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Working Out the Various Factors in iPS Cell Generation

by Jeremy Cherfas

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The excitement generated by the announcement, in November 2007, that adult human cells could be reprogrammed into stem cells, capable of differentiating into any type of cell, shows no signs of dying down. The original paper, from Shinya Yamanaka's group at Kyoto University in Japan, maintains its vice-like hold on the #1 spot despite a fundamental problem with these induced pluripotent stem (iPS) cells: one of the four triggers necessary to rejuvenate an adult cell is a retrovirus called Myc, and re-activating the c-Myc retrovirus gene unfortunately increases the likelihood of tumors developing among the descendants of iPS cells. A follow-up paper from essentially the same group, which addresses that fundamental difficulty in the original paper, has now arrived at #7.

Yamanaka's team undertook a very thorough examination of "family members" related to the four transcription factors that reprogram adult cells, each time using the standard package plus one homolog. For the first, Oct3/4, neither of the two closest homologs was able to induce iPS cells. The second factor, Sox2, has several homologs, of which six were tested. Sox1 did relatively well, and two of the others induced some iPS cells. The third factor is Klf4; three family members did induce iPS cells, but inefficiently. c-Myc, the troublesome retroviral oncogene, has two related proteins, and both N-Myc and L-Myc were able to induce pluripotency.

So far so good. But as Yamanaka's team reported, "unexpectedly" a few stem-cell like colonies were obtained in the absence of the Myc retrovirus. In the earlier studies, no iPS cells had been obtained in the absence of Myc. The team noted that one difference was in the timing of drugs to select the reprogrammed cells; previously, they had started selection after 7 days, but in the present study selection began after 14 days. As the team noted, "This suggested that iPS cell generation without Myc is slower than with Myc."

In a direct test of this hypothesis, the team started selection 7, 14, or 21 days after treating the cells with all four factors and without Myc. Four factors gave positive colonies under all three conditions, as expected, with substantially more iPS colonies when selection was delayed. Three factors gave no colonies if selection started at 7 days, again consistent with previous results, and increasing numbers with selection at 14 and 21 days. Even more interesting, the transformation was in one sense more efficient without Myc, in that the percentage of positive colonies was higher (even though the number of colonies was lower).

Injected into blastocysts (early-stage embryos), the three-factor iPS cells gave rise to complete healthy adult mice with a high proportion of tissues derived from the iPS cells. And these iPS cells were associated with fewer tumors. Of 37 animals derived from four-factor iPS cells, 6 died of tumors within 100 days of birth. By contrast all of 26 animals derived from three-factor iPS cells survived to 100 days. Of course these mice might have gone on to develop tumors later in life, as the team acknowledged.

Other experiments showed that under various scenarios the three factors, without Myc, were able to induce iPS cells at lower efficiency and higher specificity. And human cells? They too can be generated without using Myc. Colonies that resembled human embryonic stem cells were generated at a low rate from skin cells, and when these colonies were selected and expanded

Shinya Yamanaka

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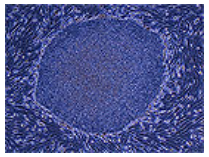

Human induced pluripotent stem cell.

Photo courtesy of Dr. Shinya Yamanaka, Kyoto University.

they generated cells that expressed markers typical of embryonic stem cells. Those cells could differentiate into at least three different cell types.

The paper's conclusion makes it clear that there are trade-offs. Without Myc there is a significantly lower risk of tumors developing. The efficiency of generating iPS cells is, however, considerably lower without Myc; in half the experiments with human cells, it was not possible to generate iPS cells in the absence of Myc.

