Science

iPS Technology Update: Gene-Free Nuclear Reprogramming and the Correction of Genetic Disease

Over the years, these notes have chronicled the ongoing developments in the stem cell wars that have dominated much public debate in the United States and elsewhere. In many ways, I do not think that this public debate will advance definitively until one technology, either human embryonic stem cells or iPS cells, is used to effectively and permanently cure a patient of a debilitating disease. This past quarter, several advances with iPS cells brings them closer to this goal.

For the first time, human skin cells have been reprogrammed into the pluripotent state without using DNA (Dohoon Kim et al., "Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins," *Cell Stem Cell*, June 5, 2009, no abstract). Instead, the reprogramming factors were made in mammalian cells such that they would be secreted into the culture media. They were also genetically modified so that they could enter the nucleus of any cells that were exposed to the culture media. When human skin cells were actually bathed in this media over the course of eight weeks, they were reprogrammed into pluripotent iPS cells. These iPS cells passed all the standard tests for pluripotency and had the genetic signature usually associated with embryonic stem cells. It is the first method that could generate patient-specific stem cells without temporarily or permanently modifying the cells' DNA. Recall that one of the most significant problems with the original iPS methodology developed by Shinya Yamanaka three years ago is that it modified the DNA of the iPS cells in such a way that recipients of those cells would have a heightened risk of developing cancer. Thus, with the advent of this gene-free technology, we now have iPS cells that could be used to treat patients without increasing their risk of cancer.

The gene-free reprogramming method accomplished in human cells described above is similar to the first DNA-free approach described by Hongyan Zhou and colleagues at the Scripps Research Institute in San Diego, California, in April of this

year ("Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins," *Cell Stem Cell*, May 8, 2009, no abstract). Working with mouse cells, the San Diego team began with the four most commonly used Yamanaka reprogramming factors. They then used genetic engineering to add poly-arginine tails to the factors, a modification that would allow them to penetrate and enter mammalian cells. After purifying these modified factors from bacteria, the research team from California used them to reprogram mouse cells to the pluripotent state. They simply exposed these cells to the purified factors in the presence of another molecule called valproic acid.

Finally, in a proof-of-concept paper, Ángel Raya and colleagues successfully used iPS technology in the laboratory to correct the genetic defect in Fanconi anemia (FA) cells ("Disease-Corrected Haematopoietic Progenitors from Fanconi Anaemia Induced Pluripotent Stem Cells," *Nature*, May 31, 2009). FA is a rare hereditary disease that affects the bone marrow in such a way that it cannot produce sufficient blood cells. The research team took hair or skin cells from patients with FA and corrected their defective genes using somatic cell gene therapy. They then successfully reprogrammed these genetically repaired cells into the pluripotent state using iPS technology. Notably, the resulting FA-iPS cells were indistinguishable from either human embryonic stem cells or iPS cells generated from healthy donors. Since the most significant symptoms of FA arise from the bone marrow's failure to give rise to the diversity of blood cells found in a healthy human being, the team concluded their study by testing their FA-iPS cells to determine if these cells could become the blood progenitor cells that are needed to regenerate healthy blood. The FA-iPS cells could, suggesting that one day these cells may be transplanted into patients to cure FA.

To recap the significance of these papers: These studies demonstrate that iPS technology can now be used to create patient-specific pluripotent stem cells that are safe enough for human transplantation and effective enough to cure genetic disease. They support the claim made by opponents of destructive embryo research that human embryonic stem cells are not necessary for the ongoing development of regenerative medicine.

Embryonic Stem Cell Update: Micro-RNAs, the Nature of Pluripotency, and the Problem of Tumor Development

One of the most important questions in the field of stem cell research is the following: What is the nature of pluripotency? Or to put it another way, what makes a pluripotent stem cell pluripotent? This past quarter, two papers suggest that the answer to this question will necessarily have to include microRNAs (miRNAs). Discovered only recently, miRNAs are small molecules that turn other genes on or off. They have been shown to be involved in the process of animal development. The two papers summarized here were the first to link them with stem cell biology and pluripotency.

