Research Highlights

Highlights from the latest articles in regenerative medicine

The common path: tumor suppression in the generation of iPS cells and cancer stem cells

Marcel M Daadi

Stanford University, Department of Neurosurgery, MSLS P304, 1201 Welch Rd, Stanford, CA 94305-5487, USA
Tel.: +1 650 724 9998;
Fax: +1 650 498 4134;
mdaadi@stanford.edu

Evaluation of: Utikal J, Polo JM, Stadtfeld M *et al.*: Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* 460, 1145–1148 (2009).
Li, H, Collado M, Villasante A *et al.*: The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature* 460, 1136–1139 (2009).
Krizhanovsky V, Lowe SW: Stem cells: the promises and perils of p53. *Nature* 460, 1085–1086 (2009).

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Somatic cell reprogramming necessitates the silencing of gene-expression programs that define a differentiated, mature somatic cell and the reactivation of embryonic genes of pluripotency. This has recently been achieved by the simultaneous over-expression of four transcription factors, the Yamanaka factors: Oct4, Sox2, c-Myc and Klf4 in adult somatic cells. This dedifferentiation process is powerful as it could be utilized to derive patient-specific pluripotent cell lines from any tissue of the body. These cell lines could be used for *in vitro* modeling of human diseases and as a source for autologous cell therapy, thus eliminating the need for immunosuppression and graft-versus-host diseases. The mechanisms by which the Yamanaka factors drive differentiated cells into a pluripotent stage are not fully understood. This inquiry is currently under intense research. In addition, new improved methods are needed for generating induced pluripotent stem (iPS) cells from differentiated adult somatic cells since they are currently inefficient (0.01–0.2%).

To address these limitations, five studies published in the 27 August 2009 issue of *Nature* showed that the inactivation of cyclin-dependant kinase (cdk) inhibitors and the tumor suppressor gene *p53* significantly improved the efficiency of iPS cell generation. Although these articles presented evidence of the implication of

p53 in cellular reprogramming, the precise mechanisms remain to be determined.

The manuscript by Utikal *et al.* [1] addressed the role of cell proliferation, senescence and the indirect involvement of p53 in iPS cell generation. The authors reported that the efficiency in the generation of iPS cells decreased even more in senescent cells and correlated with the elevated expression of the cdk inhibitors p16(INK4a), p19(ARF) and p21. Interestingly, cultures in low oxygen (4%) mitigated the senescence-induced cdk inhibitor expression, which resulted in a threefold increase in the efficiency of iPS cell generation. This efficiency in low oxygen was mediated by a decrease in the expression of p16(INK4a), p19(ARF) and p53. Using ARF-GFP knockin reporter mice, the authors confirmed that the level of p19(ARF) in the starting cell population influenced the efficiency of reprogramming. Cells expressing lower levels of p19(ARF) (ARF-GFP^{low}) are more amenable to reprogramming. The overexpression of the four reprogramming factors resulted in downregulation of ARF-GFP, while the expression of each of these reprogramming factors alone did not silence ARF-GFP expression. This suggests that the synergistic actions of at least two reprogramming factors is necessary to inhibit p19(ARF) and drive iPS cell generation.

Since they are mitotically active and less senescent, SSEA1-expressing precursor cells were presumed to be more likely to become iPS cells. However, only those SSEA1⁺ cells with low p19(ARF) levels showed a threefold increase in iPS cell efficiency. The authors used a PCR assay to demonstrate that cellular reprogramming is induced by gradual *INK4a/ARF* promoter methylation detectable after 9 days *in vitro*. Together these results suggest that the level of p19(ARF) is a better indicator of cells that are more amenable to iPS cell generation and that the four reprogramming factors act gradually by silencing the transcription of the *INK4a/ARF* locus through epigenetic modification.

As expected, the cellular immortalization induced by the complete deletion of either p19(ARF) or p53 resulted in a dramatic increase in iPS cell generation. p53-deficient fibroblasts more efficiently (three-times faster) generated iPS cells. In wild-type cells, the infection with lentiviral vectors expressing a shRNA against p53 increased the efficiency of iPS cell generation.

Together these results point out that higher levels of p19(ARF) and increased senescence negatively impact the efficiency of iPS cell generation. Yet, an increased rate of cell proliferation, which usually correlates to low cdk inhibitors and senescence, is more conducive to reprogramming. Utikal *et al.* demonstrated that during the first 9 days in culture, the reprogramming factors directly silenced the cdk inhibitors p16(INK4a) and p19(ARF) that normally activate p53. Further studies are needed to reconcile the discrepant findings that the epigenetic silencing of the *INK4a/ARF* locus is mediated by histone modifications [2], the kinetics of the repression and to clarify the direct versus indirect interactions with p53 and the reprogramming factors [3].

These findings advance our understanding in cellular dedifferentiation, reprogramming and cancer initiation. However, in regards to the use of iPS cells as a source of cells for cellular therapy, the alarming danger in manipulating p53 and p19(ARF) to increase iPS efficiency is the induction of genomic instability that may lead to deleterious aberrations, cancer stem cell generation and compromise the functionality and pluripotency of the iPS cells. Although there are common pathways activated during reprogramming and tumorigenesis, there are fundamental differences between iPS and transformed cancer stem cells. iPS cells display the unique property to form teratomas, a distinct and differentiated tumor that does not metastasize. In addition, iPS cells are responsive to exogenous signals. Before considering iPS cells for basic or translational studies, it is important to confirm these fundamental criteria in comparison to embryonic stem cells, currently the gold standard for pluripotency and cellular differentiation. Similarly, it is critical to fully characterize the *in vitro* functional properties of the iPS cell-derived tissue-specific cells and the *in vivo* reconstitution and functional integration without tumorigenicity.

References

- 1 Utikal J, Polo JM, Stadtfeld M *et al.*: Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* 460, 1145–1148 (2009).
- 2 Li H, Collado M, Villasante A *et al.*: The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature* 460, 1136–1139 (2009).
- 3 Krizhanovsky V, Lowe SW: Stem cells: the promises and perils of p53. *Nature* 460, 1085–1086 (2009).