Science

Pluripotent Stem Cells: Comparing Embryonic Stem Cells and iPS Cells

There are two types of human pluripotent stem cells. Induced pluripotent stem (iPS) cells are derived from reprogrammed adult cells, whereas embryonic stem cells are derived from the destruction of human embryos. This past quarter, two published papers reported side-by-side comparisons of iPS cells and embryonic stem cells as these cell types were coaxed into becoming various kinds of cells. First, Qiang Feng et al. compared the ability of eight human iPS cell lines and twenty-five human embryonic stem cell lines to become a variety of cells types in the human body ("Hemangioblastic Derivatives from Human Induced Pluripotent Stem Cells Exhibit Limited Expansion and Early Senescence," *Stem Cells,* February 12, 2010). Their data suggest that human embryonic stem cells are more efficient—up to one thousand times more efficient in one test—at becoming specialized cell types than their human iPS cell counterparts. Moreover, in contrast to cells obtained from embryonic stem cells, cells derived from the iPS cells started to undergo programmed cell death and aging. In a second study, a team from the University of Wisconsin, Madison, compared the ability of human embryonic stem cells and human iPS cells to become nerve cells (B. Y. Hu et al., "Neural Differentiation of Human Induced Pluripotent Stem Cells Follows Developmental Principles but with Variable Potency," *PNAS*, March 2, 2010). Both kinds of pluripotent cells were able to become nerve cells, but again, both cell types underwent specialization with different efficiencies: 90 percent of the human embryonic stem cells responded to the signals to become nerve cells, whereas 15 to 79 percent of the iPS cells did the same. There appear to be subtle but real differences between iPS cells and embryonic stem cells.

In light of these studies, I think that it is important to review the available data comparing iPS cells and embryonic stem cells. First, numerous papers have shown that iPS cells are highly similar to embryonic stem cells. Most of the iPS cell lines have normal numbers of chromosomes and comparable telomeres, the ends of chromosomes

that have been implicated in cancer and aging.[1] These data have been confirmed by a recent report that has shown that iPS cells obtained from patients with dyskeratosis congenita, a disorder of telomere maintenance, are able to restore normal telomere function (S. Agarwarl et al., "Telomere Elongation in Induced Pluripotent Stem Cells from Dyskeratosis Congenita Patients," *Nature*, March 11, 2010). Next, the behavior of the reprogramming genes in both pluripotent stem cell types are comparable.[2] However, these stem cell types do have different genetic signatures, although the significance of the differences is not known.[3] Several reports have suggested that the location of the viral inserts used to reprogram the iPS cells may be one reason for these differences.[4] Also, one report suggests that over time in cell culture, iPS cells and embryonic stem cells gradually become more similar.[5] Significantly, a recent conference report from the Hochedlinger laboratory at the Harvard Stem Cell Institute suggests that they have identified the key genetic difference between these two kinds of stem cells.[6] If this unpublished report is accurate, it would suggest that scientists will soon be able to increase the efficiency of iPS cells by further manipulation of the iPS cell genes, overcoming even the subtle differences between the induced pluripotent and the embryonic pluripotent stem cells.

Finally, I note the commentary by Bernard Lo and his colleagues on the recently published National Institutes of Health guidelines for stem cell research and gamete donors ("NIH Guidelines for Stem Cell Research and Gamete Donors," *Science*, February 19, 2010). Currently, third parties who donate sperm and ova for the use of infertile individuals sign a form giving the IVF patient unrestricted legal authority to use embryos generated with their gametes. The team of commentators from the University of California, San Francisco, wants the NIH to revise its guidelines so that gamete donors will be told that embryos generated from their sperm or eggs could be used for embryonic stem cell research so that they can give their consent. They recommend a process of disclosure made through oral discussion or brochures rather than a detailed procedure involving informed consent.

