



Data in Brief

Genome-wide transcriptional profiling of human glioblastoma cells in response to ITE treatment



Bo Kang*, Yanwen Zhou, Min Zheng, Ying-Jie Wang

State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, China

ARTICLE INFO

Article history:

Received 11 June 2015

Accepted 16 June 2015

Available online 30 June 2015

Keywords:

Aryl hydrocarbon receptor

ITE

Glioblastoma cells

Neural stem cell

ABSTRACT

A ligand-activated transcription factor aryl hydrocarbon receptor (AhR) is recently revealed to play a key role in embryogenesis and tumorigenesis (Feng et al. [1], Safe et al. [2]) and 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) (Song et al. [3]) is an endogenous AhR ligand that possesses anti-tumor activity. In order to gain insights into how ITE acts via the AhR in embryogenesis and tumorigenesis, we analyzed the genome-wide transcriptional profiles of the following three groups of cells: the human glioblastoma U87 parental cells, U87 tumor sphere cells treated with vehicle (DMSO) and U87 tumor sphere cells treated with ITE. Here, we provide the details of the sample gathering strategy and show the quality controls and the analyses associated with our gene array data deposited into the Gene Expression Omnibus (GEO) under the accession code of GSE67986.

© 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/cell line/tissue	Human glioblastoma U87 MG
Sex	Male
Sequencer or array type	Affymetrix Human Gene Expression Array GPL15207
Data format	Raw data (CEL files)
Experimental factors	parental U87 cells, U87 tumor sphere cells treated with vehicle (DMSO) and U87 tumor sphere cells treated with ITE
Experimental features	Isolation and gene expression profiling of U87 cells grown in neural stem cell medium supplemented with ITE
Consent	N/A
Sample source location	Hangzhou, China

1. Direct link to deposited data

Deposited data can be found at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67986>

* Corresponding author.

E-mail address: kangbo@zju.edu.cn (B. Kang).

2. Experimental design, materials and methods

2.1. Experimental design

To determine the global transcriptional effects of ITE (Song et al. [3]) on parental and tumor sphere U87 cells, mRNA samples from three experimental groups (i.e., parental U87 cells, U87 tumor sphere cells treated with vehicle (DMSO) and U87 tumor sphere cells treated with ITE) were analyzed by Affymetrix Human Gene Expression Array.

2.2. Sample culture and isolation procedure

For parental U87 sample, cells were cultured in DMEM (Invitrogen 21063-029, containing 16 mg/L L-tryptophan) supplemented with 10% fetal bovine serum (GIBCO 10099 or Pufe 1101-500). For tumor sphere assay (Ledur et al. [4]), U87 cells were plated at a density of 30,000 cells/ml with neural stem cell (NSC) medium which contains DMEM/F-12 (Corning R10-092-CV) supplemented with 1% (v/v) PS, 2% (v/v) B-27® Supplement Minus Vitamin A, 20 ng/mL EGF, 20 ng/mL bFGF and 10 ng/mL LIF to allow tumor sphere formation. U87 tumor sphere cells were cultured in NSC medium and treated with either 0.01% DMSO (vehicle) or 10 μM ITE for 5 days with the ITE being replenished every 12 h. After treatment, U87 tumor spheres were harvested and subjected to microarray analysis.

2.3. RNA isolation and microarray

Briefly, RNA was extracted from U87 parental and tumor sphere cells by TRIzol method according to the manufacturer's instructions. Reverse transcription to the first-strand cDNA was primed with T7 oligo (dT) primer to synthesize cDNA containing a T7 promoter sequence. Second-strand cDNA synthesis converts the single-stranded cDNA into

a double-stranded DNA (dsDNA) template for transcription. The reaction employs DNA polymerase and RNase H to simultaneously degrade the RNA and synthesize the second-strand cDNA. In vitro transcription to synthesize biotin-modified aRNA with IVT Labeling Master Mix generates multiple copies of biotin-modified aRNA from the double-stranded cDNA templates. aRNA purification removes unincorporated NTPs, salts, enzymes, and inorganic phosphate to improve the stability

