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Data in Brief

Dataset of microarray analysis to identify endoglin-dependent bone morphogenetic protein-2-responsive genes in the murine periodontal ligament cell line PDL-L2

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ABSTRACT

The periodontal ligament (PDL), connective tissue located between the cementum of teeth and alveolar bone of the mandibula, plays a crucial role in the maintenance and regeneration of periodontal tissues. We previously reported that endoglin was involved in the bone morphogenetic protein (BMP)-2-induced osteogenic differentiation of mouse PDL cells, which is associated with Smad-2 phosphorylation but not Smad-1/5/8 phosphorylation. Further, we found that the BMP-2-induced Smad-2 phosphorylation was, at least in part, dependent upon endoglin. In this study, to elucidate the detailed mechanism underlying the BMP-2-induced signaling pathway unique to PDL cells, we performed a cDNA microarray analysis to identify endoglin-dependent BMP-2-responsive genes in PDL-L2, a mouse PDL-derived cell line. Here we provide experimental methods and obtained dataset to correspond with our data in Gene Expression Omnibus (GEO) Datasets.

Introduction

Specifications	
Organism/cell line/tissue	The mouse periodontal ligament cell line PDL-L2
Strain(s)	C57BL/6-derived cell line
Sequencer or array type	Affymetrix Mouse Genome 430 2.0 Array
Data format	Raw data (CEL files)
Experimental factors	Factor-treated cells with or without siRNA-mediated gene silencing
Experimental features	Experiment to compare gene expression in PDL-L2 cells cultured in the presence or absence of BMP-2, and that to compare gene expression in BMP-2-treated PDL-L2 cells with or without endoglin knockdown.
Consent	n/a
Sample source location	n/a

Direct link to deposited data

Deposited data are available here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54220.

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Experimental design, materials and methods

Reagents

Recombinant human BMP-2 (rhBMP-2) was kindly provided by Astellas Pharma Co. Ltd. (Tokyo, Japan). Other general reagents used for DNA/RNA manipulation were purchased from Wako Chemicals (Osaka, Japan).

Cell culture and RNA isolation

Mouse PDL-derived PDL-L2 cells were cultured as described earlier [1,2]. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. RNA purity was evaluated by RNA integrity number (RIN), a representative index to assess RNA quality determined using Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA). We confirmed that RINs of all RNA samples used in this study were more than 9.9.

SiRNA-mediated knockdown

PDL-L2 cells were transfected with SMARTpool siRNA for mouse endoglin (siENG) or SMARTpool non-target (siCont) as a negative control (Thermo-Fisher Scientific, Waltham, MA) as described elsewhere [3]. The cells were cultured for 48 h after transfection to achieve the knockdown of endoglin.

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Microarray study design

We prepared RNA samples from PDL-L2 cells that were processed under the following conditions: 1) treated with SMARTpool non-target (siCont) and exposed to vehicle for 12 h (Sample #1), 2) treated with siCont and exposed to recombinant human rhBMP-2 (250 ng/ml) for 12 h (Sample #2), and 3) treated with SMARTpool siRNA for mouse endoglin (siENG) and exposed to rhBMP-2 (250 ng/ml) for 12 h (Sample #3). The RNA samples were subjected to microarray analysis using a Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA).

RNA labeling and hybridization

RNA labeling and hybridization were performed using GeneChip® One-Cycle Target Labeling and Control Reagents (Affymetrix) according to the manufacturer's instructions. Briefly, 1 μg total RNAs from PDL-L2 were subjected to double-stranded cDNA synthesis, biotin labeling of antisense cRNA, fragmentation (to approximately 35- to 200-base fragments), and hybridization to probe array on the chips. The hybridized probe array was washed and subjected to signal fluorescent signal detection using a GeneChip® Scanner 3000 (Affymetrix).

