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Identification of the proteins related to SET-mediated hepatic cytotoxicity of trichloroethylene by proteomic analysis

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HIGHLIGHTS

- Identification of 20 abnormal differentially expressed proteins caused by TCE.
- Eight proteins were found to be modulated by SET in TCE-induced cytotoxicity.
- Differential proteins validated may be involved in SET-mediated TCE hepatic cytotoxicity.

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ABSTRACT

Trichloroethylene (TCE) is an effective solvent for a variety of organic materials. Since the wide use of TCE as industrial degreasing of metals, adhesive paint and polyvinyl chloride production, TCE has turned into an environmental and occupational toxicant. Exposure to TCE could cause severe hepatotoxicity; however, the toxic mechanisms of TCE remain poorly understood. Recently, we reported that SET protein mediated TCE-induced cytotoxicity in L-02 cells. Here, we further identified the proteins related to SET-mediated hepatic cytotoxicity of TCE using the techniques of DIGE (differential gel electrophoresis) and MALDI-TOF–MS/MS. Among the 20 differential proteins identified, 8 were found to be modulated by SET in TCE-induced cytotoxicity and three of them (cofilin-1, peroxiredoxin-2 and S100-A11) were validated by Western-blot analysis. The functional analysis revealed that most of the identified SET-modulated proteins are apoptosis-associated proteins. These data indicated that these proteins may be involved in SET-mediated hepatic cytotoxicity of TCE in L-02 cells.

1. Introduction

Trichloroethylene (TCE) is widely used as a solvent in degreasing of metals, a raw material for HFC 134a production, dry cleaning, rocket engines cleaning solvent and even a component of anesthetics. Recently, EPA (U.S. Environmental Protection Agency) released a Final Health Assessment for TCE, which characterized TCE as carcinogenic to humans and as a human non-cancer health hazard. It was reported that 8 of 10 death cases from a total 457 cases of occupational diseases caused by organic solvent exposure from 1993 to 2009 was owing to TCE exposure in Shenzhen, Longgang district, China (Gang and Bing, 2005). A study showed that a toxic effect on the kidney in Chinese workers exposed to TCE could still be observed at relatively low occupational exposure levels (Vermeulen et al., 2012). Another report offered additional support

Abbreviations: TCE, trichloroethylene; FBS, fetal bovine serum; PBS, phosphate buffered saline; CBB, Coomassie Brilliant Blue; IEF, isoelectric focusing; IPG, immobilized pH gradient; PMF, peptide mass fingerprinting; DTT, dithiothreitol; DIGE, difference gel electrophoresis; IAA, iodoacetamide; TFA, trifluoroacetic acid; DMSO, dimethyl sulphoside; IC_{50}, 50% growth inhibition concentration; TEMED, N,N,N,N′-tetramethyl ehytlene diamine; Tris, trashydroxymethyl amino methane; ACN, acetonitrile; Nano-HPLC, nano-performance liquid chromatography; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio -1-propanesulfenate; MALDI-TOF-MS/MS, matrix-assisted laser desorption ionization time of flight.

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for the linkage of non-Hodgkin lymphoma (NHL) with the occupational exposure to TCE (Mandel et al., 2006). In addition, TCE exposure could not only cause hypersensitivity dermatitis (Jia et al., 2012), but also severe liver damage.

TCE is a strong mutagen activated by oxidation by cytochrome P-450, and it appears to induce frame shift in mouse liver and base substitution mutations in yeast after activation (Ensley, 1991). Metabolized in liver, TCE can form as an epoxide intermediate, which has the abilities of covalent binding to microsomal proteins and may be involved in TCE-induced liver toxicity and carcinogenesis (Van Duuren and Banerjee, 1976). Evidence showed that TCE repeatedly administered by oral gavage to mice can lead to hepatocellular proliferation, mitochondrial dysfunction and glycogen depletion (Sano et al., 2009). TCE-treated human liver cells (L-02 cells) displayed altered expression of hepatic metabolic enzyme genes including CYP1A2 and apoptosis-related genes including BAX (Xu et al., 2012).

