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Data in Brief

Molecular effect of ethanol during neural differentiation of human embryonic stem cells *in vitro*

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ABSTRACT

Potential teratogenic effects of alcohol on fetal development have been documented. Especially studies have demonstrated deleterious effect of ethanol exposure on neuronal development in animal models and on the maintenance and differentiation of neuronal precursor cells derived from stem cells. To better understand the molecular effect of alcohol on the process of neural differentiation, we have performed gene expression microarray analysis on human embryonic stem cells being directed to neural rosettes and neural precursor cells in the presence of ethanol treatment. Here we provide detailed experimental methods, analysis and information associated with our data deposited into Gene Expression Omnibus (GEO) under GSE56906. Our data provide scientific insight on potential molecular effects of fetal alcohol exposure on neural differentiation of early embryo development.

Specifications	
Organism/cell line/tissue	Neural stem cells derived from human embryonic stem cells *in vitro*
Sex	*N/A*
Sequencer or array type	Affymetrix Human Genome Plus 2.0
Data format	Raw and analyzed
Experimental factors	Treatment of hESCs with ethanol during neural differentiation
Experimental features	Alcohol exposure experiment to profile molecular effects of ethanol on neural differentiation of human embryonic stem cells
Consent	*N/A*
Sample source location	*N/A*

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56906

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Experimental design, materials and methods

Human embryonic stem cell culture and derivation of neural stem cells

Human embryonic stem cells (H1 and H9 lines) were obtained from UCLA Broad Stem Cell Research Center through license agreement with WiCell Research Institute (Madison, WI). Cells cultured on a mouse embryonic fibroblast feeder layer were transferred to mTeSR1 serum free human embryonic stem cell (hESC) culture system (STEMCELL Technologies Inc., Vancouver, Canada). Cultured cells were subjected to neural differentiation by using STEMdiff Neural System (STEMCELL Technologies Inc., Vancouver, Canada) according to the manufacturer's instruction. Briefly exponentially growing cells were washed once with PBS and dissociated by treating with 1× Accutase (STEMCELL Technologies Inc., Vancouver, Canada) for 5 min at 37 °C. Cells were collected into a 50 ml Falcon tube and spun for 5 min at 300 ×*g*. Cell pellets were washed twice with Dulbecco's Modified Eagle Medium/Ham's F-12 (DMEM/F-12) and finally resuspended in neural induction medium (NIM) containing 10 μM Y-27632 (Chemdea, Ridgewood, NJ). Cell suspension was subjected to embryoid body formation by using an AggreWell 800 plate (STEMCELL Technologies Inc., Vancouver, Canada). Each well was rinsed with 1 ml of DMEM/F-12 and aspirated

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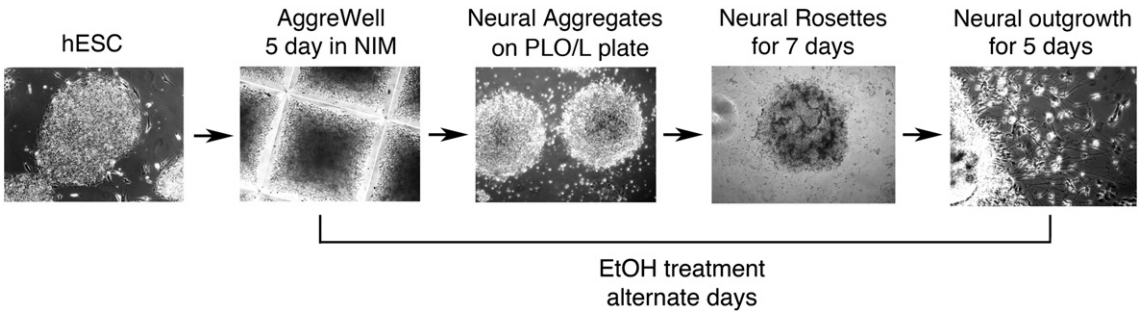

Fig. 1. Neural differentiation of human embryonic stem cells *in vitro*. Human embryonic stem cells were subjected to embryoid body formation using AggreWell for 5 days in neural induction medium. Neural aggregates were seeded on poly-L-ornithine/laminin coated plates and cultured with NIM for 7 days to develop neural rosette structure. Ethanol treatment was initiated a day after plating the neural aggregates onto PLO/L plates. For ethanol treatment cells were fed with fresh medium every day by alternating a treatment with 20 mM ethanol for 1 day and a withdrawal for 1 day. Treatment was continued till the end of neural expansion. After 7 days, the neural rosettes were dislodged and then re-plated for the expansion of neural precursor cells for 5 days.

