou [a], Haiyan Huang [a], Desheng Wu [a], Xinfeng Huang [a], Zhixiong Zhuang [a], Jianjun Liu [a,*]

[a] Key Laboratory of Modern Toxicology of Shenzhen, Medical Key Laboratory of Guangdong Province, Medical Key Laboratory of Health Toxicology of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, China
[b] School of Life Science, Shenzhen University, Shenzhen 518060, China

ARTICLE INFO

*Article history:*
Received 27 February 2014
Available online 13 March 2014

*Keywords:*
Trichloroethylene (TCE)
DNA methylation
DNA methyltrasferases (DNMT)
Mass spectrometry (MS)

ABSTRACT

Trichloroethylene (TCE), a major occupational and environmental pollutant, has been recently associated with aberrant epigenetic changes in experimental animals and cultured cells. TCE is known to cause severe hepatotoxicity; however, the association between epigenetic alterations and TCE-induced hepatotoxicity are not yet well explored. DNA methylation, catalyzed by enzymes known as DNA methyltransferases (DNMT), is a major epigenetic modification that plays a critical role in regulating many cellular processes. In this study, we analyzed the TCE-induced effect on global DNA methylation and DNMT enzymatic activity in human hepatic L-02 cells. A sensitive and quantitative method combined with liquid chromatography and electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) was validated and utilized for assessing the altered DNA methylation in TCE-induced L-02 cells. Quantification was accomplished in multiple reaction monitoring (MRM) mode by monitoring a transition pair of *m/z* 242.1 (molecular ion)/126.3 (fragment ion) for 5-mdC and *m/z* 268.1/152.3 for dG. The correlation coefficient of calibration curves between 5-mdC and dG was higher than 0.9990. The intra-day and inter-day relative standard derivation values (RSD) were on the range of 0.53–7.09% and 0.40–2.83%, respectively. We found that TCE exposure was able to significantly decrease the DNA methylation and inhibit DNMT activity in L-02 cells. Our results not only reveal the association between TCE exposure and epigenetic alterations, but also provide an alternative mass spectrometry-based method for rapid and accurate assessment of chemical-induced altered DNA methylation in mammal cells.



## 1. Introduction

Trichloroethylene (TCE), an organic solvent widely used in industry, has long been known as a major pollutant that affects both the occupational and general environments [1]. Many studies have demonstrated that TCE exposure can cause severe health effects, including neurotoxicity, hepatotoxicity and kidney damage [2–4]. In 2011, the U.S. EPA (Environmental Protection Agency) formally characterized TCE as a human carcinogen in the Final Health Assessment (http://www.epa.gov/IRIS/). TCE undergoes metabolism through two pathways, conjugation with glutathione as minor one and cytochrome P450 (P450)-dependent oxidation as a major one. Liver is principally metabolized site where TCE is oxidatively metabolized to TCE-epoxide. TCE hepatotoxicity has been well demonstrated in experimental animals (i.e. mice and rats) and culture cells [5,6]. Though remarkable progress has been made, the potential molecular mechanisms by which TCE-induced hepatotoxicity are not yet well understood.

DNA methylation, carried out by the addition of a methyl group to position 5 of cytosine by DNA methyltransferases (DNMT), is a central mammalian epigenetic control mechanism that impacts gene expression, genome stability, genetic imprinting, and cellular differentiation [7]. Consistent with these important roles, aberrant DNA methylation have been found to be associated with various human diseases such as developmental disorders, cancer and aging [8]. The possibility of using DNA methylation as a marker for disease has created a strong need for techniques to detect and measure DNA methylation. These approaches can be classified as

* Corresponding author. Address: Key Laboratory of Modern Toxicology of Shenzhen, Shenzhen Center for Disease Control and Prevention, No. 8, Longyuan Road, Nanshan District, Shenzhen 518055, China. Fax: +86 755 25508584.
*E-mail address:* bio-research@hotmail.com (J. Liu).
[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.03.015
0006-291X/

either gene-specific or global analysis [9,10]. Gene-specific assays are crucial for integrating information about DNA methylation patterns with gene expression, chromatin modification and assembly of transcription factors at gene promoters [11]. On the contrary, global analysis provide an overall picture of DNA methylation levels, which is important for understanding the relationship between genomewide alterations in DNA methylation, gene specific methylation pattern, and genome stability [12]. Currently, reported methods for the determination of genomic DNA methylation include Southern blot analysis [13], combined bisulfate restriction analysis [14], and capillary zone electrophoresis [15]. Among these, liquid chromatography (LC) in combination with mass spectrometric (MS) detection can provide a highly specific and extremely sensitive way, which has powerful potential for the quantification of global DNA methylation levels [16,17].

