Science

Demonstrating the Pluripotency of Induced Pluripotent Stem (iPS) Cells

Since induced pluripotent stem (iPS) cells were first made in the Yamanaka laboratory at Kyoto University several years ago, there have been lingering doubts among scientists about the true developmental potential of these cells. Are they truly pluripotent? Some scientists thought not. This past quarter, three laboratories, two in China and one in the United States, reported that they had used iPS cells to generate intact adult mice (X. Zhao et al., "iPS Cells Produce Viable Mice through Tetraploid Complementation," *Nature*, September 3, 2009; M. J. Boland et al., "Adult Mice generated from Induced Pluripotent Stem Cells," *Nature*, September 3, 2009; and L. Kang et al., "iPS Cells Can Support Full-Term Development of Tetraploid Blastocyst-Complemented Embryos," *Cell Stem Cell*, August 7, 2009). These groundbreaking experiments demonstrate that iPS cells, at least mouse iPS cells, like human embryonic stem cells, are truly pluripotent. In other words, they are able to produce all the cell types of the adult organism.

To illustrate the experimental approach used, we focus on one approach of these research papers. To begin, one group of Chinese scientists created iPS cells from an adult mouse that was black. Then the research team created a tetraploid embryo by fusing together two cells from an early-stage white mouse embryo. Such embryos are called tetraploid embryos because they have four (tetra) copies of each chromosome in their cells rather than the two copies, one from mom and one from dad, that mice and human beings normally have in their cells.

Tetraploid embryos are unable to develop normally. They are able to develop only into embryonic-like entities that have a placenta without the rest of the body-proper of the embryo. In fact, because of this fundamental defect in organization, it is unlikely that tetraploid embryos are bona fide embryos. However, when one Chinese team transplanted black-mouse-derived iPS cells into 624 white-mouse tetraploid embryos, some of the resulting entities (22 embryos, or 3.5 percent of the total number) were able to develop into black adult mice, many of which were fertile and able

to themselves mate and reproduce. Genetic tests revealed that the cells of the adult black mice were completely derived from the implanted iPS cells. Thus, at least in mice, iPS cells have passed the definitive test for pluripotency: like embryonic stem cells, they are able to generate all the cell types of the adult organism.

One may ask, philosophically, how do we understand what happened here? Basically, neither a tetraploid embryo-like entity nor an iPS cell on its own is a bona fide embryo. However, when both are mixed together, they are able to reconstitute an embryo with the inherent ability to undergo organismal organization and development. It is a technological innovation that generates a substantial change. The creation of these embryos from the union of tetraploid embryo-like entity and iPS cells is akin to the creation of an embryo from the union of sperm and egg. It is another way of making an organism.

Finally, and sadly, we have to also point out that these studies have unveiled another method for cloning a human being, a gravely immoral act. Previously, cloners could clone a human being only by using a somatic cell nuclear transfer technique pioneered by Ian Wilmut, who made the famous sheep named Dolly. Now future cloners can clone a human being by first obtaining his iPS cells and inserting them into a tetraploid human embryo, which would then go on to develop into a mature diploid human organism with two copies of every chromosome in the human genome.

Increasing the Efficiency and Safety of the iPS Cell Protocol

A handful of recent papers report discoveries that increase the efficiency and the safety of the nuclear reprogramming protocol at the heart of the iPS technology.

First, a team from the Schöler Laboratory at the Max Planck Institute for Molecular Biomedicine in Münster, Germany, has been able to reprogram human fetal neural stem cells to the pluripotent state using just one gene, *OCT4*, as opposed to the four factors previously needed to generate human iPS cells (J. B. Kim et al., "Direct Reprogramming of Human Neural Stem Cells by *OCT4*," *Nature*, August 28, 2009). Ten to eleven weeks after the research group introduced *OCT4* into the human fetal neural stem cells, they noticed that these cells had given rise to cell cultures that resembled pluripotent human embryonic stem cells. Further analysis showed that these colonies of reprogrammed cells not only expressed human embryonic stem cell genes but were also able to become cells of a variety of cell types from all three germ layers. This is the standard experimental test for pluripotency. Earlier this year, the same German team had succeeded in reverting mouse adult neural stem cells to the pluripotent state using the same one-gene technique. Eliminating the use of the three other Yamanaka genes, including one that could predispose the iPS cell to cancer, should make it easier for scientists to create safer stem cell therapies.

