Epigenetic Changes Correlate with Loss of Pluripotency Marker Expression During Retinoic Acid-induced Differentiation of NTERA-2 Cells

Abstract

Identifying and investigating the molecular and biochemical events associated with changes in cell fate, such as those that occur during stem cell differentiation or somatic cell reprogramming, are fundamental goals in stem cell biology research. Achieving these objectives requires the development of highly specific and sensitive reagents that have been validated in a contextually appropriate manner. At Cell Signaling Technology, we are developing a portfolio of sensitive, rigorously validated antibody reagents designed to investigate the epigenetic, molecular, and biochemical events that underlie changes in cell fate. Here, we use retinoic acid (RA)-induced differentiation of NTERA-2 cells as a model to demonstrate the utility of our antibody reagents for interrogating the epigenetic and biochemical events associated with induced neuronal differentiation. Using chromatin immunoprecipitation, fluorescent immunocytochemistry and intracellular flow cytometry, we are able to demonstrate the predicted correlation between epigenetic changes at distinct genomic loci with changes in the expression of key pluripotency factors during directed differentiation. These data confirm the utility of these antibody reagents, employed across multiple technology platforms, for investigating the molecular mechanisms underlying cellular differentiation.

Materials and Methods

Cell Culture:
NTERA-2 embryonal carcinoma cells were treated with all-trans retinoic acid (RA) for 0–14 days to induce neuronal differentiation as previously described (1). Cells were collected for analysis at multiple time points representing different stages of neuronal differentiation.

Fluorescent Immunocytochemistry:
Cells were fixed for 15 min in 4% formaldehyde, permeabilized, blocked for 1 hr, then incubated overnight in primary antibody at 4°C. After washing, cells were incubated in secondary antibody for 1-2 hr at RT in the dark. Stained cells were imaged by laser scanning confocal microscopy.

Flow Cytometry:
Cells were harvested, fixed, permeabilized, and processed according to the standard CST flow cytometry protocol. In brief, cell pellets were resuspended in 4% formaldehyde (methanol-free) for 10 min at 37°C, followed by addition of ice cold 90% methanol for at least 30 min on ice. Cells were then immunostained for 1 hr at RT by suspension in an antibody cocktail containing Oct-4A Alexa Fluor® 488, Sox2 Alexa Fluor® 647, or Nanog Alexa Fluor® 594. After washing 2x with PBS/0.5% BSA, cells were analyzed on a 4 laser Gallios™ flow cytometer (Beckman Coulter).

ChIP Immunoprecipitation:
Chromatin Immunoprecipitations were performed with cross-linked chromatin from NTERA-2 cells using the indicated antibodies, or 2 μl of Normal Rabbit IgG (p3, K2729), using the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using gene-specific primers. The amount of immunoprecipitated DNA in each sample is represented as a percentage of the total input chromatin.

Reference


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