Recently, TCE have been linked to aberrant changes in epigenetic pathway *in vivo* and *in vitro* studies [6,18,19]. TCE exposure was found to induce a global DNA hypomethylation in TK6 cells [20] and a DNA hypermethylation in the Serca2 promoter region in cardiac myoblast cells and rat embryonic cardiac tissue [19]. Exposure to TCE and its metabolites can lead to decreased methylation in the promoter regions of the c-jun and c-myc genes in mice livers [21]. As part of our long-term efforts to understand the potential molecular mechanisms of TCE-induced hepatic toxicity [22–27], we describe here the TCE-induced effect on global DNA methylation and DNMT enzymatic activity in L-02 cells. Specifically, a sensitive and quantitative method combined with liquid chromatography and electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) was used for global DNA methylation measurement. To the best of our knowledge, the current study to be the first time shows TCE-induced effect on DNA methylation events in human hepatic L-02 cells. Our results indicated that TCE exposure can cause significant alterations in global DNA methylation and DNMT enzymatic activity, which may provide helpful scientific clues for further understanding the TCE-induced hepatotoxicity through epigenetic mechanisms.

## 2. Materials and methods

### 2.1. Materials

Human hepatic L-02 cells were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences (Chinese Academy of Science, China). TCE and 5-Aza-deoxycytidine (DAC) were purchased from Sigma–Aldrich Corp (St. Louis, MO, USA). RPMI-1640 culture medium, fetal calf serum (FCS) and EDTA–trypsin were purchased from GIBCO (Billings, MT, USA). HPLC-grade methanol and acetonitrile, formic acid (HPLC), 2′-deoxycytidine (dC), 2′-deoxyguanosine (dG), 2′-deoxyadenosine (dA), 5-methyl-deoxycytidine (5-mdC), thymidine (T), cytidine (C), guanosine (G), adenosine (A), uridine (U) and nuclease P1, phosphodiesterase I were purchased from Sigma–Aldrich Corp (St. Louis, MO, USA). Alkaline phosphatase was obtained from New England Biolabs (Beverly, MA, USA).

### 2.2. Cell culture and proliferation assay

L-02 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/ml streptomycin. A Cell Counting Kit-8 (CCK-8) assay was used to investigate the effect of TCE on the viability of L-02 cells. In a previous study, we figured out an $IC_{50}$ value (approximately 16 mmol/L) for 24 h-treatment of TCE [26]. We therefore chose 1/2 $IC_{50}$ value as the maximum exposure concentration for our study. Briefly, L-02 cells were treated with medium containing various concentration of TCE (0.25, 0.50, 1.00, 2.00, 4.00, 8.00 mmol/L) and left to incubate for 24 h. The control group was treated with the solvent (DMSO, 0.5% v/v) only. A 10-μL aliquot of the CCK-8 solution was added to each well of the plate, and the plate was incubated for 2 h at 37 °C. The absorbance at 450 nm was measured with an Infinite M1000 Microplate Reader (TECAN Group Ltd, San Jose, CA, USA).

### 2.3. DNA isolation and hydrolysis

Genomic DNA was isolated from treated L-02 cells using the DNA purification kit (Sangon Biotech, China), according to the instructions of the manufacturer. Briefly, cells suspended in culture medium were pelleted and incubated with Buffer Digestion for 1 h, followed by incubation with Buffer PA for 5 min. The cell lysate was then pelleted by centrifugation, and the supernatant was transferred to a clean tube containing 100% 2-propanol. Then, the DNA pellet was washed with 75% ethanol, air-dried, and rehydrated in TE Buffer. DNA hydrolysis was performed as previous described by Crain [28]. The final DNA hydrolysate was filtrated by Microcon centrifugal filter device (10 kDa cut-off weight, Millipore, Bedford, MA, USA) to remove the proteins/enzymes added for hydrolysis.

### 2.4. HPLC/ESI/MS/MS conditions

LC analysis was performed with a Shimadzu LC-20A high performance liquid chromatography (Shimadzu Corporation, Japan). The liquid chromatography was equipped with a vacuum degasser, an autosampler, a rapid resolution binary pump, a diode array detector and a thermostated column compartment. A Waters (Milford, MA, USA) Atlantis dC18 2.1 mm × 150 mm column (5-μm particle size) protected by a Phenomenex dC18 2.1 mm × 20 mm guard column (5-μm particle size) was used for the chromatographic separation. 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B) were used as mobile phases, and the flow rate was set at 0.22 mL/min. The linear gradient elution was 0–18% of solvent B in 24 min. The sample injection volume was 10 μL. The detection wavelength for dG and 5-mdC was set at 284 nm.

Mass spectrometric detection was performed on an API 4000 Q-Trap system mass spectrometry (AB SCIEX, Framingham, MA, USA). Optimized ESI source parameters were shown in Supplementary Table 1. Quantitative determination of dG and 5-mdC were performed under multiple reaction monitoring (MRM) mode and MS data were collected corresponding to transition $m/z$ 268.1–152.3 for dG and $m/z$ 241.9–126.3 for 5-mdC, respectively. AB SCIEX Analyst 1.3 software (AB SCIEX, Framingham, MA, USA) was used for data acquisition and processing.