First, Na Xu and colleagues at the University of California, Santa Barbara, have discovered that the levels of the miRNA miR-145 change dramatically when human embryonic stem cells lose their pluripotency and become specialized cells ("MicroRNA-145 Regulates OCT4, SOX2, and KLF4 and Represses Pluripotency in Human Embryonic Stem Cells," *Cell*, April 30, 2009). Moreover, they demonstrated that this single molecule is able to turn off three important Yamanaka reprogram-

ming factors, Oct4, Sox2, and Klf4. They conclude that miR-145 is a key factor in defining pluripotency: low levels of miR-145 are associated with pluripotency while high levels of the molecule are linked to the loss of pluripotency and the process of cell specialization.

Next, a team from the University of California, San Francisco, used miRNAs usually found in embryonic stem cells to generate iPS cells in mice (R. L. Judson et al., "Embryonic Stem Cell-Specific microRNAs Promote Induced Pluripotency," *Nature Biotechnology*, May 2009). Using a combination of viral infection that introduced three reprogramming factors in the mouse skin cells and of miRNA transfection that allowed the embryonic-stem-cell-specific miRNAs to enter these same cells, they reprogrammed these cells and then showed that they could be incorporated into a mouse embryo to become every cell type in the adult animal. This is the hallmark of pluripotency. The scientists demonstrated that these miRNAs could increase the efficiency of iPS reprogramming. Moreover, unlike the original reprogramming factor, c-Myc, the miRNAs could induce a homogenous population of iPS cell colonies.

As both teams conclude, their studies should help investigators to more efficiently create pluripotent stem cells for research and for potential therapeutic interventions in the clinic.

Advances in Xenotransplantation and Transgenic Technology

The prospect of xenotransplantation—the transplantation of organs from one species into another, usually from pigs into human beings—remains an exciting possibility that could save human lives. One of the chief hurdles for this technology, however, is the hyperacute rejection that occurs within minutes when pig organs are transplanted into primates. A predominantly Israeli research team has recently demonstrated that the transplantation of *embryonic* pig tissue into two diabetic cynomolgus monkeys (*Macaca fasicularis*) gave rise to a marked reduction of exogenous insulin requirement four months after the transplantation, with the monkeys reaching complete self-sufficiency five months after transplantation (Gill Hecht et al., "Embryonic Pig Pancreatic Tissue for the Treatment of Diabetes in a Nonhuman Primate Model" *Proceedings of the National Academy of Sciences USA*, May 26, 2009). Significantly, the transplanted tissue was able to grow and proliferate in the host, was able to be vascularized by the host's blood vessels, and was able to evade the hyperacute or acute rejection. The authors conclude that their approach led to durable graft protection and as such "could offer an attractive replacement therapy for diabetes." This is an important advance that could suggest that embryonic tissue should be the tissue of choice for xenotransplantation.

In a discovery of even greater technological significance, Japanese researchers from the Central Institute for Experimental Animals have created the first genetically engineered nonhuman primates that have been able to pass on their genetic modification to their progeny (E. Sasaki et al., "Generation of Transgenic Non-Human Primates with Germline Transmission," *Nature,* May 28, 2009). To appreciate the significance of this discovery, we should note that it has now become routine to knock out genes in mice to generate models for human disease, a groundbreaking technology recognized in the Nobel Prize in Physiology or Medicine that was awarded in 2007 to researchers in this field. For many diseases, however, mice are too dissimilar from humans for the

results to be meaningful. The creation of genetically engineered marmosets (*Callithrix jacchus*)—in this case, five small monkeys that glow green under ultraviolet light because of a jellyfish gene that has been introduced into their DNA—is a milestone that would allow scientists to reproduce the course of many human diseases, particularly neurological disorders, in an animal more closely related to us than mice and other rodents. Not surprisingly, however, the authors of the paper acknowledge that their discovery raises numerous ethical questions, especially in light of the already contentious animal rights debate over the use of genetically transgenic mice.

