Chronic Trichloroethylene (TCE) exposure could induce hepatocellular carcinoma in mice, and occupational exposure in humans was suggested to be associated with liver cancer. To understand the role of non-genotoxic mechanism(s) for TCE action, we examined the gene expression and DNA methylation changes in the liver of B6C3F1 mice orally administered with TCE for 5 days. As a beginning step, we profiled gene expression alterations induced by the TCE in mouse livers. Here we describe in detail the experimental methods, quality controls, and other information associated with our data deposited into Gene Expression Omnibus (GEO) under GSE58819. Our data provide useful information for gene expression responses to TCE in mouse liver. © 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**Specifications**

| Organism          | B6C3F1 mice |
|-------------------|-------------|
| Sex               | Male        |
| Array type        | Agilent Mouse Gene Expression 8 × 60K Microarray |
| Data format (in GEO) | Raw data: TXT files; normalized data: SOFT, MINIML and TXT |
| Experimental factors | TCE exposed vs. un-exposed control |
| Experimental features | Male B6C3F1 mice were exposed to control (0 mg/kg b.w.) and Trichloroethylene (1000 mg/kg b.w.) for 5 days. Mice were sacrificed 100 min after the last treatment. Gene expression changes were analyzed using Agilent microarrays. |
| Consent           | All procedures involving animals were reviewed and approved by the Institutional Animal Care/Use Ethical Committee of Soochow University, Suzhou City, China. |
| Sample source location | Model Animal Research Center of Nanjing University (Nanjing, China). |

**Direct link to deposited data**

Deposited data can be found here: [http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58819](http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58819).

**Experimental design, materials and methods**

**Animal treatment**

Adult B6C3F1 male mice, 6–7 weeks of age, were kept in animal facility maintaining 22 ± 1 °C temperature, 50 ± 5% relative humidity and 12-hour light–dark cycles. Commercial diet (Suzhou Shuangshi Laboratory Animal Feed Science Co. Ltd., Suzhou, China) and water were provided ad libitum. All experiments were conducted ‘blind’ so that the personnel involved in randomization and TCE exposures were different from those evaluating the biological end-points. After 7 days of quarantine period, a randomized block design was used to divide the animals into 2 groups, each with 5 mice. Group 1: Oral administration of TCE (dissolved in corn oil, 1000 mg/kg b.w per day) for 5 days. Group 2: Control mice similarly administered with corn oil. Approximately 100 min after the last treatment, all mice were anesthetized adequately (chloral hydrate, 350 mg/kg body weight, intra-peritoneal injection) and then sacrificed by cervical dislocation [1]. Form each mouse, liver was excised rapidly, frozen in liquid nitrogen, and stored at −80 °C.

**RNA purification**

About 20 mg mouse liver tissue was disrupted in buffer RLT and homogenized. RNA was then purified using TIANprep DNA–RNA–Protein Isolation kit (Tiangen, Beijing, China). The concentration and the quality of RNA were assessed by ultraviolet (UV) absorbance using a NanoDrop.

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### Table 1
Quality of RNA samples.

| Sample ID | Concentration (μg/μl) | A260/A280 | Volume (μl) | Amount (μg) | 28S/18S | RIN |
|-----------|------------------------|------------|-------------|-------------|---------|-----|
| C2        | 0.7293                 | 2.10       | 95          | 69          | 1.6     | 9.3 |
| C3        | 2.0641                 | 2.10       | 95          | 196         | 1.8     | 9.0 |
| C4        | 1.3248                 | 2.10       | 95          | 126         | 1.2     | 8.1 |
| T2        | 0.4791                 | 2.10       | 95          | 46          | 1.6     | 9.3 |
| T3        | 1.8198                 | 2.10       | 95          | 173         | 1.7     | 8.6 |
| T4        | 0.5115                 | 2.10       | 95          | 87          | 1.3     | 8.4 |

**Fig. 1.** Quality control assay of RNA samples. (A) Bioanalyzer output as gel images for all six samples. (B) Bioanalyzer output as traces with RIN (RNA integrity number) shown for each sample. FU, fluorescence units; nt, nucleotide.
ND-2000 spectrophotometer (Thermo Scientific, USA) (Table 1). The integrity of RNA was determined using Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) as shown in Fig. 1. Before cDNA transcription, Turbo DNA-free™ kit (Thermo Scientific) was used to remove genomic DNA contamination in purified RNA samples.

Microarrays

The Agilent Mouse Gene Expression (8 × 60 K, Design ID:028005) was used in this study. The sample labeling, microarray hybridization and washing were performed based on the manufacturer’s standard protocols. Briefly, total RNA were transcribed to double strand cDNA, then synthesized into cRNA and labeled with Cyanine-3-CTP. The labeled cRNAs were hybridized onto the microarray. After washing, the arrays were scanned by the Agilent Scanner G2505C (Agilent Technologies).

Data analysis

Feature Extraction software (version 10.7.1.1, Agilent Technologies) was used to analyze array images to get raw data. Genespring was employed for conducting the basic analysis of the raw data. We normalized the raw data with the quantile algorithm, and performed log transformation with Robust Multi-array Average (RMA) approach (Fig. 2). The probes that at least 100% of the values in any 1 out of all conditions have flags in “Detected” were chosen for further data analysis. Differentially expressed genes were then identified through fold change as well as P value calculated with t-test. A volcano plot shows a great number of significantly different genes with an adjusted p-value ≤ 0.05 and a log2 fold change ≥ 1. The threshold set for up- and down-regulated genes was a fold change ≥ 2.0 and a P value ≤ 0.05 (Fig. 3). Afterwards, GO analysis and KEGG analysis were applied to determine the roles of these differentially expressed mRNAs. Finally, Hierarchical Clustering was performed to display the distinguishable genes’ expression pattern among samples.

Discussion

Results showed that the expression levels of 431 mRNAs were changed after TCE exposure, of which 291 were up-regulated and 140 were down-regulated. The expression changed genes were involved in key signal pathways including PPAR, proliferation, apoptosis and homologous recombination. Notably, the expression level of a number of vital genes involved in the regulation of DNA methylation, such as Utrf1, Tet2, DNMT3a and DNMT3b, were dysregulated, which were further validated by qPCR [2].

Acknowledgment

This work was supported by the start-up funding of Soochow University, National Nature Sciences Foundation of China (Grant number: 81300143) and The Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

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