Stock solution of 5-mdC and 5dG were prepared at 1.0 mg/mL in deionized water. Standard working solutions of 5-mdC and 5dG (1.0, 10, 100, 500, 1000 ng/mL) were prepared by further diluting the stock. Quality control (QC) samples were prepared by adding different concentrations of 5-mdC (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 ng/mL) into 500 ng/mL of dG. The final percentage of 5-mdC/dG were 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0% and 10.0%, respectively. All the above solutions were stored at −20 °C and allowed to equilibrate at room temperature before use.

### 2.5. Global DNA methylation measurement

The TCE-induced effect on the genomic DNA methylation was analyzed by RPLC–ESI-MS/MS. Briefly, the cells were lysed after 24 h of TCE exposure under various concentrations. The control group was treated with the solvent (DMSO, 0.5% v/v) only. Cells treated with DAC (DNA methyltransferase inhibitor) were used as hypomethylation control. DAC treatment was given to L-02 cell

lines at 20 μmol/L for 24 h. The resulting 10 μL DNA hydrolysis products were injected for LC–ESI-MS/MS analysis. Based on the assumption that [dG] = [5-mdC] + [dC] in genomic DNA [16], we expressed genomic DNA methylation as [5mdC]/[dG] and quantified this value using a calibration curve between the peak area ratio of (5-mdC/dG) versus (5-mdC/dG) (Fig. 2). dG was used as an internal standard for an easier and more accurate determination of dC methylation.

### 2.6. Measurement of DNA methyltransferases (DNMT) enzymatic activity

The TCE-induced effect on the DNMT enzymatic activity was analyzed by the colorimetric EpiQuik DNMT Activity/Inhibition Assay Kit (Epigentek Group Inc, Farmingdale, NY, USA). Briefly, the cells were harvested after 24 h of TCE exposure (0.25, 0.50, 1.00, 2.00, 4.00, 8.00 mmol/L). The control group was treated with the solvent (DMSO, 0.5% v/v) only and the hypomethylation control was treated with DAC at 20 μmol/L for 24 h. Then, the nuclear extracts were collected by using the Nuclear Extraction Kit (Key GEN Biotech, China). The concentration of nuclear proteins extracts were measured by the 2-D Quant Kit assay (GE Healthcare Life Sciences, Piscataway, NJ, USA) and any nuclear extracts not immediately used were stored at −80 °C.

To measure the DNMT activity, an equal amount of nuclear extract was reacted with enzyme substrate. Subsequently, capture antibody against 5-methylcytosine (5-mC) and then detection antibody were added. After the final washing step, the reaction was developed and absorbance values were measured with Infinite M1000 Microplate Reader (TECAN Group Ltd, San Jose, CA, USA). DNMT enzyme activity was quantified using a standard curve.

### 2.7. Statistical analysis

All experiments were performed more than three times. The data were analyzed with the statistical program Sigma Stat (Jandel Scientific, Chicago, IL, USA) and data are presented as mean ± standard error. The percentage of global DNA methylation was expressed as [5mdC]/[dG]% and quantified this value using a calibration curve between the peak area ratios of (5-mdC/dG) versus (5-mdC/dG) (Fig. 2). Statistical differences between the control and treated groups were evaluated using the Student's *t*-test or the Student–Newman–Keuls multiple comparison test. $P < 0.05$ was considered to indicate a statistically significant difference between groups.

## 3. Results

### 3.1. The TCE-induced effect on the viability of L-02 cells

We measured the viability of the cells treated with different concentrations of TCE (0, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00 mmol/L) for 24 h with a CCK-8 assay and the cell viability was assessed to 106.1 ± 4.1%, 108.0 ± 4.2%, 90.1 ± 4.2%, 78.5 ± 4.0%, 75.0 ± 4.5%, or 69.4 ± 5.0% of the control level, respectively (Supplementary Fig. 1). All of these concentrations were chosen for measurement of DNA methylation and DNMT activity.

### 3.2. Mass spectrometric characterization of dG and 5-mdC

The full scan of ESI mass spectra of dG and 5-mdC were obtained direct infusion of a standard working solution. The data indicated that protonated dG ($[M+H]^+$, *m*/*z* 268.1) and 5-mdC ($[M+H]^+$, *m*/*z* 241.9) were the predominant ion species formed by ESI. The product ion spectra of dG and 5-mdC were shown in Fig. 1A and B, and it can be found that the main product ion of dG was *m*/*z* 152.3 whereas the main product ion of 5-mdC was *m*/*z* 126.3. Thus, the precursor/product ion pairs of *m*/*z* 268.1/152.3 for dG and *m*/*z* 241.9/126.3 for 5-mdC were used as MRM transitions.

