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Data in Brief

Genome-wide ChIP-seq and RNA-seq analyses of Pou3f1 during mouse pluripotent stem cell neural fate commitment

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ABSTRACT

Appropriate neural initiation of the pluripotent stem cells in the early embryos is critical for the development of the central nervous system. This process is regulated by the coordination of extrinsic signals and intrinsic programs. However, how the coordination is achieved to ensure proper neural fate commitment is largely unknown. Here, taking advantage of genome-wide ChIP-sequencing (ChIP-seq) and RNA-sequencing (RNA-seq) analyses, we demonstrate that the transcriptional factor Pou3f1 is an upstream activator of neural-promoting genes, and it is able to repress neural-inhibitory signals as well. Further studies revealed that *Pou3f1* could directly bind neural lineage genes like *Sox2* and downstream targets of neural inhibition signaling such as BMP and Wnt. Our results thus identify Pou3f1 as a critical dual-regulator of the intrinsic transcription factors and the extrinsic cellular signals during neural fate commitment. Data were deposited in Gene Expression Omnibus (GEO) datasets under reference number GSE69865.

Specifications	
Organism/cell line/tissue	Time-course differentiation of mouse embryonic stem cells (mESCs)
Sex	Male
Sequencer or array type	Illumina HiSeq 2000 for Pou3f1 ChIP-seq, RNA-seq
Data format	Raw and analyzed
Experimental factors	mESCs under neural differention with or without Pou3f1 overexpression
Experimental features	ChIP-seq and RNA-seq analyses of Pou3f1
Consent	N/A
Sample source location	Shanghai, China

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE69865.

2. Experimental design, materials and methods

2.1. Cell culture and differentiation

Mouse embryonic stem cells (mESCs) (R1 and R1/E) were maintained on a layer of mitomycin C-treated mouse embryonic fibroblast feeder cells in standard medium supplemented with 1000 U/mL mouse leukemia inhibitory factor (LIF). Unbiased ESC differentiation in serum-containing medium was performed as described previously [1,2]. Briefly, ESCs were dissociated into single cells after treatment with 0.05% Trypsin–EDTA at 37 °C for 2 min and were seeded at a density of 1×10^5 cells/ml in Petri dishes to form embryonic bodies (EBs). EB cells are maintained in supplemented DMEM containing 10% FBS (Gibco), 1% NEAA, 1% sodium pyruvate, and 0.1 mM β-mercaptoethanol (both from GIBCO). On days 2, 4 and 6, wild-type cells and Pou3f1 stable overexpressed (Pou3f1-OE) cells were collected for RNA-seq analysis,

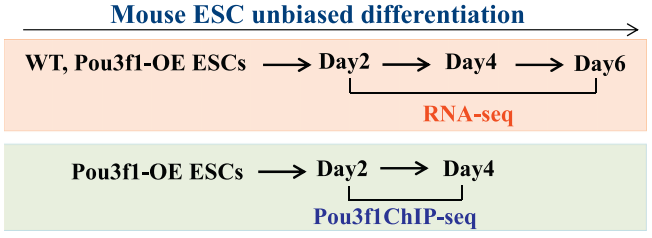

Fig. 1. RNA-seq and ChIP-seq analyses of Pou3f1 during mouse ESC unbiased differentiation. mESCs were aggregated and then differentiated into embryonic bodies for 6 days. RNA-seq samples were collected from WT or Pou3f1-OE cells during differentiation day 2 to day 6. On differentiation day 2 and day 4, Pou3f1-OE ESCs were harvested for ChIP-seq analysis. WT: wild type; Pou3f1-OE: Pou3f1-overexpressing ESC.

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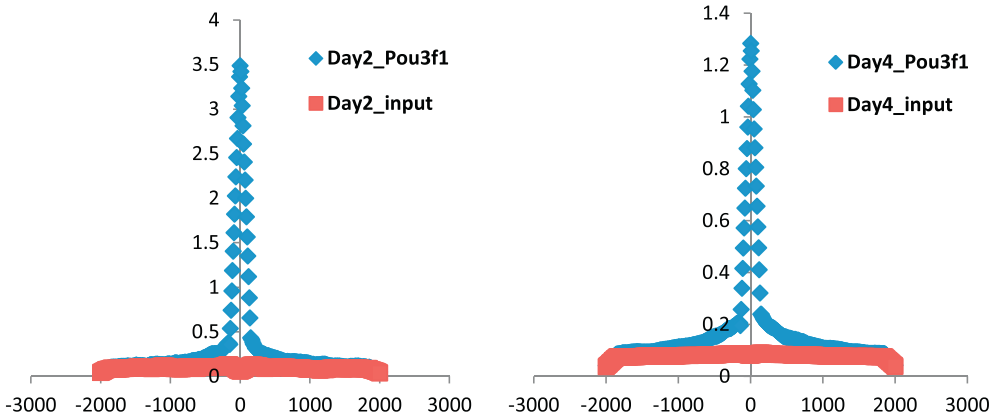

Fig. 2. Read distribution around peaks. The blue stands for Pou3f1 ChIP signals and the red for input. The x-axis represents the distance to peak centers, and the y-axis represents signal levels of binding.

