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Data Article

SATB1-regulated transcriptome datasets of Rcho-1 rat trophoblast stem cells

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ABSTRACT

SATB homeobox 1 (SATB1) and its heterodimeric partner SATB2 play an important regulatory role in maintaining proliferation of trophoblast stem (TS) cells and in inhibiting trophoblast differentiation. To identify the SATB-regulated genes in TS cells, we studied the transcriptome changes in a 'loss of function' model of Rcho-1 rat TS cell line. *Satb1* gene expression was silenced by lentiviral delivery of shRNAs targeted to exon 9 and exon 12. An *Egfp* shRNA was used as a non-targeted control. Total RNA was purified from shRNA-transduced Rcho-1 cells, and whole transcriptome was assessed by RNA-sequencing on an Illumina HiSeq X platform. Differentially expressed genes in *Satb1* shRNA-transduced cells were identified by analyses of the RNA-sequencing data using CLC Genomics Workbench. Differentially expressed genes with each of the two different shRNAs were compared to identify SATB1-target genes and to eliminate the potential off-targets of the shRNAs. These datasets can be used to identify the SATB-regulated genes in TS cells and to understand the molecular mechanisms that regulate trophoblast proliferation and inhibit differentiation.

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Specifications table

Subject	Biology
Specific subject area	Developmental biology
Type of data	Table Figure
How data were acquired	Sequencing of RNA from Rcho-1 rat trophoblast stem (TS) cells
Data format	Raw (Fastq) and analyzed (Excel tables)
Parameters for data collection	RNA-sequencing was performed on Rcho-1 cells treated with shRNAs against *Satb1* gene or control shRNA.
Description of data collection	Rcho-1 rat TS cells were stably transduced with lentivirus to deliver the shRNAs. Total RNAs were purified from the shRNA-transduced cells and analyzed by mRNA-sequencing. SATB1-regulated genes in Rcho-1 cells were identified by analyses of the transcriptome data using CLC Genomics Workbench.
Data source location	University of Kansas Medical Center, Kansas City, KS 66160, USA
Data accessibility	Repository name: SRA Data identification number: PRJNA558387 Direct URL to data: https://www.ncbi.nlm.nih.gov/sra

Value of the Data

- This article provides with the transcriptome analyses of differentially expressed genes in Rcho-1 rat TS cells treated with shRNA targeted to *Satb1* gene.
- These datasets represent the SATB1-target genes as well as the potential off-targets of the shRNAs.
- These data can be used to identify the SATB1-regulated genes that maintain proliferation and inhibit differentiation of TS cells.

1. Data

Transcriptome data generated by RNA-sequencing of Rcho-1 rat TS cells treated with shRNA targeted to *Satb1* or *Egfp* were analyzed to identify the differentially expressed genes. Analyzed data are presented in Excel format and the raw data are deposited to NCBI SRA (SRR9894784-9894789, included under PRJNA558387). SRR9894786 and SRR9894787 are raw data obtained from Rcho-1 cells treated with control shRNA (*Egfp*), whereas SRR9894784, SRR9894785, SRR9894788 and SRR9894789 are the raw data obtained from Rcho-1 cells treated with *Satb1* shRNA. Analyzed data are presented in the following tables:

Supplementary Table 1. Differentially expressed genes in Rcho-1 rat TS cells treated with shRNA targeted to *Satb1* exon 9.

Transcriptome analyses of SATB1-regulated genes in Rcho-1 cells compared to control shRNA. Of the 3699 differentially expressed genes, 2462 were $\geq$2 fold upregulated, whereas the 1237 were $\leq$2 fold downregulated.

Supplementary Table 2. Differentially expressed genes in Rcho-1 rat TS cells treated with shRNA targeted to *Satb1* exon 12.

Transcriptome analyses of SATB1-regulated genes in Rcho-1 cells compared to control shRNA. Of the 4265 differentially expressed genes, 2781 were $\geq$2 fold upregulated, whereas the 1484 were $\leq$2 fold downregulated.

Supplementary Table 3. Differentially expressed genes in Rcho-1 rat TS cells treated with shRNA targeted to either *Satb1* exon 9 or exon 12.

Differentially expressed genes in Rcho-1 cells treated with shRNA targeted to *Satb1* exon 9 were compared with those of shRNA targeted to *Satb1* exon 12. Of the 3699 differentially expressed genes with shRNA targeted to *Satb1* exon 9, and 4265 differentially expressed genes with shRNA targeted to *Satb1* exon 12, 2447 were common to both groups.

Supplementary Table 4. Differentially expressed unique genes in Rcho-1 rat TS cells treated with shRNA targeted to *Satb1* exon 9.