Attention, in the meantime, has switched from the nuts and bolts of generating iPS cells to the ways in which they can be put to use and how they affect the ethical and practical landscape surrounding the use of stem cells, embryonic and induced. Parkinson's disease, heart disease, certain types of blindness and neurological damage have all been candidates for iPS therapy. Doubts about tumorigenicity remain, however, with some researchers calling for renewed efforts to understand and use true embryonic stem cells. A recent paper describing a method for creating iPS cells free of vector and transgene sequences is attracting a lot of interest, already registering in the Hot Papers Database (J. Yu, *et al.*, *Science*, 324[5928]: 797-801, 9 May 2009, with 13 citations this period), and much of the attention seems to be on using iPS cells to create populations of differentiated cells, including cells specifically intended to exhibit certain disease symptoms, that drug companies can use to screen new compounds.

Dr. Jeremy Cherfas is Science Writer at Bioversity International, Rome, Italy.

Biology Top 10 Papers

Rank	Paper	Citations This Period (Jul-Aug 09)	Rank Last Period (May-Jun 09)
1	K. Takahashi, *et al.*, "Induction of pluripotent stem cells from adult human fibroblasts by defined factors," *Cell*, 131(5): 861-72, 30 November 2007. [Kyoto U., Japan; CREST, Kawaguchi, Japan; Gladstone Inst. Cardio. Dis., San Francisco, CA] *243MG	88	1
2	Intl. HapMap Consortium (K.A. Frazer, *et al.*), "A second generation human haplotype map of over 3.1 million SNPs," *Nature*, 449(7164): 854-61, 18 October 2007. [72 institutions worldwide] *221LY	73	2
3	V. Cherezov, *et al.*, "High-resolution crystal structure of an engineered human beta$_2$-adrenergic G protein-coupled receptor," *Science*, 318(5854): 1258-65, 23 November 2007. [Scripps Res. Inst., La Jolla, CA; Stanford U., CA] 233JG	42	6
4	N.J. Wilson, *et al.*, "Development, cytokine profile and function of human interleukin 17-producing helper T cells," *Nature Immunol.*, 8(9): 950-7, September 2007. [Schering-Plough Biopharma, Palo Alto, CA; U. de Poitiers, France] *202QN	37	†
5	E.V. Acosta-Rodriguez, *et al.*, "Interleukins 1ß and 6 but not transforming growth factor-ß are essential for the differentiation of interleukin 17-producing human T helper cells," *Nature Immunol.*, 8(9): 942-9, September 2007. [Inst. Res. Biomed., Bellinzona, Switzerland] *202QN	35	†
6	A. Grimson, *et al.*, "MicroRNA targeting specificity in mammals: Determinants beyond seed pairing," *Cell*, 27(1): 91-105, 6 July 2007. [Howard Hughes Med. Inst., MIT, Cambridge, MA; Whitehead Inst., Cambridge, MA; Rosetta Inpharmatics, Seattle, WA] *190VK	34	†
7	M. Nakagawa, *et al.*, "Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts," *Nature Biotech.*, 26(1): 101-6, January 2008. [Kyoto U., Japan; CREST, Kawaguchi, Japan; Gladstone Inst., San Francisco, CA] *249IW	34	†
8	L. Zhou, *et al.*, "IL-6 programs T_H-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways," *Nature Immunol.*, 8(9): 967-74, September 2007. [NYU Sch. Med., and Howard Hughes Med. Inst., NY; NHLBI, Bethesda, MD] *202QN	33	†
9	Intl. Consortium for SLEGEN, *et al.*, "Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in *ITGAM*, *PXK*, *KIAA1542* and other loci," *Nature Genetics*, 40(2): 204-10, February 2008. [11 institutions worldwide] *256MJ	33	†
10	L. Yang, *et al.*, "IL-21 and TGF-ß are required for differentiation of human T_H17 cells," *Nature*, 454(7202): 350-3, 17 July 2008. [Harvard Med.Sch., Boston, MA] *326ND	33	†

SOURCE: Thomson Reuters *Hot Papers Database*. Read the Legend.

KEYWORDS: STEM CELLS, INDUCED PLURIPOTENT STEM CELLS, IPS CELLS, SHINYA YAMANAKA, MYC RETROVIRUS.

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