In principle, the Catholic moral tradition is supportive of animal research. The Catholic Church teaches that God entrusted the animals to the stewardship of those whom he created in his own image and likeness, and that animals do not and cannot have the dignity ascribed to human beings. Hence it is legitimate to use animals for food, for clothing, and for biomedical research "if it remains within reasonable limits and contributes to caring for or saving human lives."[1] This use would include the genetic engineering of animals. However, all effort must be taken to minimize the suffering of the animal subjects because "it is contrary to human dignity to cause animals to suffer or die needlessly."

Recent Discoveries in Neuroscience: The Nature of Conscious Intention, Self-Control, and Long-Term Meditation

In recent years, empirical studies in neurobiology have allowed scientists to investigate human phenomenon that in the past have been reserved to the musings of philosophers and theologians. Three studies published this past quarter are representative of this trend.

First, how do people form a conscious intention to move? By directly stimulating patients' brains with electrodes and observing the effects of this intervention on their behavior and sensation, Michel Desmurget and his colleagues in Bron, France, have discovered that the feeling that one has willed his arm into motion and the realization that one has moved his arm at all are both the result of neuronal activity in an area at the back of the brain called the posterior parietal cortex ("Movement Intention after Parietal Cortex Stimulation in Humans," *Science*, May 8, 2009). This region helped produce the intention to move, and predicted what the movement would feel like, even before the patient had twitched a single muscle: When the research team stimulated the parietal cortex, the patients experienced a strong desire to move their arms, hands, feet, or lips, although they never actually did. Stronger stimulation convinced the patients that they had actually moved even though recordings of electrical activity in their muscles said otherwise. In contrast, when Desmurget and his team stimulated a different region called the premotor cortex, they discovered the opposite effect: The patients moved their limbs without desiring or realizing it. The data suggests that the activity of neurons in the parietal cortex create a sense that we initiate actions of our own accord and that our sense of motion does not depend on our actually moving. Some have suggested that this study confirms the materialistic constitution of the human being. In response, it will be important to incorporate the

[1] *Catechism of the Catholic Church* 2nd ed., trans. U.S. Conference of Catholic Bishops (Vatican City: Libreria Editrice Vaticana, 1997): n. 2418.

data of this study and others like it into a classical account of human agency that acknowledges the hylomorphic structure of the acting person.

Next, by studying a group of volunteers—all self-reported dieters—researchers at the California Institute of Technology have uncovered differences in the brains of people who are able to exercise self-control from those who are unable to do so (Todd A. Hare et al., "Self-Control in Decision-Making Involves Modulation of the vmPFC Valuation System," *Science*, May 1, 2009). The researchers picked nineteen volunteers who were able to exercise dietary self-control in their food choice, picking mostly healthy foods, regardless of taste, and eighteen additional volunteers who showed very little self-control, picking what they believed to be the tastier food most of the time, regardless of its nutritional value. Brain scans of the participants revealed significant differences in the brain activity of the group able to exercise self-control. In the case of good self-controllers, individuals who were able to reject unhealthy foods even if they liked them, activity in an area of the brain called the dorsolateral prefrontal cortex increased when subjects exercised self-control. Poor self-controllers did not manifest any neuronal activity in this part of the brain. Why is this significant? As one commentator has suggested, this study is the first that observes the brain respond to and, in some individuals, resist temptation.