Supplementary Fig. 2 showed the LC/UV chromatogram of normal and modified nucleosides from L-02 cells DNA extraction,

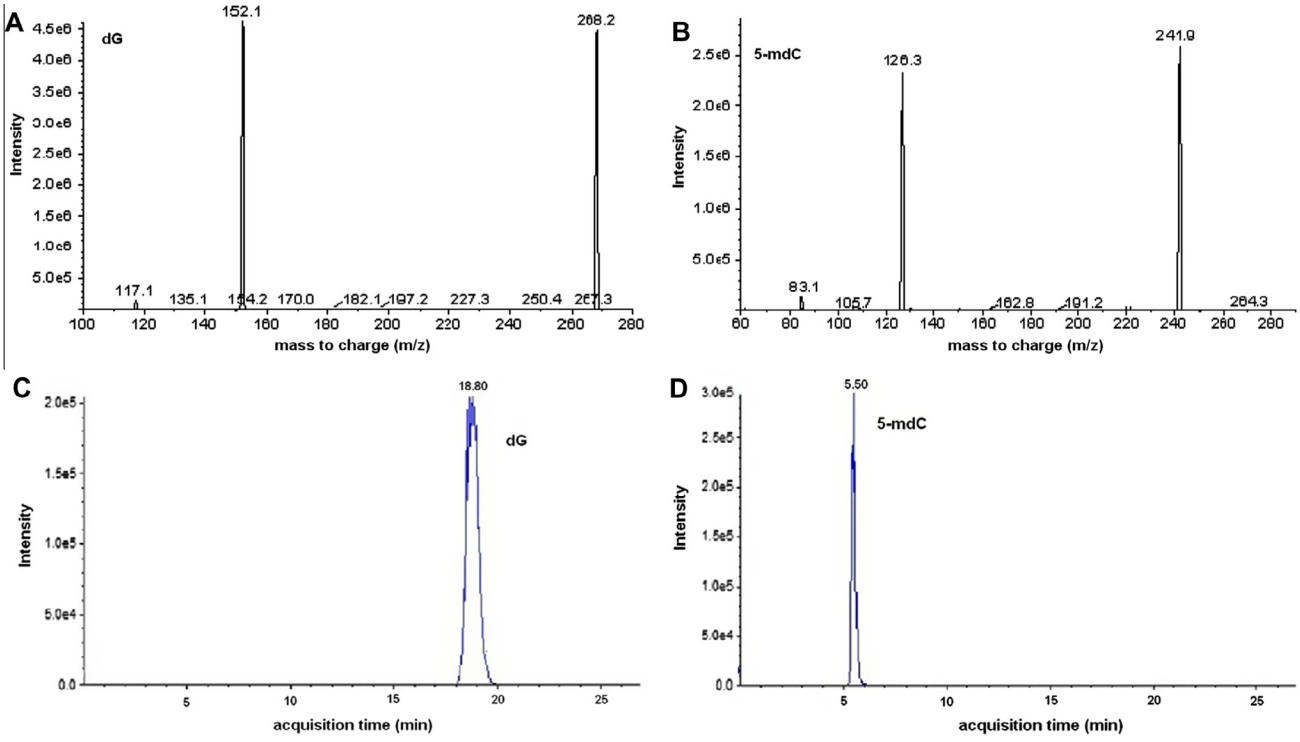


**Fig. 1.** Positive ESI product ion spectra of dG (A) and 5-mdC (B), and typical MRM chromatogram resulting from the hydrolyzed DNA sample: (C) dG (RT 18.80 min) and (D) 5-mdC (RT 5.50 min), The ESI conditions were optimized as described in Supplementary Table 1.

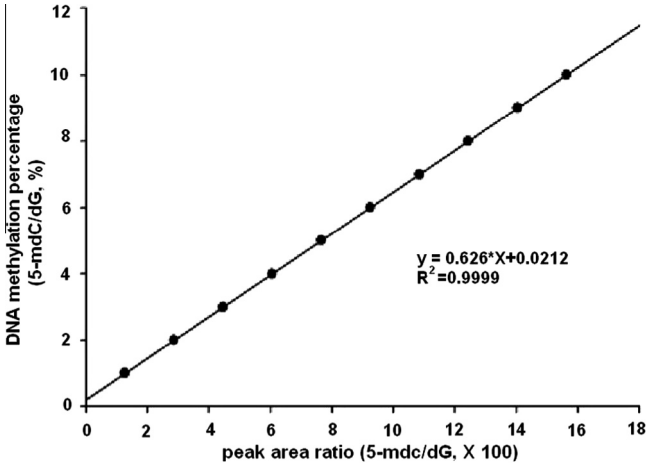


**Fig. 2.** Calibration curve of global DNA methylation value by liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS).

