Science

Primate Cloning and Nuclear Reprogramming (iPS) Technology: The End of the Stem Cell Wars

This past quarter, several landmark papers were published that forever changed the context of the stem cell wars. In a paper published online in mid November, Shoukhrat Mitalipov and his team at the Oregon National Primate Research Center reported that they were able to obtain embryonic stem cells from a cloned primate, in this case, a cloned rhesus macaque embryo, for the first time (J. A. Byrne et al., "Producing Primate Embryonic Stem Cells by Somatic Cell Nuclear Transfer," *Nature*, November 22, 2007). By using a noninvasive imaging system called Oosight to avoid damaging either the egg or the donor nucleus, the research group generated two embryonic stem cell lines by inserting the nuclei of skin cells taken from a nine-year-old male rhesus monkey into 304 eggs collected from fourteen female monkeys. Although the experiment is a proof of principle that shows that primates can now be cloned to generate embryonic stem cell lines, the low success rate of 0.7 percent suggests that the technique will have to be drastically improved before it could ever be used to generate human embryonic stem cells for therapy. Nevertheless, the study makes it likely that human embryonic stem cells will be generated from a cloned human embryo in the near future—probably at Newcastle University in the United Kingdom.

Despite widespread publicity reporting the advance in primate cloning, this major achievement was soon overshadowed by even more significant breakthroughs in nuclear reprogramming technology. On November 20, 2007, two research teams, one in Japan and the other in the United States, independently reported that they had successfully reprogrammed adult human cells into induced pluripotent stem (iPS) cells that are indistinguishable from pluripotent stem cells taken from human embryos.

First, in new work that built upon their groundbreaking paper published last year, where they reprogrammed adult mouse tail cells with four genes, *OCT4, SOX2,*

C-MYC, and *KLF4*, Shinya Yamanaka and his colleagues at Kyoto University in Japan report that they have successfully taken skin cells from a thirty-six-year-old woman and connective cells from a sixty-nine-year-old man and reprogrammed them into human iPS cells. These cells passed all the tests for pluripotency used to identify embryonic stem cells taken from human embryos. In their paper, the team used retroviruses to introduce the same four genes originally used with mice into the human cells (K. Takahashi et al., "Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors," *Cell*, November 30, 2007). Two weeks later, however, the same team reported that they were able to create human iPS cells with only three genes (M. Nakagawa et al., "Generation of Induced Pluripotent Stem Cells without Myc from Mouse and Human Fibroblasts," *Nature Biotechnology*, online November 30, 2007). Significantly, their experiments did not include the *C-MYC* gene that has been implicated in tumor formation.

Next, James A. Thompson and his team at the University of Wisconsin-Madison—this is the same research group that first identified human embryonic stem cells over a decade ago—published a paper describing their experiments to reprogram skin cells taken from a human fetus and from human foreskin (J. Yu et al., "Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells" *Science*, December 21, 2007). They showed that four genes, *OCT4, SOX2, NANOG,* and *LIN28*, were sufficient to reprogram these cells to a pluripotent state. These four genes were different from the original four used by the Yamanaka team, suggesting that different sets of genes can be used to create iPS cells. Moreover, the report also showed that the cancer-causing gene *C-MYC* is not essential for nuclear reprogramming. The team concluded, "The human iPS cells described here meet the defining criteria that we originally proposed for human embryonic stem cells, with the notable exception that the iPS cells are not derived from embryos. Similar to human embryonic stem cells, human iPS cells should prove useful for studying the development and function of human tissues, for discovering and testing new drugs, and for transplantation medicine. For transplantation therapies based on these cells, with the exception of autoimmune diseases, patient-specific iPS cell lines should largely eliminate the concern of immune rejection."

These two landmark papers and a paper published several weeks later by the Daley Laboratory at Harvard University—where George Daley and his colleagues reprogrammed somatic cells taken from a fresh skin biopsy from a volunteer (I-H Park et al., "Reprogramming of Human Somatic Cells to Pluripotency with Defined Factors," *Nature*, online December 23, 2007)—demonstrate that nuclear reprogramming technology works with human cells and with different combinations of genes.

