



Data in Brief

Gene expression profiling of Ctr9-regulated transcriptome in ER α -positive breast cancer

Hao Zeng, Wei Xu *

Graduate Program in Cellular and Molecular Biology, McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, WI 53706, USA

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ABSTRACT

Ctr9, the key scaffold subunit in human RNA polymerase II associated factor complex (PAF_c), has diverse functions in cells and has been implicated in human diseases. Recently, our study found that loss of Ctr9 led to apparent morphological change, decrease of proliferation, and reduced colony formation in ER α ⁺ breast cancer cells. Moreover, Ctr9 and ER α show positive correlation at protein levels and the high levels of Ctr9 are associated with poor survival among all women with ER α ⁺ breast cancers, and specifically among those treated with tamoxifen. To gain a molecular understanding of the role of Ctr9 in promoting ER α ⁺ breast cancer, we performed a microarray gene expression profiling of Ctr9-regulated transcriptome. Here we provide the experimental details and analysis of the microarray data, which have been deposited into Gene Expression Omnibus (GEO): GSE73388.

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Specifications

Organism/cell line/tissue	Homo sapiens/human breast cancer cell line/MCF7
Sex	Female
Sequencer or array type	Affymetrix human transcriptome array 2.0
Data format	Raw data: CEL files Processed data: CHP files
Experimental factors	Ctr9 knockdown vs. control in the presence or absence of 17 β -estradiol (E2) treatment in MCF7 breast cancer cells
Experimental features	Microarray gene expression profiling to identify transcripts that are regulated by E2 and Ctr9
Consent	Data are publicly available
Sample source location	Madison, Wisconsin, USA

1. Direct link to deposited data

The deposited data can be found at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73388>.

2. Experimental design, materials and methods

2.1. Cell culture and reagents

MCF7-tet-on-shCtr9 cells were generated and characterized previously [1,2] and maintained in DMEM supplemented with 10% FBS. Cells were cultured at 37 °C in a humidified atmosphere containing 5%

CO₂. Doxycycline (Dox) was purchased from Clontech (Mountain View, CA) and used at a final concentration of 500 ng/ml to induce Ctr9-targeting shRNA expression. Dimethyl sulfoxide (DMSO) and 17 β -estradiol (E2) were purchased from Sigma (St. Louis, MO).

2.2. Ctr9 knockdown and E2 induction

In order to globally identify E2- and Ctr9-regulated transcriptome in ER α ⁺ breast cancer cells, MCF7-tet-on-shCtr9 cells were maintained in DMEM supplemented with 10% FBS in the absence or presence of 500 ng/mL Dox for 4 days, followed by continuing culture in stripped medium with or without 500 ng/ml Dox for another 3 days. Cells were subsequently treated with DMSO or 10 nM E2 for 4 h prior to cell collection (Fig. 1).

2.3. Total RNA extraction and microarray hybridization

To ensure the biological confidence, three independent experiments were performed as illustrated in Fig. 1. Total RNA from twelve samples was extracted using a Qiagen RNeasy Plus Kit according to manufacturer's protocol. The RNA quantity was determined by NanoDrop spectrophotometer. Purified total RNA was then submitted to the University of Wisconsin Madison Biotechnology Center. RNA quality was assessed on the Agilent 2100 BioAnalyzer using a RNA 6000 Nano Chip. All twelve RNA samples look intact, with both ribosomal peaks present (Fig. 2). 400 ng of total RNA for each sample was used to synthesize double-stranded cDNA and the corresponding in vitro transcription (IVT) cRNA, using the Ambion WT Expression Kit

* Corresponding author at: McArdle Laboratory for Cancer Research, Wisconsin Institute for Medical Research, 1111 Highland Ave, Madison, WI 53705, USA.
E-mail address: w Xu@oncology.wisc.edu (W. Xu).