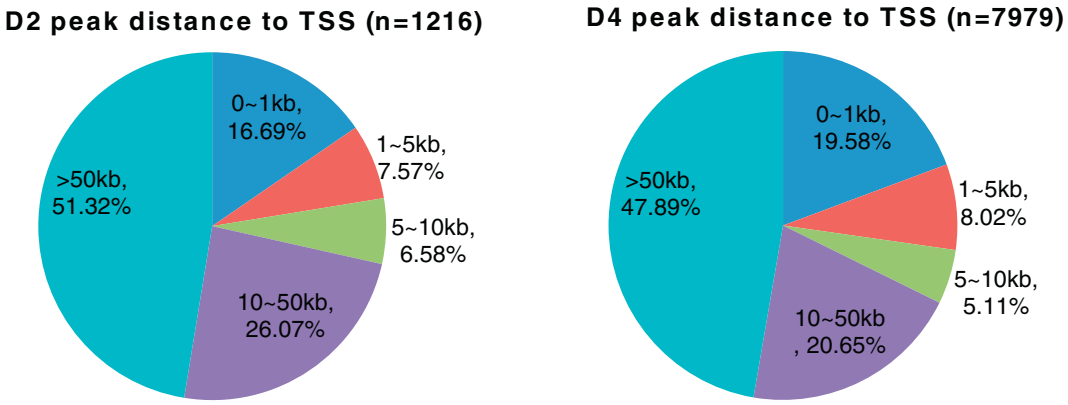

Fig. 3. Peak distance to TSS. Five categories were defined according to the distance from peak center to the nearest TSS. % represents the percentage of peaks with the distance to TSS belonging to one category. D2: day 2; D4: day 4; TSS: transcription start site.

and Pou3f1 ChIP-seq was performed on day 2 and day 4 with Pou3f1-overexpression cells (Fig. 1).

2.2. Chromatin immunoprecipitation (ChIP)

ChIP assays were performed as described in the manufacturer's manuals (Dynabeads Protein A/G (Invitrogen) and Protein A/G Agarose/Salmon Sperm DNA (Upstate Biotech)), and modified as previously reported [3]. Briefly, differentiated EB cells were dissociated into single cells by Trypsin and subjected to cross-linking. The cells were lysed and crude extracts were sonicated into DNA fragments of an average size of 200 bp. Cell lysate was cleared and subsequently incubated with antibodies against Pou3f1 or directly used as ChIP input. Immunoprecipated DNA was reverse cross-linked and recovered. The input DNA and 10–15 ng IP DNA were used to construct sequencing library by ChIP-seq Sample Prep Kit (Illumina). Enriched DNA sequencing was performed on Illumine HiSeq 2000. The high-throughput sequencing was performed by the Computational Biology Omics Core, PICB, Shanghai.

2.3. ChIP-seq data processing

The ChIP-seq reads were mapped into the mouse genome build mm9 using the SOAP version 2.20 [4]. The uniquely mapped reads with less than two mismatches were kept for subsequent analysis. We then used findpeaks in Homer package to call Pou3f1 binding peaks, which required 4-fold greater normalized reads in ChIP sample comparing with the control. The binding peaks of Pou3f1 were very narrow as typical transcription factors (Fig. 2) [5]. Furthermore, the distance of peak center to the nearest transcription start site (TSS) was calculated for all peaks, and most of them were distal to TSS (Fig. 3).

2.4. RNA-Seq data processing

TopHat (version 1.4.1) alignment tool was used to map raw reads to mm9 [6], and Cufflinks (version 1.3.0) was used to calculate FPKM (fragment per kilo base per million) as an expression value for each gene [7]. Then, we identified differentially expressed genes between treatment and control samples using Cuffdiff software [8], and performed k-means clustering using Euclidean distance as the distance between genes.

Acknowledgments

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References

[1] T. Zhang, et al., The zinc finger transcription factor Ovol2 acts downstream of the bone morphogenetic protein pathway to regulate the cell fate decision between neuroectoderm and mesendoderm. J. Biol. Chem. 288 (9) (2013) 6166–6177.
[2] Q. Zhu, et al., The transcription factor Pou3f1 promotes neural fate commitment via activation of neural lineage genes and inhibition of external signaling pathways. Elife 3 (2014).

[3] Z. Jin, et al., Different transcription factors regulate nestin gene expression during P19 cell neural differentiation and central nervous system development. J. Biol. Chem. 284 (12) (2009) 8160–8173.
[4] R. Li, et al., SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 25 (15) (2009) 1966–1967.
[5] S. Heinz, et al., Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell 38 (4) (2010) 576–589.
[6] C. Trapnell, L. Pachter, S.L. Salzberg, TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25 (9) (2009) 1105–1111.
[7] C. Trapnell, et al., Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28 (5) (2010) 511–515.
[8] C. Trapnell, et al., Differential analysis of gene regulation at transcript resolution with RNA-seq. Nat. Biotechnol. 31 (1) (2013) 46–53.