Finally, a group of researchers at the University of California, Los Angeles, who used high-resolution magnetic resonance imaging (MRI) to scan the brains of individuals who meditate report that specific parts of the brain were larger in long-term meditators as compared to non-meditator controls ("The Underlying Anatomical Correlates of Long-Term Meditation: Larger Hippocampal and Frontal Volumes of Gray Matter," *Neuroimage,* January 13, 2009). Dr. Eileen Luders and her colleagues examined forty-four people, twenty-two control subjects and twenty-two experimental subjects, who had practiced various forms of Eastern meditation for an average of twenty-four years. The researchers found significantly larger cerebral measurements in meditators than in controls, including larger volumes of the right hippocampus and increased gray matter in the right orbitofrontal cortex, the right thalamus, and the left inferior temporal lobe. In contrast, there were no regions of the brains in the control subjects that were significantly larger than those of the meditators. Since the increased regions in the meditators are associated with the regulation of emotions, the scientists conclude that observed differences in brain anatomy might help us understand why these individuals have a singular ability to cultivate positive emotions, retain emotional stability, and engage in mindful behavior. In the end, though the experiment is an interesting one, its implications remain unclear. For instance, it is not clear whether the meditation process changed the brains of the meditators or whether a larger brain predisposed individuals to engage in meditation. Distinguishing these two possibilities—in other words, distinguishing cause from effect—will have to be a goal for any future research in this field. Nonetheless, the study illustrates a growing trend in cognitive neuroscience to try to reduce human behavior and experience to neuronal activity in the brain.

Gender Specification, Female Reproduction, and Early Embryo Loss

What makes a man, a man, or a woman, a woman? Is it the presence or absence of a Y chromosome, or the presence or absence of sex-specific genitalia? This past quarter, a team from Zurich led by Dr. Anna Biason-Lauber described a girl who

is chromosomally XY but who is a completely normal female with a uterus and histologically normal ovaries ("Ovaries and Female Phenotype in a Girl with 46,XY Karyotype and Mutations in the CBX2 Gene," *American Journal of Human Genetics,* May 2009). The girl with the X and Y chromosomes is female because she carries mutations in the Chromobox homolog 2 (CBX2) gene on human Chromosome 17, a gene whose mouse counterpart has also been implicated in male-to-female sex reversals in mice. This genetic study suggests that the male-determining gene on the Y chromosome, called SRY, is not a "male master switch," because it works only in the presence of functional CBX2. As such, any definition of human sex that relies solely on the presence of SRY on the Y chromosome would be inadequate. Rather, human sexuality needs to be understood within a more holistic systems perspective that acknowledges that sexual development in mammals requires not one, but a network of functioning genes, many of which remain unidentified.

Next, as any college biology student can tell you, the accepted dogma in reproductive biology is that female mammals, including women, have lost the capacity to make eggs after birth. This orthodoxy has been challenged by recent experimental findings that suggest that the ovaries of young and adult female mice have growing cells that can give rise to new eggs. However, with these earlier studies, it is not clear whether these dividing cells come from the ovary or from elsewhere in the body, and whether these growing cells could give rise to eggs that could be fertilized to produce healthy offspring. A few months ago, Professor Ji Wu and colleagues from the Shanghai Jiao Tong University in China reported that they have been able to successfully transplant stem cells from immature and mature ovaries into infertile female mice ("Production of Offspring from a Germline Stem Cell Line Derived from Neonatal Ovaries," *Nature Cell Biology,* May 2009). After transplantation, the recipient mice went on to produce healthy offspring after mating. This paper is the first to describe cells from ovaries of both juvenile and adult mice that have the characteristics of egg-producing germline stem cells. This discovery opens up medical possibilities that have ethical implications. First, women may one day be able to delay menopause because of this paper: They would simply rejuvenate their ovaries by transplanting germline stem cells from younger donors. Next, it may be possible to isolate these stem cells from a woman earlier in life so that she could have children later. Finally, this technology could provide a means to restore fertility to women who have few eggs or who have to undergo chemotherapy, by isolating these germline stem cells, expanding their numbers, and keeping them frozen until they are needed for in vitro fertilization.

Finally, we briefly mention the report by Evelyne Vanneste and her colleagues who have discovered that chromosome instability, a phenomenon found in human cancers, is also common in cleavage-stage—that is, very young—human embryos ("Chromosome Instability is Common in Human Cleavage-Stage Embryos," *Nature Medicine*, May 2009). It is not clear why early embryos manifest this phenomenon though it could explain the high rates of death seen during early embryogenesis.

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