which indicated a complete separation for C, dC, 5-mdC, U, dA, A, G, dG, T in the retention times of 2.39, 3.06, 5.50, 5.95, 10.02, 11.63, 14.94, 18.80, 24.26 min, respectively. It can be clearly seen that the detection of dG and 5-mdC would not be influenced from other nucleosides. Typical MRM ion chromatograms for dG and 5-mdC were shown in Fig. 1C and D. Calibration curves of 5-mdC and dG in different percentage ranging from 0% to 10% were shown in Fig. 2, with the correlation coefficient $R^2$ was 0.9999. To determine the precision of the present method, QC samples of dG and 5-mdC were analyzed three times a day for five consecutive days to obtain the intra-day precision ($n = 3$) and the inter-day precision ($n = 15$). The accepted criteria for each quality control was that the relative standard deviation (RSD, %) value should not exceed 10%. In this study, the intra-day and inter-day precision values were in the range of 0.53–7.09% and 0.40–2.83%, respectively (Supplementary Table 2).

### 3.3. TCE-induced effect on the genomic DNA methylation in L-02 cells

The genomic DNA methylation was measured by LC–ESI-MS/MS with TCE exposure in various concentrations (0, 0.25, 0.50, 1.00, 2.00, 4.00 and 8.00 mmol/L). Fig. 3A and B showed that TCE exposure can caused a significant DNA hypomethylation in L-02 cells.

### 3.4. TCE-induced effect on DNMT enzymatic activity in L-02 cells

DNMT enzymatic activity was determined in nuclear extracts of L-02 cells with various TCE concentrations for 24 h. DAC treatment was used as hypomethylation control. TCE exposure was able to significantly decrease the DNMT enzymatic activity in L-02 cells (Fig. 4).

## 4. Discussion

Environmental and occupational chemicals can modify multiple biological processes that affect epigenetic mechanisms, including DNA methylation, histone codes, and miRNA expression [29]. In the last few years, more and more investigations have examined the relation between epigenetic and exposure to chemicals, including metals, air pollution, endocrine-disrupting chemical and reproductive toxicant [20,30]. As major pollutant that affects both occupational and general environments [1], exposure to TCE was recently associated abnormal epigenetic changes such as global DNA hypomethylation and hypermethylation of tumor suppressor genes [18,20]. However, the potential associations between TCE

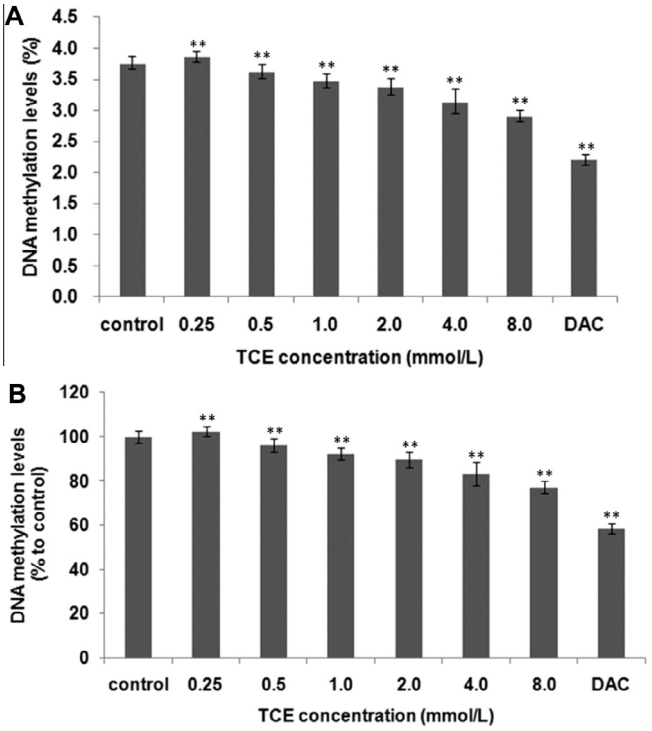


**Fig. 3.** The measurement of global DNA methylation in L-02 liver cells exposed in various concentration of TCE. (A) DNA methylation value and (B) percentage to control level. 5-Aza-deoxycytidine (DAC), a DNA methyltransferase inhibitor, was used as hypomethylation control. The data represent the mean ± SEM of at least three independent experiments. $^{**}P < 0.01$ compared with the control.

hepatotoxicity and epigenetic alternations are not well explored *in vivo* and *in vitro*.

