

Proceedings of German Society for Stem Cell Research (PGSSCR)

Defining the end of pluripotency in murine embryonic stem cells

Choi SW, Obier N, Dinger TC, Vallabhapurapu D, Muller AM

Institute of Medical Radiation and Cell Research, University of Wuerzburg, Versbacherstr. 5, 97078 Wuerzburg, Germany

Published on 16 May 2007

Introduction:

Pluripotency describes the ability of stem cells to self-renew and to give rise to all cell types of the developing embryo. Pluripotent embryonic stem cells (ESCs) are established from the inner cell mass of blastocysts and can be maintained *in vitro* as stable cell lines in the presence of LIF whereas they start differentiation upon LIF withdrawal. To further study molecular processes at the end of pluripotency we analysed ESCs during early *in vitro* differentiation with respect to gene expression and chromatin state.

Materials and Methods:

CCE and BL6 ESCs were routinely grown on murine embryonic fibroblasts (MEFs) and E14 ESCs without MEFs on gelatine-coated dishes in the presence of LIF and 15% FCS. Upon FCS reduction (10%) and MEF- as well as LIF-removal differentiation was induced. Loss of pluripotency was measured by CFC assay, for which appropriate numbers of differentiated ESCs were plated onto MEFs and colonies were counted. Gene expression of pluripotency- and differentiation-associated

genes was determined via *Real Time* PCR. Besides Western Blot we established an intranuclear FACS-based analysis to detect global changes of histone H4 acetylation levels during ESC differentiation.

Results:

Upon differentiation induction ESCs quickly lose their pluripotent character and their potential to form colonies on MEFs. The expression of pluripotency-associated genes *Rex1* and *FGF4* was downregulated, whereas the expression of mesodermal or entodermal marker genes *brachyury* and *?*-*Fetoprotein* was increased during differentiation. Western Blot analysis on whole cell protein lysates revealed that histone H4 acetylation levels in early differentiating ESCs do not change significantly. However, in flow-cytometric studies - implementing two different staining strategies - we constantly observed coordinated patterns of histone H4 acetylation, increasing from day 0 to day 1 and decreasing from day 2 of differentiation induction. In parallel, cell proliferation rates were only marginally reduced, as analysed by CFSE label retention studies.

Discussion and Conclusion:

Together, these data indicate that the end of pluripotency is characterized by an unexpectedly low level of heterogeneity within the pool of differentiating ES cells regarding histone H4 acetylation, proliferation and gene expression. Further studies shall address the question whether the end of pluripotency is a defined point of no return or whether there is a time window of ambiguity.

JSRM
www.pubstemcell.com