Bypassing Pluripotency with Direct Cell-Type-to-Cell-Type Reprogramming

In recent months, two papers have described nuclear reprogramming strategies that have bypassed the need for pluripotent stem cells. First, Thomas Vierbuchen and

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[1]Rosa M. Marion et al., "Telomeres Acquire Embryonic Stem Cell Characteristics in Induced Pluripotent Stem Cell," *Cell Stem Cell* 4.2 (February 6, 2009): 141–154.

[2]Rupa Sridharan et al., "Role of the Murine Reprogramming Factors in the Induction of Pluripotency," *Cell* 136.2 (January 2009): 364–377.

[3]Mark H. Chin, "Induced Pluripotent Stem Cells and Embryonic Stem Cells Are Distinguished by Gene Expression Signatures," *Cell Stem Cell* 5.1 (July 2, 2009): 111–123.

[4]See Frank Soldner et al., "Parkinson's Disease Patient-Derived Induced Pluripotent Stem Cells Free of Viral Reprogramming Factors," *Cell* 136.5 (March 6, 2009): 964–977; and Junying Yu et al., "Human Induced Pluripotent Stem Cells Free of Vector and Transgene Sequences," *Science* 324.5928 (May 8, 2009): 797–801.

[5]Chin, "Induced Pluripotent Stem Cells and Embryonic Stem Cells."

[6]See Elie Dolgin, "Gene Flaw Found in Induced Stem Cells," *Nature* 464.7289 (April 1, 2010): 663.

his colleagues at the Institute for Stem Cell Biology and Regenerative Medicine at Stanford University have identified three genes, *Ascl1*, *Brn2*, and *Myt1l*, which are able to convert skin cells called fibroblasts directly into nerve cells in mice ("Direct Conversion of Fibroblasts to Functional Neurons by Defined Factors," *Nature*, February 25, 2010). They began by identifying nineteen genes that were turned on in developing and adult nerve tissues. By selectively expressing different subsets of these genes, they were able to identify the three critical genes necessary to create the induced neuronal or nerve cells. This discovery bypasses the need for stem cells, providing scientists with another approach for disease modeling and drug discovery. It also illustrates a general experimental approach that would allow other research groups to identify those genes required for the direct transformation of one cell type to another cell type.

Next, N. Uhlenhaut and colleagues at the European Molecular Biology Laboratory in Germany have been able to reprogram the female ovaries of mice and transform them directly into testes by simply turning off the single gene called *Foxl2* ("Somatic Sex Reprogramming of Adult Ovaries to Testes by *FOXL2* Ablation," *Cell*, December 11, 2009). Previous work with goats had already implicated *Foxl2* in the specification of sex. Animals with abnormal *Foxl2* gene expression developed polled intersex syndrome, or PIS, where they became male despite carrying two X chromosomes, while female animals lacking the gene failed to develop ovaries. Interestingly, online commentators have suggested that manipulating this gene and others like it may lead to the development of gene therapies for patients who wish to change their sex/gender without undergoing sex reassignment surgery.

Emergency Contraception: A New Drug and an Old Controversy

Ulipristal acetate (also known as CDB-2914 and sold under the trade name ellaOne) is an emergency contraceptive granted marketing authorization by the European Medicines Agency in March 2009. Two recent studies have shown that ulipristal acetate is an effective emergency contraceptive even when a single dose of the drug (30 mg) is taken up to five days after unprotected intercourse (P. Fine et al., "Ulipristal Acetate Taken 48–120 Hours after Intercourse for Emergency Contraception," *Obstetrics and Gynecology*, February 2010; and A. F. Glasier et al., "Ulipristal Acetate versus Levonorgestrel for Emergency Contraception: A Randomised Non-Inferiority Trial and Meta-analysis," *Lancet*, February 13, 2010). In the first study, 1,241 women who presented for emergency contraception at forty-five Planned Parenthood clinics were treated with ulipristal acetate. Twenty-six became pregnant with a pregnancy rate of 2.1 percent, which was significantly below the expected pregnancy rate of 5.5 percent, meaning sixty-nine expected pregnancies. In the second study, the research team studied 2,221 women who were randomly assigned to receive a single dose of ulipristal acetate or the standard dose of levonorgestrel (trade name PlanB), the most widely used emergency contraceptive in the United States. Among the 1,700 women who received emergency contraception within seventy-two hours of intercourse, there were thirty-seven pregnancies—fifteen in the ulipristal acetate group and twenty-two in the levonorgestrel group. In the 203 women who received emergency contraception between seventy-two hours and one hundred twenty hours after unprotected intercourse, there were three pregnancies,