to remove. STEMdiff NIM supplemented with 10 μM Y-27632 (0.5 ml per well) was added to each well. The plate was briefly centrifuged at 2000 ×*g* for 5 min to remove any air bubbles from the microwells and observed under a microscope to make sure that bubbles have been removed. Cells in single suspension ($2–3 \times 10^6$ cells) will be added per well and the plate was centrifuged at 100 ×*g* for 3 min to capture cells in the microwells. The plate was examined under a microscope to confirm that cells were evenly distributed among the microwells. On the next day, cells were fed with fresh NIM without Y-27632. Ethanol exposure was done by forming embryoid bodies with complete NIM containing predetermined concentration of ethanol. Neural aggregate formation was done for 5 days (without or with 20 mM ethanol) at 37 °C and 5% CO_2 with a partial medium (3/4 of culture medium) change every day.

For culture of neural aggregates 6-well culture plates were coated with poly-L-ornithine (15 μg/ml in PBS, Sigma Catalog #P4957) for 2 h at room temperature and washed twice with PBS and once with DMEM/F-12. The plates were then coated with laminin (10 μg/ml in ice-cold DMEM/F-12, Sigma Catalog #L2020) overnight at 4 °C. The laminin solution was aspirated and the neural aggregates harvested were transferred into the well coated with PLO/L. The cells were cultured at 37 °C with 5% CO_2 and 95% humidity with a full medium change daily for 7 days with STEMdiff NIM (without or with 20 mM ethanol). Morphological assessment and scoring of neural rosettes were done to ensure that 50% or more of the area of each aggregate was filled with neural rosettes (as shown in Fig. 1).

On day 7 of attached neural aggregate culture, neural rosettes were selected away from contaminating flat cells. The medium was removed from each well and washed with 1 ml of DMEM/F12 per well. STEMdiff Neural Rosette Selection Reagent (1 ml) was added per well and incubated for 1 h at 37 °C. The STEMdiff Neural Rosette Selection Reagent was removed by using a micropipette outfitted with a disposable 1 ml tip. The attached aggregates were detached from the plates by expelling pre-warmed DMEM/F12 onto the rosette clusters using a micropipette outfitted with a disposable 1 mL tip. Detached neural rosettes were collected and centrifuged for 5 min at 350 ×*g*. The rosettes were resuspended in pre-warmed NIM and briefly pipetted up and down and plated onto 6-well plates precoated with PLO/L. Cells were cultured at 37 °C with 5% CO_2 and 95% humidity with daily full medium changes using pre-warmed STEMdiff NIM (without or with 20 mM ethanol) for 5 days. To ensure

