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Data in Brief

Gene expression response to EWS–FLI1 in mouse embryonic cartilage

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ABSTRACT

Ewing's sarcoma is a rare bone tumor that affects children and adolescents. We have recently succeeded to induce Ewing's sarcoma-like small round cell tumor in mice by expression of EWS–ETS fusion genes in murine embryonic osteochondrogenic progenitors. The Ewing's sarcoma precursors are enriched in embryonic superficial zone (eSZ) cells of long bone. To get insights into the mechanisms of Ewing's sarcoma development, gene expression profiles between EWS–FLI1-sensitive eSZ cells and EWS–FLI1-resistant embryonic growth plate (eGP) cells were compared using DNA microarrays. Gene expression of eSZ and eGP cells (total, 30 samples) was evaluated with or without *EWS–FLI1* expression 0, 8 or 48 h after gene transduction. Our data provide useful information for gene expression responses to fusion oncogenes in human sarcoma.

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Specifications	
Organism/cell line/tissue	*Mus musculus*
Strain	BALB/c, dpc 18.5
Sex	Both male and female
Array type	Affymetrix MOE430 2.0 array
Data format	Raw data: CEL files, processed data: Excel table
Experimental factors	Tissue
Experimental features	Gene expression in eSZ cells and eGP cells with or without EWS–FLI1 expression was compared
Consent	n/a

Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32618.

Experimental design, materials and methods

Preparation of mouse embryonic superficial zone (eSZ) and growth plate (eGP) cells

Femoral and humeral bones of BALB/c mouse embryos were removed aseptically on 18.5 dpc, and they were microdissected into eSZ and growth plate (eGP) under a stereomicroscope (Zeiss Stemi 2000-C, Carl Zeiss MicroImaging). Each region was minced and gently digested with 2 mg/mL of collagenase (Wako Pure Chemical) at 37 °C for 2 h. They were cultured in growth medium composed of Iscove's Modified Dulbecco's Medium (Invitrogen) supplemented with 15% fetal bovine serum, and subjected immediately to retroviral infection.

Retroviral infection

N-terminal FLAG-tagged *EWS–FLI1* was introduced into the pMYs-IRES-GFP vector. The full length *EWS–FLI1* cDNA was a kind gift from Dr. Susanne Baker. Retroviral infections of eSZ, eGP or shaft cells were performed as described [1]. Infection efficiency was examined using a FACSCalibur flow cytometer (Beckton Dickinson). Cells were harvested after fourty-eight hours of infection.

RNA isolation and microarray

GeneChip analysis was conducted to determine gene expression profiles. The per cell normalization method (Percellome method) was applied to eSZ and eGP samples [2]. Briefly, cellular lysates were prepared with RLT buffer (QIAGEN). A 10 μL aliquot of each lysate was treated with DNAse-free RNase A (Nippon Gene Inc., Japan) for 30 min at 37 °C, followed by Proteinase K (Roche Diagnostics GmbH., Germany) for 3 h at 55 °C. The aliquot was then transferred to a 96-well black plate. PicoGreen fluorescent dye (Molecular Probes Inc., USA) was added to each well, shaken for 10 s four times and then incubated for 2 min at 30 °C. DNA concentration was measured using a 96

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well fluorescence plate reader with excitation at 485 nm and emission at 538 nm. λ phage DNA (PicoGreen Kit, Molecular Probes Inc., USA) was used as standard. As reported previously [2], the grade-dosed spike cocktails (GSCs) made of the *Bacillus subtilis* RNAs corresponding to the sequences in the Affymetrix GeneChip arrays (AFFX-ThrX-3_at, AFFX-LysX-3_at, AFFX-PheX-3_at, AFFX-DapX-3_at, and AFFX-TrpnX-3_at) were prepared, and GSCs were added to the sample homogenates in proportion to their DNA concentrations. Total RNA was extracted using the RNeasy Mini Kit (QIAGEN). The GeneChip Mouse Genome 430 2.0 Array (Affymetrix) was hybridized with the cRNA generated from eSZ and eGP cells, and murine Ewing's sarcoma tissue (Table 1). After staining with streptavidin–phycoerythrin conjugates, arrays were scanned using an Affymetrix GeneChip Scanner 3000 and analyzed using Affymetrix GeneChip Command Console Software (AGCC, Affymetrix) and GeneSpring GX 11.0.2 (Agilent Technologies) as described previously [3]. The expression data for eSZ and eGP cells were converted to Percellome data, i.e., absolute copy numbers of mRNA per one cell, by the homemade software SCal4 (Spike Calculation version 4). This software also graphically indicates the efficiency of in vitro transcription, the dose–response linearity of the five GSC spikes and the location of spike probe sets in the histogram of all probe sets (Fig. 1A). From the same treatment group ($n = 3$), all the pairs were plotted to a scatter graph as red (expression above detection level) or green dots (below detection level) with the data of five yellow spike probe sets (Fig. 1B). If any samples did not draw a symmetric scatter plot with yellow dot on the diagonal line, the sample were rejected for evaluation, and they were subjected to additional analyses.

