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Data in Brief

Snai1 represses Nanog to promote embryonic stem cell differentiation

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ABSTRACT

Embryonic stem cell (ESC) self-renewal and pluripotency is maintained by an external signaling pathways and intrinsic regulatory networks involving ESC-specific transcriptional complexes (mainly formed by OCT3/4, Sox2 and Nanog proteins), the Polycomb repressive complex 2 (PRC2) and DNA methylation [1–8]. Among these, Nanog represents the more ESC specific factor and its repression correlates with the loss of pluripotency and ESC differentiation [9–11]. During ESC early differentiation, many development-associated genes become upregulated and although, in general, much is known about the pluripotency self-renewal circuitry, the molecular events that lead ESCs to exit from pluripotency and begin differentiation are largely unknown. Snai1 is one the most early induced genes during ESC differentiation in vitro and in vivo [12,13]. Here we show that Snai1 is able to directly repress several stemness-associated genes including Nanog. We use a ESC stable-line expressing a inducible Snai1 protein. We here show microarray analysis of embryonic stem cells (ESC) expressing Snail-ER at various time points of induction with 4-OH. Data were deposited in Gene Expression Omnibus (GEO) datasets under reference GSE57854 and here: http://epigenetics.hugef-research.org/data.php.

Specifications	
Organism/cell line/tissue	*Mouse E14 embryonic stem cells*
Sex	*Male*
Sequencer or array type	*Illumina MouseWG-6 v2.0 expression beadchip*
Data format	*Raw and analyzed*
Experimental factors	*ESCs were treated with OHT at various time points*
Experimental features	*microarray analysis of embryonic stem cells (ESC) expressing Snail-ER*
Consent	*N/A*
Sample source location	*Torino, Italy and Siena, Italy*

Direct link to deposited data

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE57854
http://epigenetics.hugef-research.org/data.php

Experimental design, materials and methods

cDNA of human Snai1 coding region without stop codon, in frame with cDNA of mouse ERα ligand-binding region was obtained by PCR from pBabePuro- hSnai1.ER.NoTag (Addgene plasmid 19292) using following oligonucleotides: 5′-GAGAGGATCCSCCATGCCGCGCTCTTTCCTC-3′ and 5′-GAGAGTCGACTCAGATCGTGTTGGGGAA-3′, and cloned in the lentiviral vector pCCLsin.hPGK.GFP.pre. The plasmid was confirmed by sequencing. Lentiviral vector were co-transfected with pMD2.VSVG, pMDLg/pRRE, pRSV-Rev in Lenti-X TM 293 cell line (Clonetech) to produce lentivirus particles.

Embryonic stem cells were cultured as previously described [14]. Briefly, mESC were grown in DMEM high glucose medium (Invitrogen) supplemented with 15% FBS (Millipore), 0.1 mM nonessential amino acids (Invitrogen), 1 mM sodium pyruvate (Invitrogen), 0.1 mM 2-mercaptoethanol, 1500 U/ml LIF (Millipore), 25 U of penicillin/ml, and 25 μg of streptomycin/ml. Cells were induced with 4-OHT at the indicated times (0 h, 2 h, 4 h, 8 h, 12 h, 24 h) for SNAIL-ER nuclear localization before RNA extraction (Fig. 1). Nuclear localization was confirmed by Western blotting analysis by performing nuclear extracts as previously described [15]. RNA was extracted as previously described [16] by using TRIzol reagent from Invitrogen following the manufacturing protocol.

Microarray analysis was performed as in Ref. [17]. Briefly, RNA library were performed using Illumina Total Prep RNA Amplification Kit following the manufacturing protocol using Cy3 fluorescent label. Direct Hybridization of RNA library was performed using Standard Operating Procedures in Whole-Genome Gene Expression Direct Hybridization Assay Guide (Illumina). Illumina MouseWG-6 v2.0 expression beadchip chips were scanned using Illumina HiScanSq instrument. Data were

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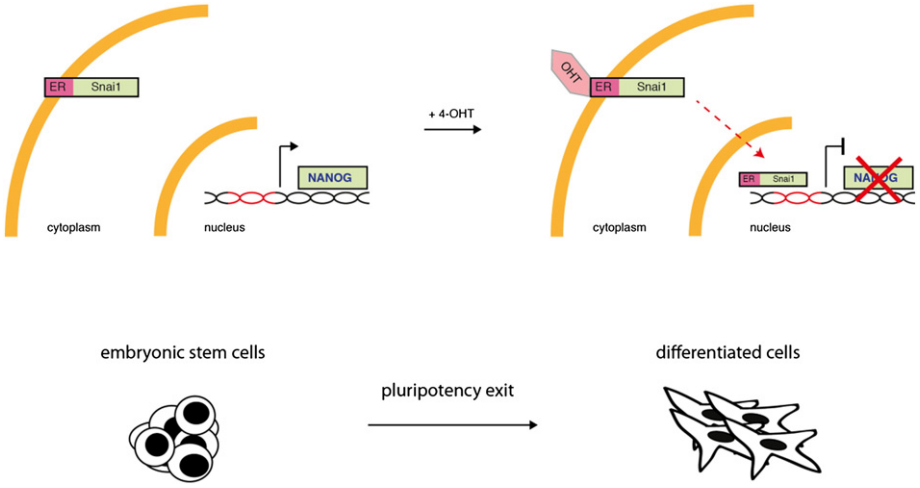

Fig. 1. Scheme of the Snai1 induction system in mouse embryonic stem cells.

analyzed by using Genome Studio software (Illumina). Analyzed data were normalized using quantile normalization and background subtraction by default parameters in Genome Studio.

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