SET protein, also called template activating factor (TAF1β) or phosphatase 2A inhibitor 2 (I2PP2A), is a specific and potent inhibitor of protein phosphatase 2A (PP2A); On the contrary, SET can stimulate phosphatase 1 activity in the presence of Mn^{2+} (Katayose et al., 2000). Moreover, SET functions like a template activating factor and is also an important component of the histone acetyl-transferase inhibition complex (INHATs), which can inhibit histone acetylation (Kato et al., 2007). Through this histone chaperone ability, SET also can inhibit DNA methylation and affect the transcriptional silencing in HEK cells (Cervoni et al., 2002). In our earlier studies, we reported that TCE up-regulated SET protein expression in L-02 cells (Liu et al., 2007), and that knockdown of SET significantly attenuated TCE-induced apoptosis in L-02 cells (Yang et al., 2012). In addition, we also demonstrated that TCE induced the alterations in the distribution, expression and interact-proteins of SET in L-02 cells (Hong et al., 2012). However, the toxic mechanisms underlying SET-mediated hepatic cytotoxicity of TCE remain poorly understood.

This study is undertaken to identify the proteins related to SET-mediated hepatic cytotoxity using 2D fluorescence difference gel electrophoresis (2-D DIGE) and MALDI-TOF-MS/MS. The concentration of TCE (8 mM) chosen in this study is from our previous research of hepatotoxicity induced by TCE. The findings suggest that under this concentration (half of IC_{50}), SET is up-regulated most significantly. Thus this concentration is considered appropriate for the purpose of this proteomic study.

2. Materials and methods

2.1. Reagents

L-02 (also named HL-7702) cell line was obtained from the Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). All the reagents for DIGE were purchased from GE Healthcare (Pittsburgh, PA, USA). The antibodies against cofilin-1, GAPDH, SET and S100A11 were purchased from Santa Cruz (Santa Cruz, CA, USA). The peroxiredoxin-2 antibody was purchased from Abcam (Cambridge, MA, USA). The secondary antibodies and electrogenerated chemilummescence substrates were purchased from Pierce (Rockford, IL, USA). Ammonium bicarbonate, trifluoroacetic acid, acetonitrile and trichloroethylene were purchased from Sigma–Aldrich (Louis, MO, USA).

2.2. Cell culture and the treatment

L-02 cells and SET siRNA-transfected L-02 cells were cultured in RPMI-1640 medium supplemented with 12% fetal bovine serum (GIBCO), 100 units/mL penicillin and 100 μg/mL streptomycin. 7.2 μl TCE (Sigma) was solved in 50 μl DMSO (final concentration, 8 mM), and then the mixture was added to 10 mL RPMI-1640 media. The control cells were treated with DMSO (50 μl DMSO in 10 mL RPMI-1640 media. The cells were incubated for 24 h in a 37 °C incubator with 5% CO_2.

2.3. Protein extraction, quantification and labeling

After the treatment, cells were collected in 1.5 mL micro tubes (0.25% trypsin-EDTA) and washed three times by PBS, then lysed on ice by lysis buffer (30 mM Tris–HCl, 2 M Thiourea, 7 M Urea, 4% CHAPS) for 15 min. The samples were then sonicated for 4 min and centrifuged at 14,000 × *g* for 20 min. The samples were stored at −80 °C for further analysis. Quantification of the proteins was performed as described in the instruction of 2-D Quant Kit (GE healthcare). The biological replicates were labeled and reversely labeled by Cy3 and Cy5 fluorescence dyes from CyDye DIGE Fluor Labeling Kit (GE healthcare), respectively. A total of 50 μg of all individual samples were pooled as internal standard and labeled by Cy2 Dye. Each sample (internal standard included) was labeled with 400 pmol/L working solution on ice and protected from light for 30 min. The labeling reaction was stopped by adding 1 μl lysine (10 mM) to the protein mixture.