The generation of patient-specific human embryonic stem cells with cloning technology has been promoted in the public square to accomplish two goals. First, this approach could be used to obtain disease-specific embryonic stem cells that could be used as laboratory reagents to study and better understand disease. Although they did not use cloning technology (SCNT) to obtain their disease-specific embryos, Rachel Eiges et al. illustrate this research interest in a paper describing their efforts to study fragile X syndrome by creating pluripotent embryonic stem cell lines from IVF embryos that carried the mutation for the genetic condition ("Developmental

Study of Fragile X Syndrome Using Human Embryonic Stem Cells Derived from Preimplantation Genetically Diagnosed Embryos," *Cell Stem Cell,* November 2007). Using the fragile-X embryonic stem cells, they were able to study the early stages of the disease. They conclude that their study "illustrates the importance of [human embryonic stem cells] in unraveling developmentally regulated mechanisms associated with human genetic disorders." Similar work has already been done with eighteen different genetic disorders by the Verlinksy Laboratory at the Reproductive Genetics Institute in Chicago. Nuclear reprogramming (iPS) technology can be used to accomplish this first goal. It should now be relatively straightforward to reprogram cells taken from patients with Lou Gehrigs disease, fragile X syndrome, or any other debilitating illness to created disease-specific pluripotent stem cells.

Second, patient-specific embryonic stem cells obtained with cloning technology could be used to treat different diseases using regenerative medicine. At the present time, nuclear reprogramming (iPS) technology, because of its reliance on the use of retroviruses, cannot be used to accomplish this second goal. Retroviruses randomly insert themselves into a cell's DNA, leading to an increased risk of cancer. Thus, as every stem cell researcher has acknowledged, the challenge in the near future will be to discover a new technique to reprogram a cell without using these viruses. Other options include using viruses that do not integrate into the host cell's DNA and using small molecules—drugs—to activate the endogenous reprogramming genes. Only then will iPS technology be safe enough for patient use.

Nevertheless, in a stunning paper, Rudolf Jaenisch and his colleagues at MIT report that they have used iPS technology to cure sickle cell anemia in mice ("Treatment of Sickle Cell Anemia Mouse Model with iPS Cells Generated from Autologous Skin," *Science,* online December 6, 2007). Sickle cell anemia is a disease of the blood caused by a defect in a single gene. To create the iPS cells, the research team began with cells from the diseased mice. These cells were then reprogrammed with retroviruses and the four genes identified by the Yamanaka team. To decrease or eliminate possible cancer in the treated mice, however, the MIT group removed the *C-MYC* gene after the reprogramming was complete. Next, the scientists transformed the iPS cells into bone marrow precursor cells, replaced the defective gene in the bone marrow cells with a normal version of the gene, and then injected the resulting altered cells back into the diseased mice. Later testing showed that the mice had been cured of their disease. Surprisingly, none of the mice developed cancer after twenty weeks. However, most scientists believe that the eventual development of cancer is inevitable because of the retroviruses used to cure the mice. The researchers conclude, "Our results provide proof of principle for using transcription factor-induced reprogramming combined with gene and cell therapy for disease treatment in mice." Indeed, the pace of discovery with nuclear reprogramming (iPS) technology has been breathtaking.

In toto, these papers should signal the beginning of the end of the stem cell wars. It is not surprising that Ian Wilmut, creator of Dolly, the first cloned mammal, has announced that his laboratory has given up on cloning technology. Instead, his team will focus all their efforts on perfecting the nuclear reprogramming (iPS) approach. Similarly, in a perspective published in *Science* ("Is Therapeutic Cloning

Dead?" December 21, 2007), stem cell biologist Jose Cibelli, commenting on the breakthroughs in nuclear reprogramming technology, writes, "Is human therapeutic cloning no longer needed? The short answer is no, but it is likely a matter of time until all the hypothetical advantages of therapeutic cloning will be implemented with induced pluripotent stem cells. More importantly, the controversial issues (ethical and technical) specific to human therapeutic cloning may well be left behind along with the procedure itself, a refreshing change for the field, indeed."

Finally, I would like to comment briefly on a recent press release from the pro-life and Catholic group Children of God for Life ("Adult Stem Cells Reprogrammed Using Aborted Fetal and HES Cells," January 8, 2008, www.cogforlife.org). In their statement, the group points out that the experiments with the human iPS cells used cell lines originally derived from the tissues taken from an aborted human being. It is important to note that the cell lines in question are standard cell lines used in laboratories throughout the world, and most scientists who use them are unaware of their origin. Furthermore, nuclear reprogramming (iPS) technology can easily be accomplished using cell lines not tainted by the grave immorality of abortion.