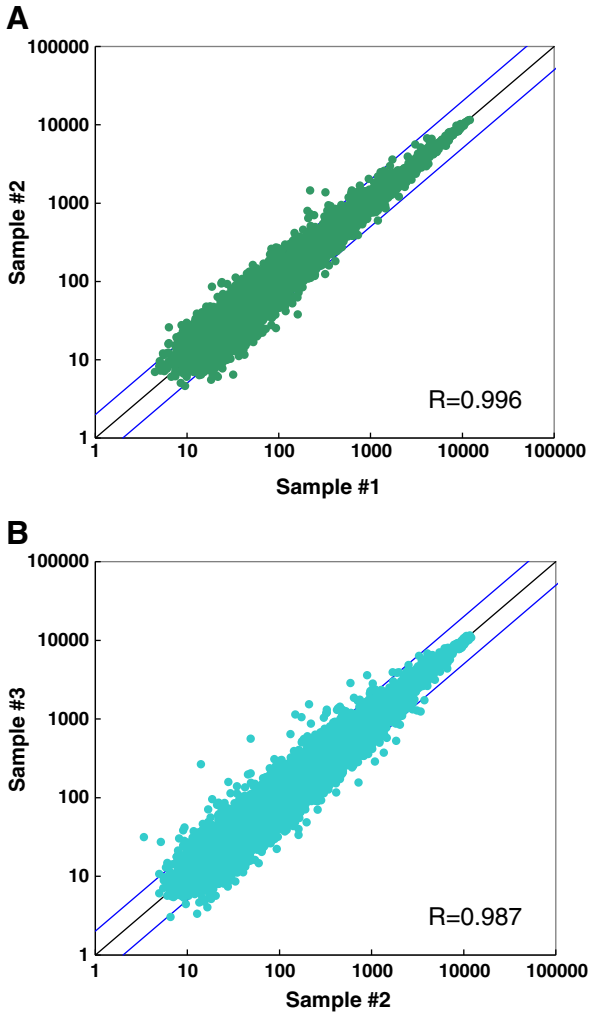

Fig. 1. Scatter plots showing correlation of signal values between two samples. Data assigned to an absent call were omitted. (A) Sample #1 (horizontal axis) vs Sample #2 (vertical axis). (B) Sample #2 (horizontal axis) vs Sample #3 (vertical axis). R values are indicated in the graphs.

Data normalization and analysis

Raw data (CEL files) were produced for the three samples using Affymetrix GeneChip Command Console Software (AGCC) and processed using Affymetrix Expression Console Software. The CEL files are registered as GEO accession no. GSE54220. A detection call algorithm was used to filter and remove missing expression values based on absent/present calls. Using this algorithm, present, marginal, or absent call was obtained for each probe set in each array. A scaling factor was applied to the normalized data from the CEL files to bring the average intensity for all probes on the array to 500, generating CHP files by the use of Microarray Suite 5 software. For the comparison analysis of gene expression, data assigned to an absent call were omitted. By comparing the data obtained from Sample #1 and Sample #2 (Set #1), it is expected to identify BMP-2-responsive genes in PDL-L2 cells. By comparing the data obtained from Sample #2 and Sample #3 (Set #2), it is expected to identify genes that are affected by endoglin knockdown in the presence of BMP-2. Thus, by combination of these comparative analyses, it is expected to identify endoglin-dependent BMP-2-responsive genes in PDL-L2 cells. Scatter plots of normalized signal values in Set #1 and Set #2 are shown in Fig. 1. Overall, signal intensities from these sample sets are well correlated (R values are 0.996 and 0.987 for Set #1 and Set #2, respectively), suggesting that relatively small numbers of genes are differentially expressed between the samples. Value distribution of signals from microarrays is shown in Fig. 2. It should be noted that median values are almost identical among the three samples, which supports the high quality of our dataset.

Discussion

Herein we describe information about a unique microarray dataset of vehicle- or BMP2-treated PDL-derived cells with or without endoglin knockdown. This dataset will provide a clue to elucidate detailed

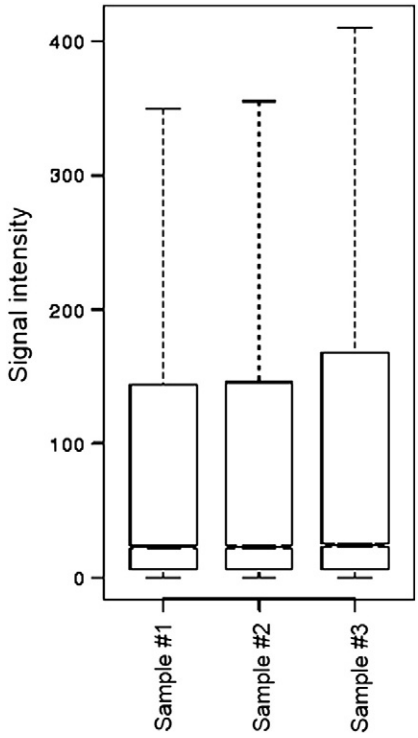

Fig. 2. Value distribution of signals from microarrays. The boxes mark the interval between the 25th and 75th percentiles. The lines inside the boxes denote medians.

mechanisms underlying the BMP-2-induced signaling pathway unique to PDL cells.

Conflict of interest

The authors have no conflicts of interest.

Acknowledgments

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