Next, several research groups have discovered that specialized adult cells made immortal with the inactivation of an antitumor genetic pathway regulated by the antitumor gene *P53* can be reprogrammed into pluripotent cells quickly and efficiently (H. Hong et al., "Suppression of Induced Pluripotent Stem Cell Generation by the p53-p21 Pathway," *Nature*, August 27, 2009; H. Li et al., "The Ink4/Arf Locus Is a Barrier for iPS Cell Reprogramming," *Nature*, August 27, 2009; T. Kawamura et al.,

"Linking the p53 Tumour Suppressor Pathway to Somatic Cell Reprogramming," *Nature*, August 27, 2009; R. M. Marión, "A p53-Mediated DNA Damage Response Limits Reprogramming to Ensure iPS Cell Genomic Integrity," *Nature*, August 27, 2009; and J. Utikal et al., "Immortalization Eliminates a Roadblock During Cellular Reprogramming into iPS Cells," *Nature*, August 27, 2009). The five teams of scientists were able to reprogram up to 10 percent of skin cells that had a silenced *P53* pathway, an improvement in the efficiency of the iPS protocol of at least one hundredfold. Although the data suggest that nuclear reprogramming will be easier, the methods still involve genetic manipulation of genes associated with cancer. The research teams are now trying to identify chemicals that only *transiently* silence *P53* so that the pluripotent stem cells generated by this process are not themselves prone to becoming cancer cells.

Finally, a research team based at Worcester Polytechnic Institute's Life Sciences and Bioengineering Center has shown that skin cells can develop pluripotent-cell-like characteristics if they are grown under special culture conditions (R. L. Page et al., "Induction of Stem Cell Gene Expression in Adult Human Fibroblasts without Transgenes," *Cloning Stem Cells,* September 2009). The team simply grew the skin cells under conditions with lower atmospheric oxygen and in the presence of fibroblast growth factor 2 (FGF2), a naturally occurring protein that is known to be involved in the maintenance of pluripotent human embryonic stem cells. This could become a significant advance, because it suggests that it may be possible to induce and to manipulate the activity of different pluripotency genes without introducing any genes into these cells, a complicated and potentially cancer-causing process.

Using iPS Cells to Understand Normal and Diseased Biological Processes

Induced pluripotent stem cells are exciting because of their potential use in biomedical research and in medicine. Several papers published this quarter continue to illustrate the power of these cells. First, Doug Melton and his colleagues at the Harvard Stem Cell Institute have taken skin cells from patients with type I diabetes and reprogrammed them to the pluripotent state using the iPS protocol (R. Maehr et al., "Generation of Pluripotent Stem Cells from Patients with Type 1 Diabetes," *Proceedings of the National Academy of Sciences USA*, August 31, 2009). These iPS cells were then manipulated and transformed into cells capable of making insulin. Recall that patients with type I diabetes are unable to produce their own insulin. These cells will be important for modeling and for understanding type I diabetes. Eventually, this method may also allow scientists to develop insulin-producing cells that could be used to treat diabetes with cell replacement therapy.

Next, a team from the University of Wisconsin School of Medicine and Public Health has successfully grown multiple types of retina cells—the cells important for sight in the eye—from both human embryonic stem cells and iPS cells (J. S. Meyer et al., "Modeling Early Retinal Development with Human Embryonic and Induced Pluripotent Stem Cells," *Proceedings of the National Academy of Sciences USA*, August 25, 2009). This discovery should help scientists not only understand the complex process of retina development but also treat patients with retinitis pigmentosa, an inherited condition that leads to blindness. For example, skin cells from patients

with retinitis pigmentosa could be reprogrammed into iPS cells and then into retina cells, which could then be used by researchers to identify drugs that could treat or cure this condition.