In a previous study, we analyzed the TCE-induced cytotoxicity in L-02 cells, including cell proliferation and cellular apoptosis etc. We further found that TCE exposure altered the expression and subcellular localization of specific oncoproteins [25,26]. In this study, a sensitive and quantitative method combined with liquid chromatography (LC) and mass spectrometry (MS) was used for global DNA methylation measurement [16,31]. Quantification was accomplished in multiple reaction monitoring (MRM) mode by monitoring a transition pair of $m/z$ 242.1 (molecular ion)/126.3 (fragment ion) for 5-mdC and $m/z$ 268.1/152.3 for dG. MRM is a mass spectrometry-based technique for small molecules detection with high sensitivity and selectivity, which have been successfully applied to a variety of biological applications [32]. The use of dG as an internal standard allows an easier and more accurate determination of dC methylation and avoids the use of expensive isotope-labeled internal standards [16]. The intra-day and inter-day relative standard derivative values (RSD) were on the range of 0.53–7.09% and 0.40–2.83%, respectively. The correlation coefficient of calibration curves was higher than 0.999 (Fig. 3). TCE exposure caused a significant global DNA hypomethylation in liver L-02 cells, which is consistent with published research in other cell lines [20]. In addition, we observed that DNMT activity significantly declined under TCE exposure in L-02 cells by ELISA-based assays.

In mammal, approximately 4% of cytosine residues in DNA are methylated [33]. DNA methylation is essential for normal development and is associated with a number of key processes including genomic imprinting, X-chromosome inactivation, suppression of repetitive elements, and carcinogenesis [34]. Aberrant DNA methylation can disrupt cellular function, whereas global DNA hypomethylation events can result in chromosomal instability

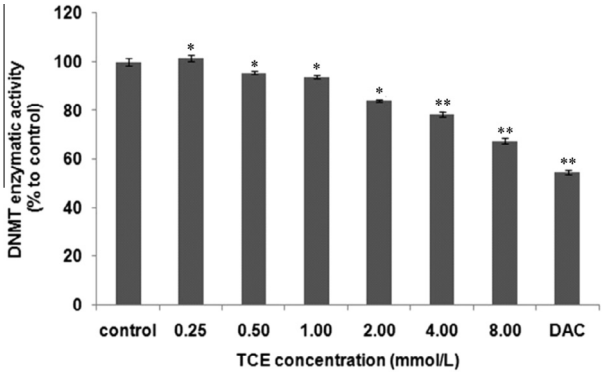


**Fig. 4.** The effect of TCE exposure on DNMT activity in L-02 cells. L-02 cells were treated with various TCE concentration for 24 h. Nuclear extracts were prepared, and the DNMT activity was measured by colorimetric methods. 5-Aza-deoxycytidine (DAC), a DNA methyltransferase inhibitor, was used as hypomethylation control. The data was normalized and compared with control level, representing the mean ± SEM of at least three independent experiments. $^{*}P < 0.05$ compared with the control, $^{**}P < 0.01$ compared with the control.

and oncogene activation. Hypomethylation of the global genome can lead to genomic instability that is exemplified by misalignments, DNA breakage, deletions and duplications during DNA replication. It is clear that disease by itself can induce hypomethylation of DNA; by contrary, a decrease in DNA methylation can have an impact on the predisposition to disease development. Exposure of chemicals has been linked to aberrant changes in epigenetic pathways both in experimental and epidemiological studies. In addition, DNA hypomethylation may mediate specific mechanisms of toxicity and responses to certain chemicals [29]. DNA methylation is regulated and balanced by DNA methyltransferases through *de novo* methylation of unmethylated cytosines and maintenance of methylation after DNA replication [35]. We thus speculate that decreased DNA methylation and declined DNMT activity may contribute to the mechanisms of TCE-induced toxicity in L-02 cells. Subtle epigenetic effect, such as gene specific DNA methylation and histone modifications, in response to TCE exposure needs to be further explored and fully understood.

In conclusion, we have demonstrated that TCE exposure induced a genomic DNA hypomethylation and a DNMT activity inhibition in L-02 cells. A novel method for determining the global DNA methylation was utilized with liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS). The quantification of DNA methylation levels was accomplished in MRM mode by monitoring the ion pair between 5-mdC and dG. Our findings not only reveal the association between TCE exposure and epigenetic alterations, but also validated an alternative liquid chromatography tandem mass spectrometry (LC–MS/MS) method for rapid and accurate assessment of chemical-induced altered DNA methylation in mammal cells.

## Conflict of interest

The authors declare no potential conflicts of interest or financial interests to disclose.

## Acknowledgments

This work was supported by NSFC (the National Natural Science Foundation of China) [81273126, 30972454, 21102094], the Key Project of Guangdong Natural Science Foundation [S2012020010903], the Project of Shenzhen Basic Research Plan [JCYJ20120616154222545, JCYJ20130329161137543], the Upgrade Scheme of Shenzhen Municipal Key Laboratory [ZDSY20120615085804889] and the Medical Scientific Research Foundation of Guangdong Province [A2012577].

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.015.

