Scientists Make Stem Cells that Are Accepted by the Ethical Community

By Colby Chiang '10 In November, two teams of scientists published methods of generating embryonic-like stem cells without destroying an embryo, a finding that could quell the ethical controversy surrounding stem cell research. The two independent research teams, headed by James Thomson of the University of Wisconsin-Madison and Shinya Yamanaka of Kyoto University in Japan, developed methods to induce pluripotency, the quality that allows stem cells to develop into any kind of body cells. The “induced pluripotent stem cells” (iPS cells) can then be differentiated into any of the body's 220 somatic cell types. Until now, the only way to acquire pluripotent cells was to harvest them from a fertilized embryo, destroying the embryo in the process.

The technique to reprogram the cells is surprisingly simple. Scientists must only add four genes, which in turn activate a cascade of other factors that rewind cell development. To introduce the genes into the cells, they use retrovirus vectors, which have the ability to insert short sequences into DNA. Once the relevant DNA is inducted into the cell, it activates genetic pathways that revert the cell to its pluripotent state.

This discovery in human cells was preceded by similar findings in July of 2006, when Shinya Yamanaka created iPS cell lines from the tail cells of mice (1). Now Yamanaka's team has managed to apply the same technique to human cells, even using the same set of genes. The group has generated iPS cell lines both from the facial dermal fibroblast cells of a 36-year-old woman and from the connective tissue of a 69-year-old man.

Yamanaka discovered the genes necessary to induce pluripotency by systematically testing various combinations from a pool of candidate genes known to be highly expressed in embryonic stem cells. He began with over a thousand candidate genes, but slowly narrowed the search to a few dozen. Eventually, he arrived at a set of four: OCT3/4, SOX2, KLF4, and c-MYC. While the exact role of each gene is unclear, OCT3/4 and SOX2 are believed to be master switches that control other genes that are crucial to stem cell behavior. KLF4 is known to alter histone acetylation, and may reconfigure the chromatin structure to allow other genes to reach their targets. c-MYC may stimulate cell growth and reproduction (2).

James Thomson and his colleagues simultaneously achieved the same result as Yamanaka's group using cells from a newborn's foreskin. But in an interesting twist, Thomson used a slightly different cocktail of genes. Like Yamanaka's group, Thomson used OCT3/4 and SOX2, but he accompanied them with NANOG and LIN28. NANOG is associated with stem cell pluripotency, and LIN28 is involved in messenger RNA processing. Thomson says he tried Yamanaka's genes without success, and Yamanaka did not test LIN28, as it was not in his original candidate pool

(3, 4). The reason for the different results may lie in the varying relative doses of the genes or the different retroviruses used. Clarifying the differences and similarities between the two techniques may help scientists to gain a deeper understanding for the functions of these genes.

While scientists are not yet certain that the iPS cells are actually embryonic stem cells, they appear identical in almost every measurable way. Yamanaka reports that "[t]he established human iPS cells are similar to [human embryonic stem] cells in many aspects, including morphology, proliferation, feeder dependence, surface markers, gene expression, promoter activities, telomerase activities, in vitro differentiation, and teratoma formation" (2). Scientists must investigate further to determine whether the iPS cells differ on the epigenetically or in their RNA profiles (2, 4). But even if there are subtle differences, the iPS cells seem to have every quality of embryonic stem cells that makes them medically useful.

The discovery shows several promising avenues for future medical inquiry. The most direct use for iPS cells is to grow new tissues for transplants. Since these tissues would be derived from the patient's own cells, the grafted tissues would not face transplant rejection. Until now, the only way to achieve exact matches was through cloning.

Already, scientists at the Whitehead Institute in Cambridge, Massachusetts have successfully used iPS cells to treat a mouse model of sickle cell anemia (5). Sickle cell anemia is a genetic disorder in which red blood cells are misshapen, causing them to stick to blood vessels and carry oxygen poorly. The scientists treated fibroblasts from the tail of a mouse model of sickle cell anemia with Yamanaka's technique to turn them into iPS cells. They then differentiated the iPS cells into hematopoietic progenitors (HPs), cells which have the ability to create new blood cells in vivo. The scientists treated the HPs to correct the defective gene and reinserted them into the mouse to generate healthy blood cells that would replace the abnormal ones. In the future, scientists look to expanding this research to other diseases and applying them to human treatment.

A more immediate prospect of iPS cells may be their use in studying disease progression and etiology. Researchers who want to study the brain of a patient who is afflicted with a genetic condition can now simply create iPS cells derived from the patient's tissue and differentiate them into neural cells. Along the way, the scientists can study the development of the cells under different treatments and conditions. This technique may prove especially useful in studying degenerative conditions such as Parkinson's or Alzheimer's disease.

But these new stem cells face a series of challenges before they can be considered for use in humans. First, scientists must find a way to prevent iPS cells from causing cancer. Because of stem cells' high growth rate, they are prone to producing tumors if not properly controlled. The problem is exacerbated in iPS cells, where the retrovirus vector that introduces the genes inserts them randomly into the genome, unpredictably altering the DNA. Furthermore, one of the genes

used by the Yamanaka team (c-MYC) is a proto-oncogene, meaning that it is especially prone to cancerous mutations (2, 3, 4).

Moreover, before they can transplant stem cells into humans, scientists must further investigate how the transplants will integrate themselves into the body. Sometimes a treatment that is effective locally wreaks havoc on other systems in an organism. For example, researchers at the Karolinska Institute in Sweden successfully used stem cell grafts to repair spinal cord damage in mice. The mice, however, became hypersensitive to pain in other parts of their bodies (6).

Scientists remain optimistic in overcoming these challenges. Shinya Yamanaka and others are currently exploring methods of activating the four genes with chemicals or benign viruses. These experiments are seeing early promise, and if they succeed, would eliminate the need for dangerous retroviruses.

The new discovery symbolizes a resurrection of sorts for stem cell research in the United States, where it has been hindered by ethical controversy. In August of 2001, President George W. Bush restricted federal funds to stem cells that were already in existence. This constraint limited researchers to only 21 cell lines. Many of these lines, however, grow too slowly to be useful and others have become contaminated or mutated over the years (7). The iPS cells created using Thomson or Yamanaka's technique would bypass these restrictions, since they can be generated without harming embryos. If the technique can be perfected it would provide scientists with a virtually unlimited supply of raw material for stem cell research.

Still, scientists warn that traditional embryonic stem cell research should not be abandoned. As exciting as the iPS discovery is, researchers must still clear significant hurdles before iPS cells are on equal footing with embryonic stem cells. But even with the challenges that lie ahead, Yamanaka and Thomson have solved a crucial problem for stem cell research, leaving the field open for a vast potential of medical advances in years to come.

1. K. Takahashi and S. Yamanaka, Cell 126, 663 (2006).
2. K. Takahashi et al., Cell 131, 861 (2007).
3. G. Vogel and C. Holden, Science 318, 1224 (2007).
4. M. Stojkovic and D. Phinney, Stem Cells 26, 1 (2008).
5. J. Hanna et al., Science 318, 1920 (2007).
6. C. Hofstetter et al., Nature Neuroscience 8, 346 (2005).
7. A. Abbott et al., Nature 442, 336 (2007).