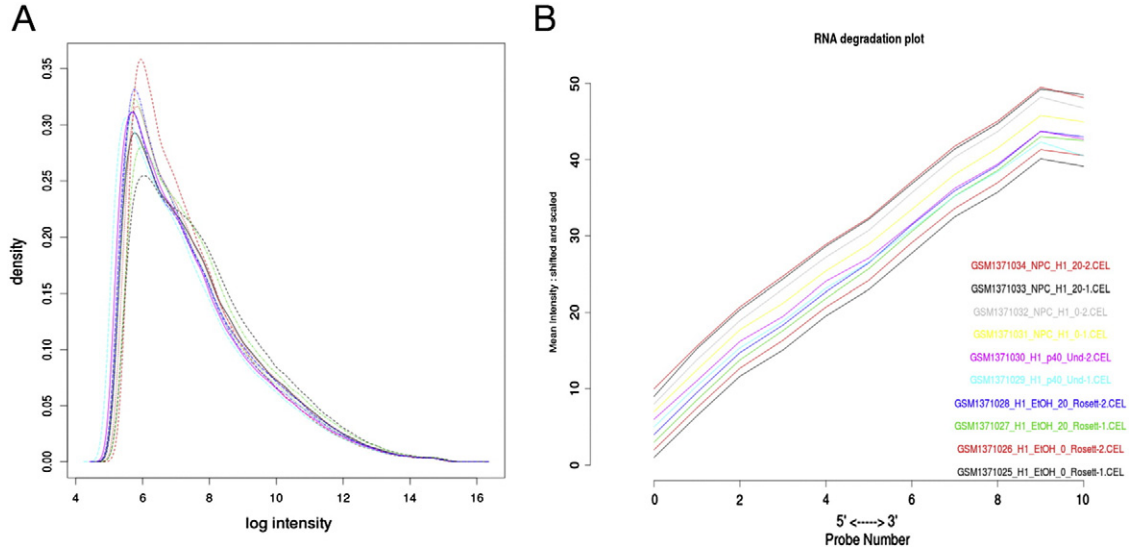

Fig. 2. (A) Log density estimates (histograms) of the data across arrays. (B) Degradation plot: Each curve corresponds to a single chip and visualizes the chip-averaged dependency between probe intensity and probe position. We performed background correction (Fig. 3), quantile normalization and log transformation with Robust Multi-array Average (RMA) approach on Affymetrix gene expression data using "Affy" R package (Fig. 4) [1].

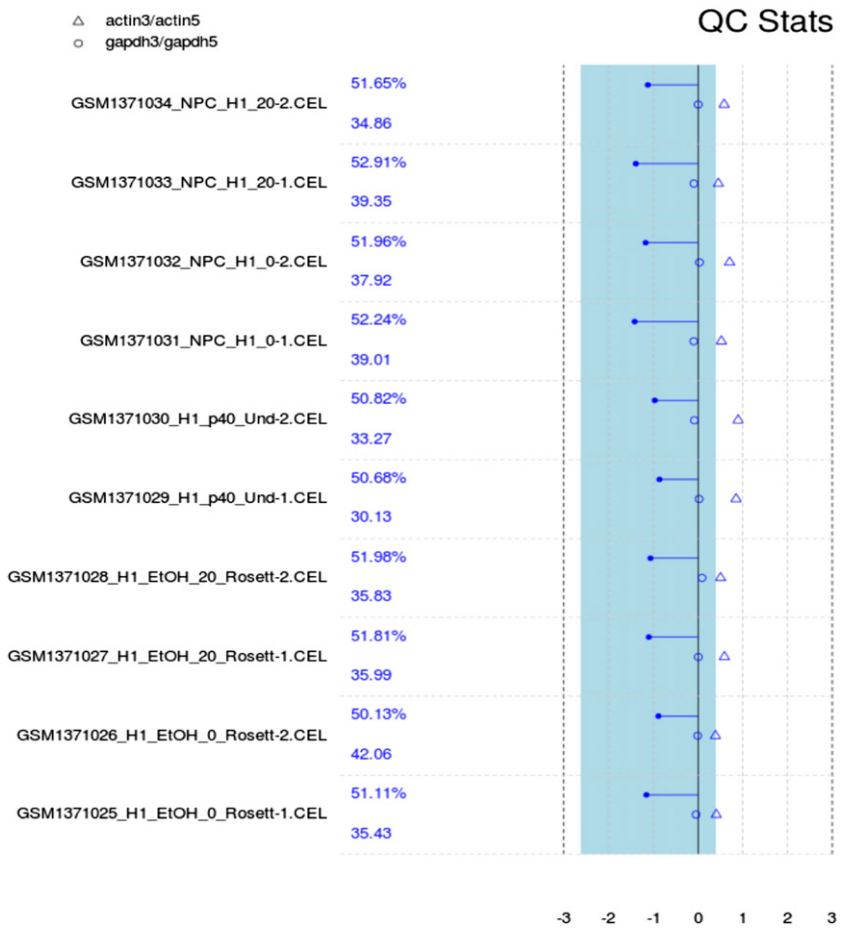

Fig. 3. Quality control statistics. Each array is presented by a separated line. The blue bar represents the region where all scale factors fall within 3 fold of the mean scale factor for all chips. The chips passed all the QC metrics, indicating good quality data. We removed probes with expression lower than the overall sample median; 22,337 out of 54,675 probes were kept for further analysis (Fig. 4).

proper neural differentiation of hESCs, the same experimental procedure was applied to a set of cells plated on the coverslips. The level of neural markers (Nestin, Sox2, Musashi and βIII tubulin) was assessed by immunofluorescence microscopy and quantitative RT-PCR analysis.