## References

[1] C. Wu, J. Schaum, Exposure assessment of trichloroethylene, Environ. Health Perspect. 108 (Suppl. 2) (2000) 359–363.
[2] D.M. Gash, K. Rutland, N.L. Hudson, P.G. Sullivan, G. Bing, W.A. Cass, J.D. Pandya, M. Liu, D.Y. Choi, R.L. Hunter, G.A. Gerhardt, C.D. Smith, J.T. Slevin, T.S. Prince, Trichloroethylene: Parkinsonism and complex 1 mitochondrial neurotoxicity, Ann. Neurol. 63 (2008) 184–192.
[3] D.H. Ramdhan, M. Kamijima, N. Yamada, Y. Ito, Y. Yanagiba, D. Nakamura, A. Okamura, G. Ichihara, T. Aoyama, F.J. Gonzalez, T. Nakajima, Molecular mechanism of trichloroethylene-induced hepatotoxicity mediated by CYP2E1, Toxicol. Appl. Pharmacol. 231 (2008) 300–307.
[4] W.A. Chiu, J. Jinot, C.S. Scott, S.L. Makris, G.S. Cooper, R.C. Dzubow, A.S. Bale, M.V. Evans, K.Z. Guyton, N. Keshava, J.C. Lipscomb, S. Barone Jr., J.F. Fox, M.R. Gwinn, J. Schaum, J.C. Caldwell, Human health effects of trichloroethylene: key findings and scientific issues, Environ. Health Perspect. 121 (2013) 303–311.
[5] L.H. Lash, J.W. Fisher, J.C. Lipscomb, J.C. Parker, Metabolism of trichloroethylene, Environ. Health Perspect. 108 (Suppl. 2) (2000) 177–200.
[6] K.M. Gilbert, A.R. Nelson, C.A. Cooney, B. Reisfeld, S.J. Blossom, Epigenetic alterations may regulate temporary reversal of CD4(+) T cell activation caused by trichloroethylene exposure, Toxicol. Sci. 127 (2012) 169–178.
[7] P.A. Jones, S.B. Baylin, The fundamental role of epigenetic events in cancer, Nat. Rev. Genet. 3 (2002) 415–428.
[8] K.D. Robertson, DNA methylation and human disease, Nat. Rev. Genet. 6 (2005) 597–610.
[9] T. Rein, M.L. DePamphilis, H. Zorbas, Identifying 5-methylcytosine and related modifications in DNA genomes, Nucleic Acids Res. 26 (1998) 2255–2264.
[10] C. Dahl, P. Guldberg, DNA methylation analysis techniques, Biogerontology 4 (2003) 233–250.
[11] A.R. Karpf, D.A. Jones, Reactivating the expression of methylation silenced genes in human cancer, Oncogene 21 (2002) 5496–5503.
[12] M. Ehrlich, DNA methylation in cancer: too much, but also too little, Oncogene 21 (2002) 5400–5413.
[13] M. Widschwendter, G. Jiang, C. Woods, H.M. Muller, H. Fiegl, G. Goebel, C. Marth, E. Muller-Holzner, A.G. Zeimet, P.W. Laird, M. Ehrlich, DNA hypomethylation and ovarian cancer biology, Cancer Res. 64 (2004) 4472–4480.
[14] Z. Xiong, P.W. Laird, COBRA: a sensitive and quantitative DNA methylation assay, Nucleic Acids Res. 25 (1997) 2532–2534.
[15] S. Sotgia, C. Carru, F. Franconi, P.B. Fiori, S. Manca, S. Pettinato, S. Magliona, R. Ginanneschi, L. Deiana, A. Zinellu, Rapid quantification of total genomic DNA methylation degree by short-end injection capillary zone electrophoresis, J. Chromatogr. A 1185 (2008) 145–150.
[16] L. Song, S.R. James, L. Kazim, A.R. Karpf, Specific method for the determination of genomic DNA methylation by liquid chromatography–electrospray ionization tandem mass spectrometry, Anal. Chem. 77 (2005) 504–510.
[17] I. Yang, M.C. Fortin, J.R. Richardson, B. Buckley, Fused-core silica column ultra-performance liquid chromatography–ion trap tandem mass spectrometry for determination of global DNA methylation status, Anal. Biochem. 409 (2011) 138–143.
[18] L. Tao, S. Yang, M. Xie, P.M. Kramer, M.A. Pereira, Effect of trichloroethylene and its metabolites, dichloroacetic acid and trichloroacetic acid, on the methylation and expression of c-Jun and c-Myc protooncogenes in mouse liver: prevention by methionine, Toxicol. Sci. 54 (2000) 399–407.
[19] B. Palbykin, J. Borg, P.T. Caldwell, J. Rowles, A.J. Papoutsis, D.F. Romagnolo, O.I. Selmin, Trichloroethylene induces methylation of the Serca2 promoter in H9c2 cells and embryonic heart, Cardiovasc. Toxicol. 11 (2011) 204–214.
[20] A.M. Tabish, K. Poels, P. Hoet, L. Godderis, Epigenetic factors in cancer risk: effect of chemical carcinogens on global DNA methylation pattern in human TK6 cells, PLoS ONE 7 (2012) e34674.
[21] L. Tao, R. Ge, M. Xie, P.M. Kramer, M.A. Pereira, Effect of trichloroethylene on DNA methylation and expression of early-intermediate protooncogenes in the liver of B6C3F1 mice, J. Biochem. Mol. Toxicol. 13 (1999) 231–237.
[22] J. Liu, H. Huang, X. Xing, R. Xi, Z. Zhuang, J. Yuan, F. Yang, J. Zhao, Comparative proteomic analysis on human L-02 liver cells treated with varying concentrations of trichloroethylene, Toxicol. Ind. Health 23 (2007) 91–101.
[23] H.Y. Huang, J.J. Liu, R.R. Xi, X.M. Xing, J.H. Yuan, L.Q. Yang, G.H. Tao, C.M. Gong, Z.X. Zhuang, An investigation of hormesis of trichloroethylene in L-02 liver cells by differential proteomic analysis, Mol. Biol. Rep. 36 (2009) 2119–2129.
[24] J. Liu, X. Xing, H. Huang, Y. Jiang, H. He, X. Xu, J. Yuan, L. Zhou, L. Yang, Z. Zhuang, Identification of antigenic proteins associated with trichloroethylene-induced autoimmune disease by serological proteome analysis, Toxicol. Appl. Pharmacol. 240 (2009) 393–400.
[25] W.X. Hong, L. Yang, M. Chen, X. Yang, X. Ren, S. Fang, J. Ye, H. Huang, C. Peng, L. Zhou, X. Huang, F. Yang, D. Wu, Z. Zhuang, J. Liu, Proteomic analysis of