Differentially expressed genes in Rcho-1 cells treated with shRNA targeted to *Satb1* exon 9 were compared with those of shRNA targeted to *Satb1* exon 12. Of the 3699 differentially expressed genes

with shRNA targeted to *Satb1* exon 9, 1252 were unique, which represent potential off-targets of the shRNA.

Supplementary Table 5. Differentially expressed unique genes in Rcho-1 rat TS cells treated with shRNA targeted to *Satb1* exon 12.

Differentially expressed genes in Rcho-1 cells treated with shRNA targeted to *Satb1* exon 9 were compared with those of shRNA targeted to *Satb1* exon 12. Of the 4265 differentially expressed genes with shRNA targeted to *Satb1* exon 12, 1818 were unique, which represent potential off-targets of the shRNA.

2. Experimental design, materials and methods

2.1. Culture of Rcho-1 cells

Rcho-1 rat TS cells were maintained in stem-state by culturing them in appropriate culture conditions as reported previously [1,2].

2.2. Satb1 knockdown

Rcho-1 cells were transduced with lentivirus carrying shRNA-encoding pLKO.1-puro vectors [3]. Stably transduced cells were established by selection with puromycin for sustained knockdown of *Satb1* expression [4]. Two effective *Satb1* shRNAs (shRNA targeted to exon 9 and exon 12) were used for the targeted knockdown [4]. A shRNA targeted to *Egfp* was used as a non-targeted control [4].

2.3. Purification of RNA, library preparation and RNA-sequencing

Total RNA was extracted from the shRNA transduced Rcho-1 cells using TRI Reagent (Sigma-Aldrich, St. Louis, MO) following the manufacturer's instruction. RNA quality was assessed by a Bioanalyzer, and samples with a RIN value over 9 were selected for mRNA-sequencing library preparation. Approximately 500 ng of total RNA was used for the RNA-sequencing library preparation using a TruSeq Standard mRNA kit (Illumina, San Diego, CA) following the manufacturer's instruction [5]. Briefly, mRNA was first enriched from total RNA by oligo-dT magnetic beads and then purified and chemically fragmented. The first strand of cDNA was synthesized by using random hexamer primers and reverse transcriptase. Then double stranded cDNA (ds cDNA) was generated by removing the RNA template and then synthesizing a replacement strand, which incorporated dUTP instead of dTTP. ds cDNAs were purified using AMPure XP beads (Beckman Coulter, Brea, CA). The cDNA ends were blunted and a poly (A) tail was added to the 3′ ends. Finally, after ligation of index adapters (Illumina), the suitable DNA fragments were selected for 15 cycles of PCR amplification. Each cDNA library was prepared from pooling three RNA samples. Two replicated of cDNA libraries were prepared for each of the control (*Egfp*) and *Satb1* shRNA treated groups. The paired-end cDNA libraries were initially evaluated for quality and then sequenced on an Illumina HiSeq X sequencer with 2 x 100 cycles (Novogene Corporation, Sacramento, CA). Fig. 1.

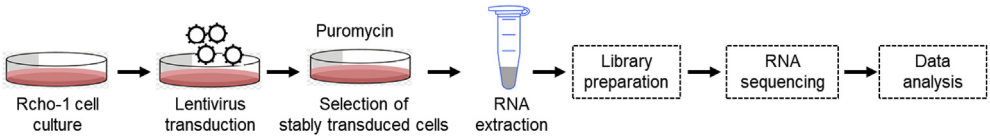

Fig. 1. Schematic presentation of the experimental design. Rcho-1 rat TS cells were grown in proliferating condition. Cells were transduced with lentivirus encoding shRNAs, and stably transduced cells were selected by adding puromycin to Rcho-1 culture media. Total RNAs were extracted from the stably transduced cells, assessed by Bioanalyzer, and used for mRNA-sequencing. RNA-sequencing data were analyzed by using CLC Genomics Workbench.

2.4. RNA-seq data analyses

RNA-sequencing data were demultiplexed, trimmed, aligned and analyzed using CLC Genomics Workbench 12.2 (Qiagen Bioinformatics, Germantown, MD). Trimming was performed to remove the low-quality reads, and good-quality reads were aligned with *Rattus norvegicus* genome (Rnor_6.0) using default parameters: (a) maximum number of allowable mismatches was 2, (b) minimum length and similarity fraction was set at 0.8, and (c) minimum number of hits per read was 10. Expression values were measured in TPM (transcripts per million). The threshold *p*-value was determined according to the false discovery rate (FDR). Differentially expressed genes were determined if the absolute fold change in expression was 2 with an FDR *p*-value of $\leq$0.05.

2.5. Statistical analysis

Each RNA-sequencing library was prepared using pooled RNA samples from three independent culture samples. Each group of RNA-sequencing consisted of two libraries. Differentially expressed genes were identified by CLC Genomics Workbench as described previously [5].

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104749.

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