Data analysis

Homemade software named RSort (Roughness Sort) [4] was used. This program sorts the probe sets as upward or downward peaks in a 3D isobologram (Fig. 2). To avoid biologically nonsense probe sets

Table 1
Summary of processed samples.

GEO accession no.	Cell types	Gene transfer	Time (h)
GSM808581	eSZ	No	0
GSM808582	eSZ	No	0
GSM808583	eSZ	No	0
GSM808584	eGP	No	0
GSM808585	eGP	No	0
GSM808586	eGP	No	0
GSM808587	eSZ	Empty vector	8
GSM808588	eSZ	Empty vector	8
GSM808589	eSZ	Empty vector	8
GSM808590	eGP	Empty vector	8
GSM808591	eGP	Empty vector	8
GSM808592	eGP	Empty vector	8
GSM808593	eSZ	EWS–FLI1	8
GSM808594	eSZ	EWS–FLI1	8
GSM808595	eSZ	EWS–FLI1	8
GSM808596	eGP	EWS–FLI1	8
GSM808597	eGP	EWS–FLI1	8
GSM808598	eGP	EWS–FLI1	8
GSM808599	eSZ	Empty vector	48
GSM808600	eSZ	Empty vector	48
GSM808601	eSZ	Empty vector	48
GSM808602	eGP	Empty vector	48
GSM808603	eGP	Empty vector	48
GSM808604	eGP	Empty vector	48
GSM808605	eSZ	EWS–FLI1	48
GSM808606	eSZ	EWS–FLI1	48
GSM808607	eSZ	EWS–FLI1	48
GSM808608	eGP	EWS–FLI1	48
GSM808609	eGP	EWS–FLI1	48
GSM808610	eGP	EWS–FLI1	48

eSZ, embryonic superficial zone; GP, growth plate.