RNA isolation and microarray analysis

Samples for gene expression microarray analysis were collected at D10 (5 days after seeding the neural aggregates for the formation of rosettes) and D15 (5 days after replating the rosette clusters for the expansion of neural precursor cells). Cells were briefly washed with PBS and subjected to the isolation of total RNA by using RNeasy mini kit (Qiagen, Valencia, CA). Total RNA was extracted using RNeasy purification kit, following the manufacturer's instruction (Qiagen, Valencia, CA). Isolated RNA was further purified by DNase treatment (Ambion/Life Technologies, Grand Island, NY). RNA purity and concentration was determined by NanoDrop, an ND-1000 spectrophotometer (Thermo Scientific, Indianapolis, IN) and a microfluidics-based platform 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA concentration ranged from 206.9 ng/μl to 366.5 ng/μl. RNA concentration ≥ 50 ng/μl is recommended. 260/280 ratio ranged from 2.03 to 2.1. Ideal 260/280 ratio for pure RNA is 2.0.

Biological duplicate samples were hybridized to Affymetrix Human Genome Plus 2.0 (Cat. # 900469). We set target intensity (TGT) at 500. The sensitivity of the system was measured by %P using the 3′ biased Affymetrix HG-U133A 2.0 arrays. %P ranged from 45.7 to 48.4% demonstrating the ability to detect a large number of transcripts across a wide range of abundance. All 10 arrays were assessed for recommended standard quality control metrics by Affymetrix including image quality, signal distribution and pair wise scatter plots and passed. mas5.CHP files were generated for each array by MAS 5.0 (Affymetrix, Santa Clara, CA) and combined to a final RESULTS.MAS5.TXT file.

Data analysis

Raw data was initially analyzed for the quality of microarray analysis by log density estimates of the data across all arrays (Fig. 2A). A degradation plot was prepared with each curve corresponding to a single chip and visualizing the chip-averaged dependency between probe intensity and probe position (Fig. 2B).

Acknowledgment

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Reference

[1] L. Gautier, L. Cope, B.M. Bolstad, R.A. Irizarry, affy–analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20 (2004) 307–315.