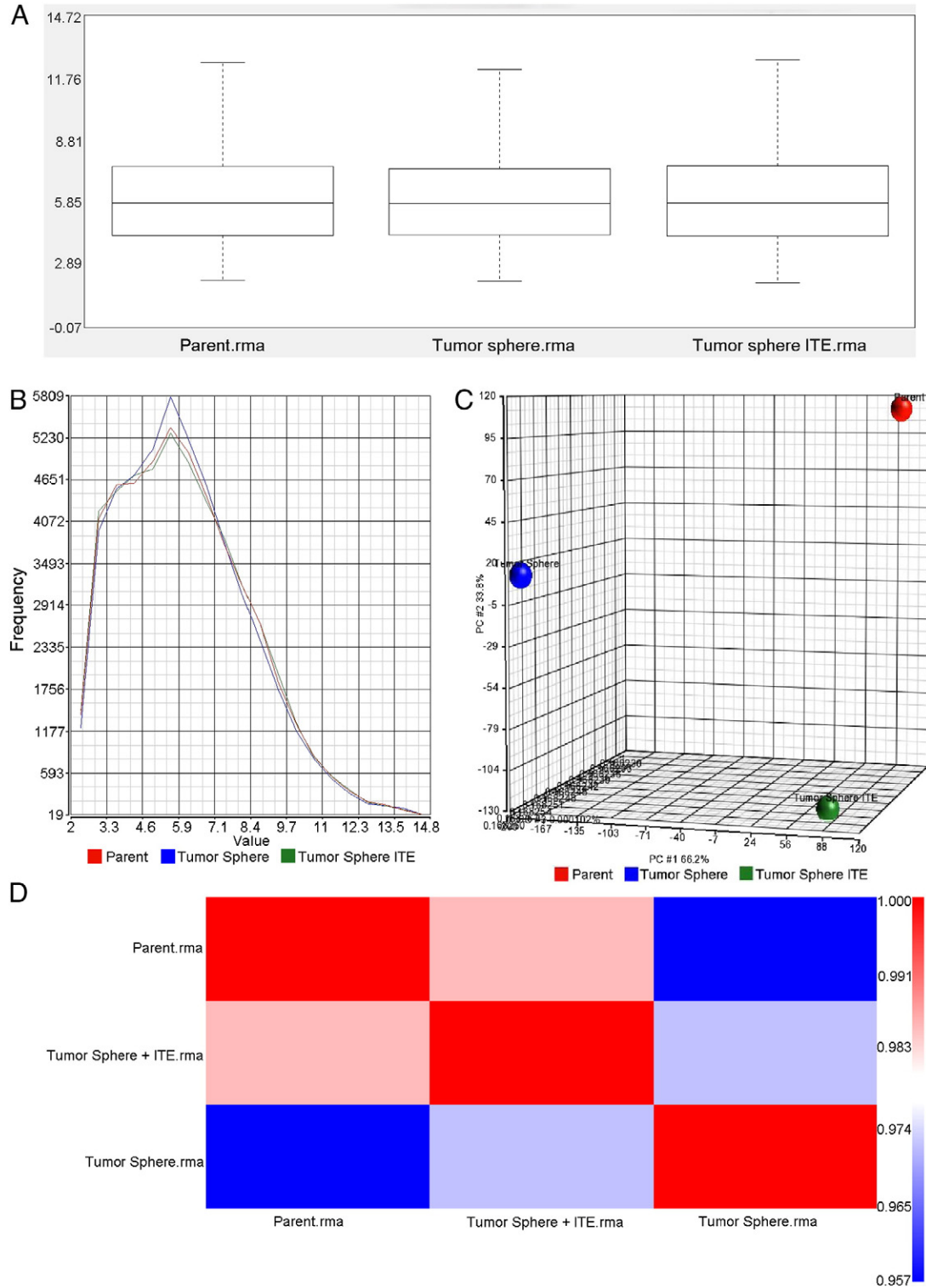


Fig. 1. Quality controls and analysis of microarrays. A) boxplots of log2 transformed probe intensity after RMA processing. B) Histograms of log2 transformed probe intensity after RMA processing. C) Principal component analysis. The percentage of variability explained by each component is indicated in brackets. D) Hierarchical clustering by Pearson correlation distance and Ward's aggregation showed the clustering of the replicates and the close genetic relationship between three subsets.

of the biotin-modified aRNA. Fragmentation of the labeled aRNA prepares the target for hybridization to GeneChip® 3' expression arrays. Above procedures were carried out by GeneChip IVT express Kits (Affymetrix: 901229). The hybridization was carried out by GeneChip Hybridization, Wash, and Stain kit (affymetrix: 900720). The arrays were scanned at 570 nm with a confocal scanner from Affymetrix.

2.4. Quality control and data analysis

Analysis of the arrays was performed using the Partek GS 6.5. Normalization of the array was performed using a robust multiarray analysis (RMA). A p-value cut off of 0.05 was used to filter genes that were significantly expressed between the two samples. A fold change of 2 was used as a criterion for primary selection for differentially expressed genes. Boxplot and histograms of RMA processed probe intensities across all arrays shown in (Fig. 1A and B) indicated similar distribution of signal intensities between arrays. Principal component analysis (PCA, Fig. 1C) and hierarchical clustering (HC) by Pearson correlation distance and Ward's aggregation (Fig. 1D) were performed after selection of probes with a differential expression in any combination of array > 1.5.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 31271465 and No. 81472722 to Y.-J.W., grant No. 81071767 to M.Z.) and an intramural grant from the First Affiliated Hospital, School of Medicine, Zhejiang University (Grant No. b2697 to Y.-J.W.).

References

- [1] S. Feng, Z. Cao, X. Wang, Role of aryl hydrocarbon receptor in cancer. *Biochim. Biophys. Acta* 1836 (2013) 197–210.
- [2] S. Safe, S.O. Lee, U.H. Jin, Role of the aryl hydrocarbon receptor in carcinogenesis and potential as a drug target. *Toxicol. Sci.* 135 (2013) 1–16.
- [3] J. Song, M. Clagett-Dame, R.E. Peterson, M.E. Hahn, W.M. Westler, R.R. Sicsinski, H.F. DeLuca, A ligand for the aryl hydrocarbon receptor isolated from lung. *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 14694–14699.
- [4] P.F. Ledur, E.S. Villodre, R. Paulus, L.A. Cruz, D.G. Flores, G. Lenz, Extracellular ATP reduces tumor sphere growth and cancer stem cell population in glioblastoma cells. *Purinergic Signal* 8 (2012) 39–48.