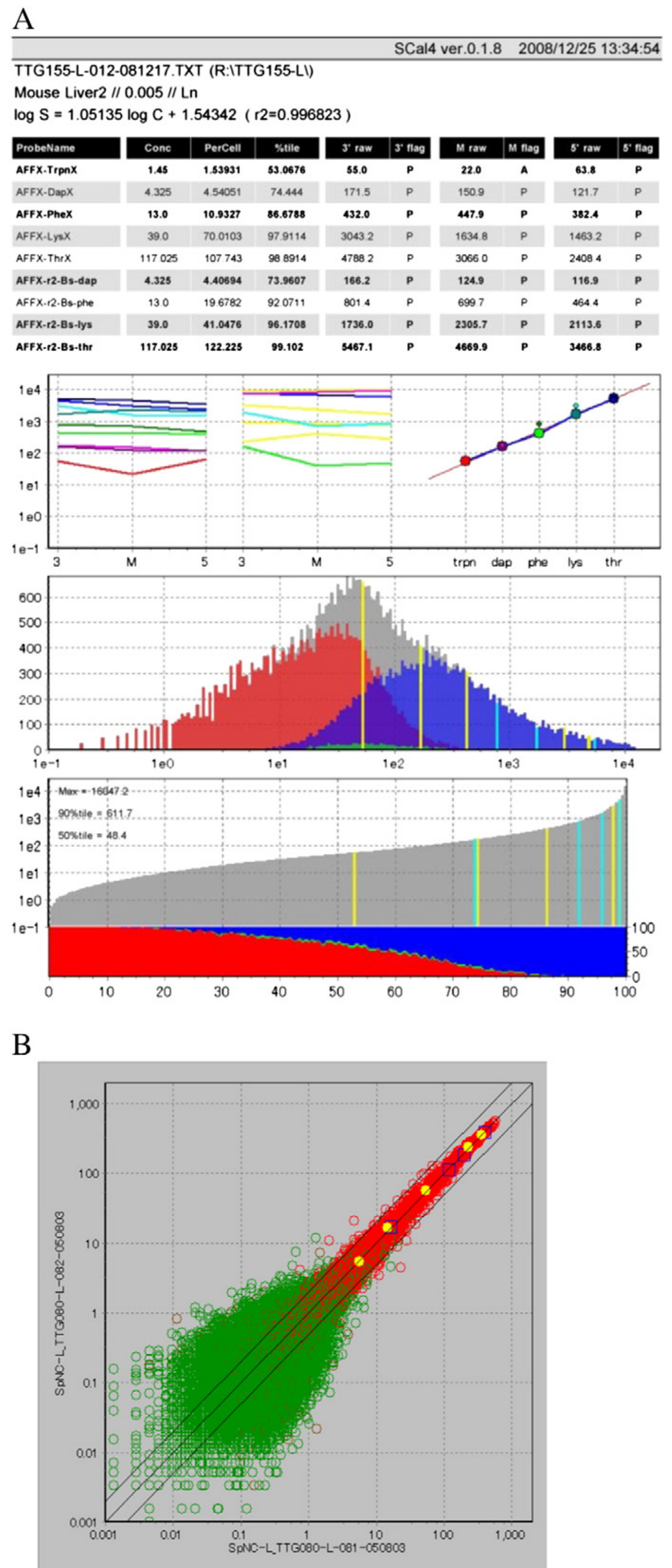

Fig. 1. Evaluation of the microarray data according to the PerCellome method. (A) An example of the SCal4 software report. SCal4 graphically indicates the efficiency of in vitro transcription, the dose–response linearity of the five GSC spikes and the location of spike probe sets in the histogram of all probe sets. (B) A scatter plot of gene expression between two experimental groups. All the pairs of probe sets were plotted to a scatter graph as red (expression above detection level) or green dots (below detection level) with the data of five yellow spike probe sets.

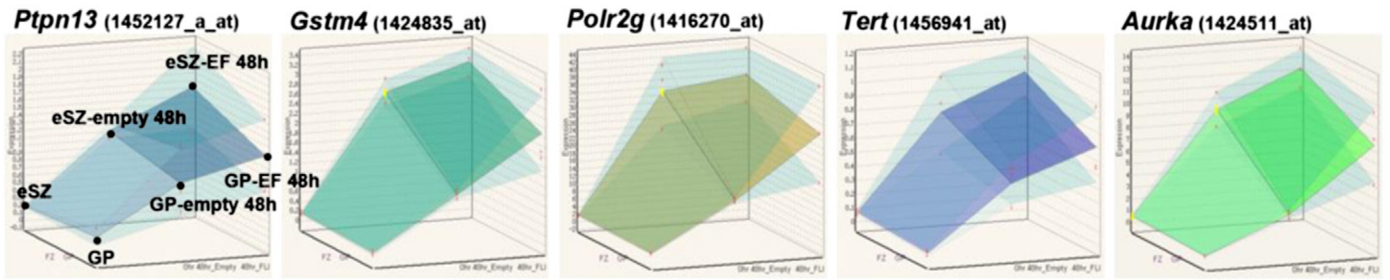

Fig. 2. Modulation of gene expression following introduction of *EWS–FLI1*. Three-dimensional grid plots of the expression of five representative genes in eSZ and eGP cells with or without *EWS–FLI1* were generated by GeneChip analysis (first two rows). The averages of each group (n = 3) were calculated and plotted as three layers of isobolograms on three-dimensional graphs as described previously [2,4].

such as ones with expression below the detection level, the data were visually checked for their 3D isobologram shape.

Discussion

We describe a unique dataset of mouse embryonic cartilage with or without the Ewing's sarcoma fusion oncogene, *EWS–FLI1*. Significantly different responses of gene expression between eSZ and eGP cells were observed. The dataset was used in the study published recently [5] and was informative to understand the tumorigenic mechanisms of Ewing's sarcoma.

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