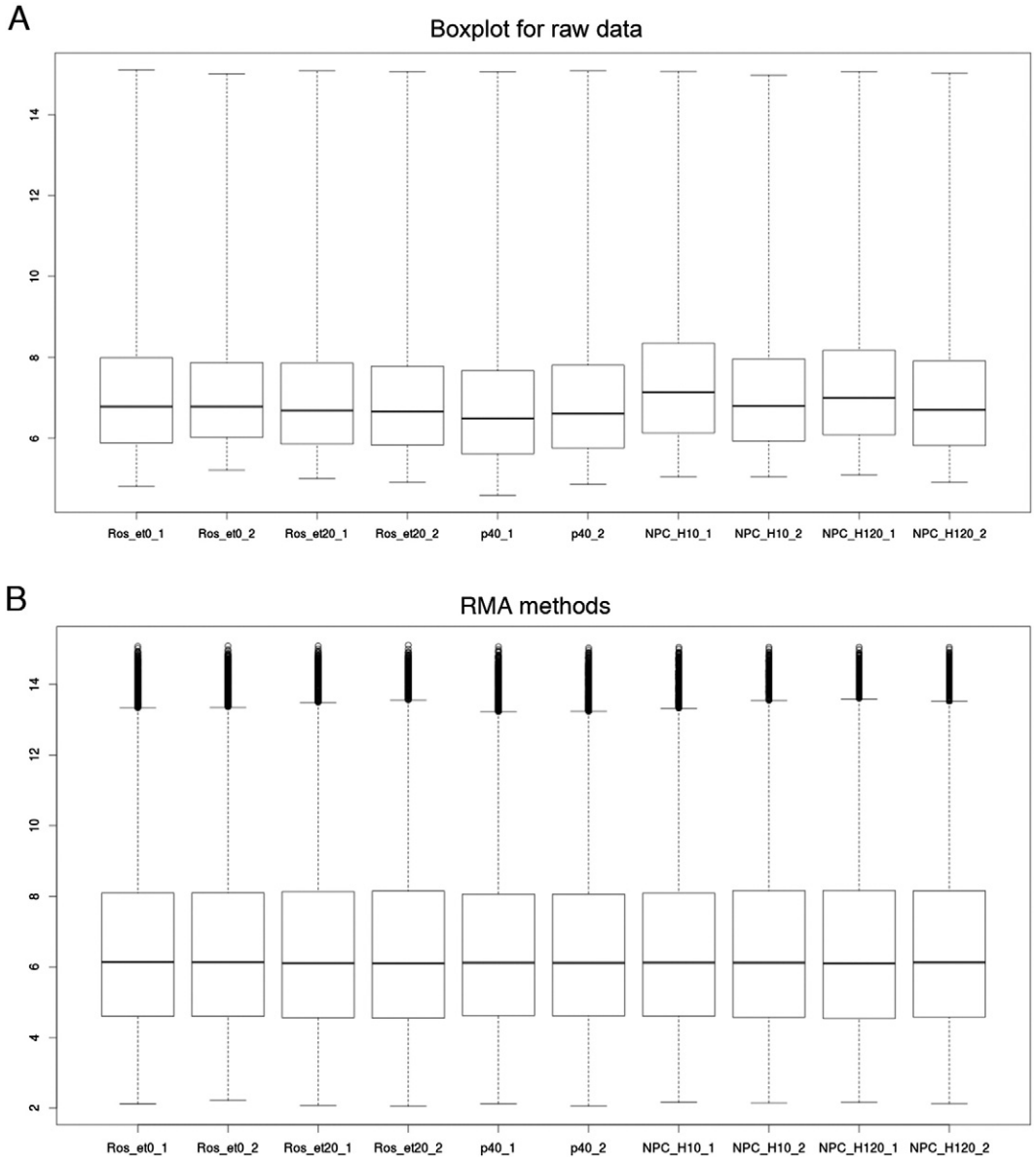

Fig. 4. Boxplot of intensity for each sample (A) after normalization and (B) log transformation using Robust Multi-array Average (RMA) method. Principal Component Analysis (PCA) was performed to detect expression data separation by EtOH treatment and also neural differentiation into rosette and NPC (Fig. 5). Data shows clear separation between undifferentiated H1 (p40), rosette and NPC before (Fig. 5A) and after (Fig. 5B) filtering probes. This demonstrates that molecular changes during neural differentiation into rosette structure and NPC are differential and distinctive.

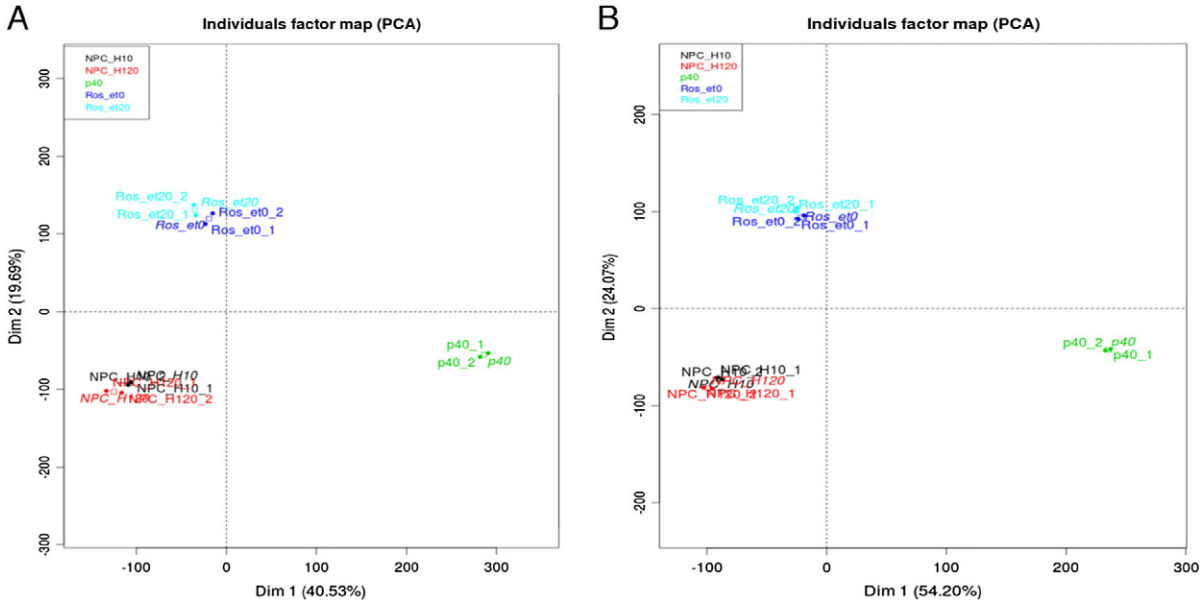

Fig. 5. Principal Component Analysis (PCA). PCA was performed on dataset (A) before filtering 54,675 probes and (B) after filtering 22,337 probes.