all among women in the levonorgestrel group. Both studies show that ulipristal acetate is at least as effective as levonorgestrel in preventing pregnancy when taken up to seventy-two hours after sexual intercourse. They also provide evidence that ulipristal acetate is an effective means for emergency contraception that can be used up to five days after intercourse. Significantly, previous studies with guinea pigs and monkeys described in the manufacturer's report to the European Medicines Agency have demonstrated that ulipristal acetate can act as an abortifacient.[7]

There has been an ongoing debate over the mechanism of action of levonorgestrel taken as an emergency contraceptive: Is it an abortifacient? Two recent papers have suggested that levonorgestrel does not affect or change the endometrium of the uterus. A third study from the Noe Laboratory in Chile reports that levonorgestrel taken as an emergency contraceptive "prevents pregnancy only when taken before fertilization of the ovum has occurred." These papers provide further evidence in support of the claim made by myself and by others that levonorgestrel does not have a post-fertilization effect when it is taken as an emergency contraceptive. In other words, all the data taken together indicate that Plan B is not an abortifacient.

First, Chun-Xia Meng and colleagues took endometrial biopsies from women who had taken levonorgestrel orally several times or had been administered a single vaginal dose of levonorgestrel, and compared them with controls ("Effects of Oral and Vaginal Administration of Levonorgestrel Emergency Contraception on Markers of Endometrial Receptivity," *Human Reproduction,* April 2010). They examined the levels of sex steroid receptors, interleukin 1-beta, leukemia-inhibitory factor, vascular endothelial growth factor, cyclooxygenase 2, tumor necrosis factor-alpha, integrin alpha-5 beta-3, and mucin 1 in endometrial cells. The paper concludes, "The two regimens of levonorgestrel caused either only minor or no alterations in markers of endometrial receptivity."

Next, a second paper, by Wilder Alberto Palomino, Paulina Kohen, and Luigi Devoto from the University of Chile, also sought to examine the effects of levonorgestrel on the endometrial receptivity phenotype through the oral or vaginal route ("A Single Midcycle Dose of Levonorgestrel Similar to Emergency Contraceptive Does Not Alter the Expression of the L-Selectin Ligand or Molecular Markers of Endometrial Receptivity," *Fertility and Sterility*, November 10, 2009). This second team compared levels of progesterone receptor, L-selectin ligand, integrin alpha-5 beta-3, and glycodelin-A in endometrial biopsies taken from women with tubal ligations who had taken levonorgestrel. Again, like the authors of the paper described above, they saw no differences in expression patterns between endometrial biopsies taken from women who had taken levonorgestrel and those from no-levonorgestrel controls. In sum, both of these independent studies taken together indicate that it is highly unlikely that levonorgestrel could act as an abortifacient by changing the endometrial lining of the uterus.

[7]European Medicines Agency, "CHMP Assessment Report for Ellaone," London, 2009, http://www.emea.europa.eu/humndocs/PDFs/EPAR/ellone/H-1027-en6.pdf.