Finally, iPS cells generated from patients with familial dysautonomia, a rare disease that affects those nerve cells that are involved in touch, blood pressure, and tear flow, have been used to test drugs for their effect against the disease (G. Lee et al., "Modelling Pathogenesis and Treatment of Familial Dysautonomia Using Patient-Specific iPSCs," *Nature*, September 17, 2009). One drug, called kinetin, showed promise in treating these diseased cells. Clinical trials of the drug are scheduled to start soon. Moreover, the team discovered that familial dysautonomia cells lacked the ability to become neurons and did not migrate as easily as normal cells in a culture dish. The study realizes one of the major goals of stem cell research: it demonstrates that iPS cells can be used to study the effects of disease in a patient's own cells and to identify drugs to treat that disease.

Recent Advances in Gene Therapy: Blindness and Mitochondrial Disease

I close this narrative by reporting advances in gene therapy, protocols that could be used eventually to alter the genetic constitution of human patients. Results of a recent study have demonstrated that it is possible to use gene therapy to cure monkeys with color blindness (K. Mancuso et al., "Gene Therapy for Red-Green Colour Blindness in Adult Primates," *Nature,* September 16, 2009). Scientists working at the University of Washington and the University of Florida were able to cure two squirrel monkeys of their color blindness by injecting viruses that contain the genes that produce color-detecting proteins into the eyes of these animals. Male squirrel monkeys are unable to distinguish between red and green. Five months after the injections, however, the two experimental subjects were able to distinguish red and green dots randomly displayed against a background of grey dots. Incredibly, it suggests that gene therapies for severe forms of human color blindness and for age-related macular degeneration could also correct these conditions in patients. The authors of the paper also speculate that this technology could be used to engineer eyes with remarkable capabilities like enhanced night vision or the ability to see new wavelengths of light.

Finally, in a paper that made headlines throughout the world, a research team from the Oregon Health and Science University has used technology originally developed for somatic cell nuclear cloning to correct a defect in the mitochondrial genes of an organism (M. Tachibana et al., "Mitochondrial Gene Replacement in Primate Offspring and Embryonic Stem Cells," *Nature*, August 26, 2009).

To understand the experiment, we need to review some basic cell biology: The genes in an animal or plant cell can be found in one of two locations. Most of a cell's genes—99 percent or more of the total—can be found in its control center called the nucleus. However, a small number of genes can be found in the mitochondria, the part of the cell involved in generating the chemical energy needed for life. In humans, if an egg with genetically defective mitochondria is fertilized, the child who is conceived could have any one of a large number of diseases including dementia, hypertension, Alzheimer's disease, or cancer.

To prevent this from happening, the research group developed a technique in monkeys to transfer the nucleus of the defective egg into another egg that contained healthy mitochondria but lacked its own nucleus. Reconstituted eggs with both healthy nuclear DNA and mitochondrial DNA were then fertilized with sperm. The embryos developed normally into healthy offspring with no apparent birth defects. The authors of the scientific research paper note that their procedure can be used to prevent the transmission of defective mitochondria in families with hereditary mitochondrial DNA disease. In the end, the team from Oregon has invented a technique that can be used to produce a child with three genetic parents: A parent who contributed the nuclear genes from the egg, a parent who contributed the nuclear genes from the sperm, and a third parent who contributed the healthy mitochondrial genes.

Some bioethicists have suggested that changing an individual's mitochondrial DNA does not really change the individual's genetic makeup. I disagree. Variations in the mitochondrial DNA called mitochondrial genetic polymorphisms have been associated with different ethnic groups and with predilections to different diseases. One recent study has even suggested that specific mitochondrial genetic variations may be associated with the prowess of elite Kenyan athletes.[1] How can one's ethic roots, one's disposition to disease, and one's athletic ability (or lack of it) not be integral dimensions of one's genetic makeup?

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[1]R. A. Scott et al., "Mitochondrial Haplogroups Associated with Elite Kenyan Athlete Status," *Medicine and Science in Sports and Exercise* 41.1 (January 2009): 123–128.