2.4. 2D electrophoresis and imaging

The labeled samples were mixed and focused on 24 cm, 3-11 NL immobilized pH gradient (IPG) strips (GE healthcare) using an IPGphor focusing apparatus (Amersham Biosciences). IEF steps were as follows: 30 V 12 h; 300 V 1 h; 1000 V 1 h; 3000 V 1 h; gradient 8000 V 4 h; 8000 V 5 h. After IEF IPG strips were equilibrated in equilibration buffer (6 M urea, 75 mM Tris–HCl, 30% glycerol, 2% SDS), 1% DTT (Sigma–Aldrich) and 2.5% IAA (Sigma–Aldrich) for 15 min, respectively. The strips were then put on the top of 12.5% Tris–Glycine–SDS gels, and the power was set as 1 W for each gel for 50 min, and 11 W for each gel for about 5 h, respectively. The gels were scanned using a Typhoon TRIO Imager (GE Healthcare) at excitation/emission wavelengths 488/520, 532/580, and 633/670 nm for Cy2, Cy3, and Cy5, respectively. The unlabeled pooled samples (1 mg) were also run under the same condition and stained with Coomassie for protein identification.

2.5. Quantitative analysis

Relative quantitation of the proteins was performed using DeCyder (Version 6.5, GE Healthcare). The internal standard was used to normalize the samples in different groups. A total of 2500 spots were analyzed across samples. Differential in-gel analysis and biological variance analysis were performed sequentially according to the normalized spot volumes/protein abundance. The Student's *t*-test and one-way ANOVA were used to determine the statistical differences in the differentially expressed protein spots.

2.6. In gel digestion

The protein spots were cut by 1 mL tips from Coomassie-stained gels and washed three times by milli-Q water. The spots were then destained by using destaining solution (25 mM ammonium bicarbonate/50% acetonitrile) and incubated at 37 °C for 30 min. The destained gels were washed again by milli-Q water and 50% acetonitrile, respectively. The gels were dehydrated using 100% acetonitrile. Then, 0.01 mg/mL trypsin (in 25 mM ammonium bicarbonate) was added to each gel and absorbed at 4 °C for 15 min. 10–15 μL ammonium bicarbonate (25 mM) was added to each gel and incubated at 37 °C overnight. The samples were centrifuged and the supernatant was isolated for further analysis.

2.7. Protein identification

All mass spectrums were acquired on an AutoFlex Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF/TOF) with LIFT technology (Bruker Daltonics, Bremen, Germany). Peptide samples were prepared on an anchor chip steel target plate, and the matrix (0.4 mg/mL α-cyano-4-hydroxycinnamic acid in 30% acetonitrile/0.06% trifluoroacetic acid) was mixed with each sample in the target plate. Peptide calibration standard (Starter Kit, Bruker Daltonics) was prepared in the plate according to the manufacture. MS data were acquired with a 355 nm nitrogen laser at a 20–50% power rate. MS/MS data were acquired based on the S/N (signal/noise, threshold was set to 15). FlexAnalysis v3.5 was used to produce peak lists and then transferred to BioTools (Bruker Daltonics) for MS/MS MASCOT searches. Search parameters were set as below: Taxonomy: Homo Spain; Enzyme: Trypsin; Missed Cleavage: 1; Fixed Modification: Carbamidomethyl (C); Variable Modification: Oxidation (M); Peptide Tolerance: ±100 ppm; Mass Tolerance: ±0.3 Da; Ions: [M+H]. Only significant hits, as defined by the MASCOT probability analysis ($p < 0.05$), were accepted. Protein scores >35 are considered statistically significant ($p < 0.05$).

2.8. Validation of the differentially expressed proteins by Western-blot analysis

The proteins from L-02 cells and SET siRNA-transfected L-02 cells with or without TCE-treatment were extracted. After electrophoresis, the proteins were then transferred into PVDF membranes. The anti-SET, anti-cofilin-1, anti-PRDX-2, anti-GAPDH and anti-S100A11 antibodies were diluted 1:1000 in TBST buffer (50 mM Tris, 150 mM NaCl, 0.03% Tween-20) and incubated at room temperature for 90 min. The secondary goat anti-mouse and goat anti-rabbit antibodies were diluted 1:2000 in TBST and incubated for 60 min at room temperature. The protein bands were developed with horseradish peroxidase-conjugated secondary antibodies and visualized using ECL substrate and an ImageQuant RT ECL System (GE Healthcare). The relative quantification of the proteins was performed using ImageQuant TL-1D Analysis Tool.