Finally, Noe et al. report that they have undertaken a study with 388 Chilean women who took levonorgestrel as an emergency contraceptive ("Contraceptive Efficacy of Emergency Contraception with Levonorgestrel Given Before or After Ovulation," *Contraception*, Volume 81, May 2010). In 122 women, intercourse occurred in one of the six fertile days of the menstrual cycle. Eighty-seven of these 122 women took the levonorgestrel during the five days prior to ovulation, while 35 women took the drug on the day of ovulation or thereafter. In the first group, 13.2 pregnancies could be expected and no pregnancy occurred, whereas in the second group, 7.1 pregnancies could be expected and 6 pregnancies occurred. Thus, the overall contraceptive efficacy of Plan B use in this study was 70 percent. (It prevented 14.3 of 20.3 pregnancies.) However, the data also show that levonorgestrel is ineffective when it is taken after ovulation, suggesting that it does not have a post-fertilization effect. To put it another way, the data show that Plan B is not an abortifacient. This study reproduces the findings of the earlier report by Novikova et al.,[8] who had found that levonorgestrel does not have a post-fertilization effect. Significantly, this is a much larger study with better statistical power. Again, the cumulative evidence indicates that Plan B is not an abortifacient.

Storing, Manipulating, and "Reading" Memories

How are memories stored in the brain? Two studies published in the past few months suggest that the acquisition of memories in animals is linked to the physical reorganization of the synaptic connections between nerve cells in the brain (T. Xu et al., "Rapid Formation and Selective Stabilization of Synapses for Enduring Motor Memories," *Nature*, December 17, 2009; and G. Yang, F. Pan, and W. B. Gan, "Stably Maintained Dendritic Spines Are Associated with Lifelong Memories," *Nature*, December 17, 2009). These papers report that learning new motor tasks and acquiring new sensory experiences is associated with the formation of new sets of connections between nerve cells, suggesting that the creation of cell–cell connections rather than the creation of new cells is the elementary unit of memory formation. Both studies revealed that by the end of the first and second days of training, twice as many new connections had been made in the brain of trained mice as compared with untrained controls. Yang, Pan, and Gan showed that mice exposed to an enriched environment consisting of altered bead strings hanging from their cage tops led to brain remodeling. Clearly, memory formation involves structural changes in the brain.

Next, it appears that these structural changes associated with memories or something like them can be picked up using functional magnetic resonance imaging. British scientists from University College London discovered that specific memories light up particular regions of the brain (M. J. Chadwick et al., "Decoding Individual Episodic Memory Traces in the Human Hippocampus," *Current Biology*, March 23, 2010). After showing each of ten people three very short films before brain scanning, they were then able to identify patterns in brain activity associated with each film.

[8] N. Novikova et al., "Effectiveness of Levonorgestrel Emergency Contraception Given Before or After Ovulation: A Pilot Study," *Contraception* 75.2 (February 2007): 112–118.

Strikingly, using this information the British research team was able to accurately predict which film a given person was thinking about when he or she was being scanned. Newspapers around the world reported this discovery as the invention of a protocol to read the minds of people! However, this certainly is not the case, because scientists are still unable to take brain scans and decode them without reference to a prior stimulus.

Recovering Formal Causes in Biology

Finally, I note two papers that signal the ongoing recovery of the formal cause in biology. First, Michael Costanzo and his colleagues working at the University of Toronto have identified a global map of all the interactions among about 75 percent of the six thousand or so genes in budding yeast ("The Genetic Landscape of a Cell," *Science*, January 22, 2010). This systems perspective of the yeast cell describes the cell in terms of its overall "shape" determined by the dynamic interactions of its molecular components.

Next, University of Chicago scientists in the Mrksich Laboratory have used geometrically patterned surfaces to influence the development of adult mesenchymal stem cells (B. Y. Hu et al., "Geometric Cues for Directing the Differentiation of Mesenchymal Stem Cells," *PNAS*, March 2, 2010). The team found that culturing the cells in a special mold that forces cells to grow in a star shape made them turn into bone cells. In contrast, cells grown in molds that forced them to grow in a flower shape turned into fat. Significantly, both groups of cells were cultured in identical media, the cocktail of molecules that nourish and signal cells, suggesting that it is solely the shape—the form—of the cells that determined their fate. Taken together, these papers suggest that manipulating the shape, and thus, the overall interactions among the molecules that make up the system that is the living cell—its form—can alter its behavior and its destiny.

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