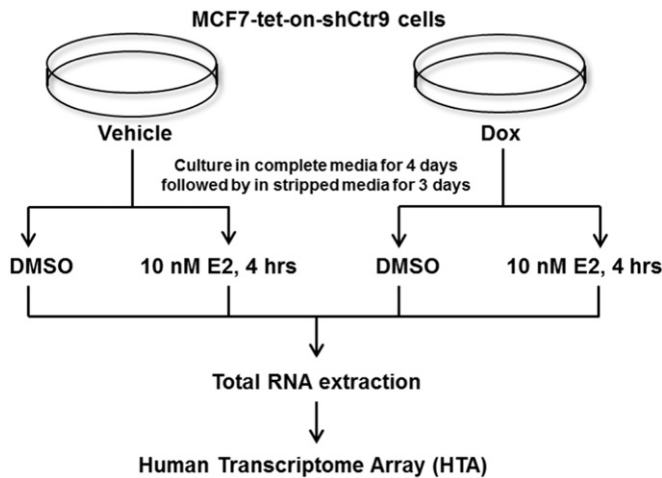


Fig. 1. Experimental design for global identification of E2- and Ctr9-regulated transcriptome.

per the manufacturer's instructions. In order to monitor labeling and hybridization quality, 400 ng of polyA control RNA was spiked in for each sample. Subsequently, cRNA was purified and quantified and then subject to single strand (ss) cDNA synthesis. The purified ss cDNA was then fragmented to <150 bp and end-terminus labeled using Affymetrix GeneChip WT Terminal Labeling Kit following manufacturer's guidelines. The labeled samples were then hybridized to GeneChip Human Transcriptome Array 2.0 containing >6.0 million distinct probes covering 44,699 protein coding genes and 22,829 non-protein coding genes at 45 °C for 16 h, according to the guidelines in the Affymetrix GeneChip WT Terminal Labeling and Hybridization User Manual.

2.4. Data process and analysis

After hybridization, the GeneChip arrays were washed and then stained (streptavidin–phycoerythrin) on an Affymetrix Fluidics Station 450 followed by scanning on a GeneChip 3000 G7 Scanner. The scanned images were analyzed to produce raw data files saved as CEL files. Data analysis and visualization were performed using the Affymetrix Transcriptome Analysis Console (TAC) Software per the manufacturer's protocol. The criteria for selecting differentially expressed genes was 1.5-fold change cutoff and a false discovery rate <0.05. Based on this criteria, we found that over 1600 genes were regulated by estrogen treatment, and of those 1600, only 50 genes respond to E2 after Ctr9 knockdown, suggesting the essential role of Ctr9 in regulating ER α -dependent gene transcription [2].

3. Discussion

We described here a dataset composed of microarray gene expression profiling of E2- and Ctr9-regulated genes in MCF7 human breast

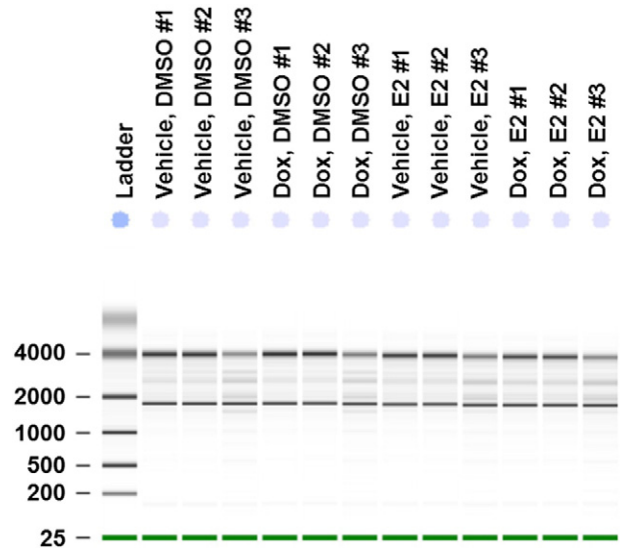


Fig. 2. Assessment of RNA quality on the Agilent 2100 BioAnalyzer.

cancer cells. With this genome-wide transcriptome analysis, we were able to demonstrate Ctr9, the key scaffold subunit in human PAFc, as a master-regulator of estrogen signaling in ER α ⁺ breast cancer [2]. We believe that this dataset would provide valuable information for researchers who are interested in studying the estrogen response hierarchy in ER α ⁺ breast cancer.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgments

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