Role of core transcription factors in differentiation, maintenance and induction of pluripotent stem cells

Abstract:

Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have tremendous potential in regenerative medicine, drug toxicity screening and disease modeling due to their unlimited self-renewal and broad differentiation capacity. Understanding the molecular mechanism of core transcription factors, namely UTF1, Nanog and Oct4 during induction and maintenance of pluripotency is an important goal in developmental biology. Our results show that human UTF1 is a tightly DNA-associated protein with transcriptional repressor activity. In addition, our data show that UTF1 is a key chromatin component in ES cells with core histone-like characteristics, preventing ES cell chromatin decondensation, and aberrant gene expression; both essential for proper initiation of lineage-specific differentiation of ES cells. Using an inducible gain-of-function system that allows precise control over time and dosage we have reported that recombinant protein Nanog enhances proliferation and self-renewal of ESCs in the absence of leukemia inhibitory factor (LIF). Moreover Nanog-TAT, in the absence of LIF, promotes pluripotency by inhibiting endodermal specification in a Stat3-independent manner. Furthermore, Nanog enhances proliferation of both, NIH 3T3 as well as primary fibroblast cells. Investigation of cell cycle factors revealed that transient activation of Nanog correlates with consistent down-regulation of cell cycle inhibitor p27\(^{kip1}\). By chromatin immunoprecipitation analysis we found bona fide Nanog binding sites upstream to the p27\(^{kip1}\) gene, establishing a direct link between physical occupancy and functional regulation. In a cellular reprogramming paradigm cell-permeant Nanog is able to enhance iPS cell generation by 3-fold when applied at day 10 post infection. Finally, we have also generated recombinant Oct4 protein to derive transgene-free iPS cells in combination with small molecules that could be a key for the development of biomedical applications of stem cells. In conclusion, our results demonstrate important functions of these transcription factors that will help us avail practical use of pluripotent stem cells.