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Data Article

Transcriptomic changes in mouse embryonic stem cells exposed to thalidomide during spontaneous differentiation

Xiugong Gao*, Robert L. Sprando, Jeffrey J. Yourick

Division of Toxicology, Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA

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ABSTRACT

Thalidomide is a potent developmental toxicant that induces a range of birth defects, notably severe limb malformations. To unravel the molecular mechanisms underpinning the teratogenic effects of thalidomide, we used microarrays to study transcriptomic changes induced by thalidomide in an in vitro model based on the differentiation of mouse embryonic stem cells (mESCs), and published the major findings in a research article entitled "Thalidomide induced early gene expression perturbations indicative of human embryopathy in mouse embryonic stem cells" [1]. The data presented herein contains complementary information related to the aforementioned research article.

Specifications table

Subject area	Biology
More specific subject area	Toxicogenomics
Type of data	1) Processed microarray data in .CEL format 2) Excel spreadsheet files listing identified genes, GO terms and functional clusters

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* Correspondence to: 8301 Muirkirk Road, Laurel, MD 20708, USA. Tel.: +1 301 796 0580.
E-mail address: xiugong.gao@fda.hhs.gov (X. Gao).

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How data was acquired	Microarray data generated on Affymetrix Mouse Gene 2.0 ST Array
Data format	Processed or analyzed
Experimental factors	Induction of spontaneous differentiation was achieved through embryoid body (EB) formation in hanging drop culture following a procedure adapted from De Smedt et al.[2]
Experimental features	Cells were collected at 24, 48, and 72 h after exposure to 0.25 mM thalidomide. Total RNA (50 ng) were preprocessed using the Affymetrix GeneChip WT PLUS Reagent Kit and hybridized onto the Affymetrix Mouse Gene 2.0 ST Array
Data source location	Laurel, MD, USA
Data accessibility	The analyzed data is with this article. Processed microarray data (.CEL files) can be accessed at Gene Expression Omnibus with accession number GSE61306 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61306)

Value of the data

- The data represent the first toxicogenomic study on thalidomide using mouse embryonic stem cells [1].
- The gene expression data provide insights into mechanisms of thalidomide embryotoxicity [1].
- The functions and pathways associated with thalidomide-impacted genes conform to known thalidomide clinical outcomes [1].
- The data suggest that transcriptomics coupled with mouse embryonic stem cells is a valuable model for developmental toxicity testing [1].

1. Experimental design, materials and methods

1.1. Materials

(±)-Thalidomide ((RS)-2-(2,6-dioxopiperidin-3-yl)-1H-isoindole-1,3(2H)-dione) and all other chemicals used in this study were of molecular biology grade and were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

1.2. Pluripotent mouse embryonic stem cell culture

Pluripotent ESGRO Complete Adapted C57BL/6 mESCs, which have been pre-adapted to serum-free and feeder-free culture condition, were obtained from EMD Millipore (Billerica, MA) at passage 12 (with 80% normal male mouse karyotype). The cells were seeded in cell culture flasks (Nunc, Roskilde, Denmark) coated with 0.1% gelatin solution (EMD Millipore), and maintained at 37 °C in a 5% CO_2 humidified incubator at standard densities (i.e., between $5 \times 10^4/cm^2$ and $5 \times 10^5/cm^2$) in ESGRO Complete Plus Clonal Grade Medium (EMD Millipore). The medium contains leukemia inhibitory factor (LIF), bone morphogenic protein 4 (BMP-4), and a glycogen synthase kinase-3b inhibitor (GSK3b-I) to help maintain pluripotency and self-renewal of the ESCs. Cells were passaged every 2–3 days (when reaching 60% confluence) with ESGRO Complete Accutase (EMD Millipore) at about 1:6 ratio. C57BL/6 mESCs maintain a stable karyotype under the above passaging condition. The cells used in the current study were at passage 18.

1.3. Mouse embryonic stem cell differentiation through embryoid body formation

Induction of differentiation was achieved through embryoid body (EB) formation via hanging drop culture following a procedure adapted from De Smedt et al. [2]. In brief, stem cells were thawed and a suspension was prepared at a concentration of 3.75×10^4 cells/ml in ESGRO Complete Basal Medium (EMD Millipore), which does not contain LIP, BMP-4, or GSK3b-I. About 50 drops (each of 20 μl) of the cell suspension were placed onto the inner side of the lid of a 10-cm Petri dish filled with 5 ml phosphate buffered saline (PBS; EMD Millipore) and incubated at 37 °C and 5% CO_2 in a humidified atmosphere. After 3 days, EBs formed in the hanging drops (Ø330–350 μm) were subsequently