trichloroethylene-induced alterations in expression, distribution, and interactions of SET/TAF-Ialpha and two SET/TAF-Ialpha-binding proteins, eEF1A1 and eEF1A2, in hepatic L-02 cells, Toxicol. Appl. Pharmacol. 263 (2012) 259–272.
[26] X. Yang, Y. Jiang, J. Li, W.X. Hong, D. Wu, X. Huang, H. Huang, L. Zhou, L. Yang, J. Yuan, Z. Zhuang, J. Liu, Lentivirus-mediated silencing of I2PP2A through RNA interference attenuates trichloroethylene-induced cytotoxicity in human hepatic L-02 cells, Toxicol. Lett. 209 (2012) 232–238.
[27] W.X. Hong, W. Liu, Y. Zhang, P. Huang, X. Yang, X. Ren, J. Ye, H. Huang, H. Tang, G. Zhou, X. Huang, Z. Zhuang, J. Liu, Identification of serum biomarkers for occupational medicamentosa-like dermatitis induced by trichloroethylene using mass spectrometry, Toxicol. Appl. Pharmacol. 273 (2013) 121–129.
[28] P.F. Crain, Preparation and enzymatic hydrolysis of DNA and RNA for mass spectrometry, Methods Enzymol. 193 (1990) 782–790.
[29] A. Baccarelli, V. Bollati, Epigenetics and environmental chemicals, Curr. Opin. Pediatr. 21 (2009) 243–251.
[30] B.G. Janssen, L. Godderis, N. Pieters, K. Poels, M. Kici Ski, A. Cuypers, F. Fierens, J. Penders, M. Plusquin, W. Gyselaers, T.S. Nawrot, Placental DNA hypomethylation in association with particulate air pollution in early life, Part. Fibre Toxicol. 10 (2013) 22.
[31] X. Li, A.A. Franke, High-throughput and cost-effective global DNA methylation assay by liquid chromatography–mass spectrometry, Anal. Chim. Acta 703 (2011) 58–63.
[32] A. Wolf-Yadlin, S. Hautaniemi, D.A. Lauffenburger, F.M. White, Multiple reaction monitoring for robust quantitative proteomic analysis of cellular signaling networks, Proc. Natl. Acad. Sci. USA 104 (2007) 5860–5865.
[33] R.E. Watson, J.I. Goodman, Epigenetics and DNA methylation come of age in toxicology, Toxicol. Sci. 67 (2002) 11–16.
[34] V.K. Rakyan, J. Preis, H.D. Morgan, E. Whitelaw, The marks, mechanisms and memory of epigenetic states in mammals, Biochem. J. 356 (2001) 1–10.
[35] G. Jiang, L. Xu, S. Song, C. Zhu, Q. Wu, L. Zhang, L. Wu, Effects of long-term low-dose cadmium exposure on genomic DNA methylation in human embryo lung fibroblast cells, Toxicology 244 (2008) 49–55.