Gene Therapy

Efforts to use genetic therapy to cure disease have often raised moral questions for bioethicists. In a paper published this past quarter, Andrew Feigin and colleagues report that brain scans used to evaluate patients who received an experimental gene therapy for Parkinson's disease show that the treatment normalizes brain function and that this improvement is still present a year later ("Modulation of Metabolic Brain Networks after Subthalamic Gene Therapy for Parkinson's Disease," *PNAS*, December 4, 2007). The research team at Weill Cornell Medical College in New York City used a virus to introduce genes that increase the production of an inhibitory chemical called GABA, which lowers the activity of nerve cells involved in Parkinson's, into the brains of patients with the disease. The patients received the gene therapy only in the one side of their brain that controlled the side of their bodies most affected by the disease. Brain scans revealed that the nerve cells on the treated side got better while the nerve cells on the untreated side got worse. The improvement in the nerve cells correlated well with improvements in patients' motor skills evaluated in the clinic. Efforts are now under way to include a larger number of patients in a phase II clinical trial.

HIV Infectivity

In light of the debate among Catholic moral theologians over the morality of condom use to prevent the transmission of HIV between married couples, it is interesting to note a paper by Jan Münch et al. ("Semen-Derived Amyloid Fibrils Drastically Enhance HIV Infection," *Cell*, December 14, 2007), who show that semen dramatically increases the infectivity of HIV. The research team in Germany discovered that a protein found in semen, called prostatic acidic phosphatase (PAP), allows the virus to better infect the cells of its human host. This protein, especially in an acidic environment that mimics the vaginal tract, increases the rate of HIV infection by up to one hundred thousand times by forming microscopic fibers that

entrap the virus and facilitate its attachment to host target cells. The protein is now a potential target for drugs that could be used to prevent or at least severely diminish the transmission of HIV during sexual intercourse.

The Biological Basis of Homosexuality

Finally, David Featherstone and his colleagues at the University of Illinois at Chicago report that they have discovered a gene in fruit flies, which they call *genderblind*, or GB, a mutation which turns flies bisexual ("A Glial Amino-Acid Transporter Controls Synapse Strength and Homosexual Courtship in *Drosophila*," *Nature Neuroscience,* January 2008). GB regulates the transport of the neurotransmitter glutamate in the brain, altering the strength of nerve cell connections called synapses. It appears that *genderblind* mutant flies were no longer recognizing male pheromones as a repulsive stimulus. Significantly, the paper also shows that homosexual behavior in flies could be turned on and off by drugs that alter synapse strength. These results demonstrate that sexual orientation—at least in the fruit fly—is not genetically determined or hard-wired. Rather, it is shaped by both genetic and environmental factors that influence synapse strength. In humans, the process of strengthening or weakening synapse strength in the absence of drugs is called learning.

Breakthrough of the Year: Personalized Genomics

At the end of every year, the editors and news staff of the journal *Science* look back at the big science stories of the past twelve months and choose one as the breakthrough of the year. For 2007, they pinned the blue ribbon on the emerging field of personal genomics, citing papers like the one by Samuel Levy and his colleagues for the revolution that they herald in medicine. In their fascinating paper, published in *PLoS Biology*, Levy et al. describe the genomic sequence of J. Craig Venter, lead researcher on the team, who donated his DNA for analysis ("The Diploid Genome Sequence of an Individual Human," *PLoS Biology*, September 4, 2007). This study and others like it are revealing the extent to which we as individual human beings differ from each other. They are also helping scientists to better understand the genetic basis behind disease on a case-by-case basis. These papers are only a foreshadowing of the research that will be upon us when genome scientists attain their goal of the $1,000 genome. (The X Prize Foundation has promised $10 million to the first person who can sequence a genome for $1,000.) It will change how we do medicine when each one of our genomes can be deciphered in all its guts and glory, intensifying the bioethical questions that come with diseased-gene testing. Interestingly, however, these studies could also help us to better answer a perennial question: What makes me, me?

Rev. Nicanor Pier Giorgio Austriaco, O.P., Ph.D.
Providence College
Providence, Rhode Island