Science

Characterizing and Defining Stem Cells: What Is Pluripotency?

A pluripotent stem cell is a cell that can become any of the many cell types found in the adult organism. One question that has intrigued biologists is, *why* is a pluripotent stem cell the way it is? Or to put it another way, *what* makes a pluripotent stem cell a pluripotent stem cell? This quarter, five papers described experiments that have contributed to our understanding of stem cells at the molecular level.

First, two papers identify the molecular machines that are important in determining the identity of pluripotent stem cells. Fuchou Tang and colleagues describe how they were able to investigate the behavior of individual cells as the cells converted from embryonic cells within the mouse inner cell to pluripotent embryonic stem cells, revealing the molecular changes necessary for this still mysterious transformation ("Tracing the Derivation of Embryonic Stem Cells from the Inner Cell Mass by Single-Cell RNA-Seq Analysis," *Cell Stem Cell*, May 7, 2010). By monitoring 385 genes in 74 individual cells, the research team at the Wellcome Trust/Cancer Research in the United Kingdom was able to identify those changes in key genes required to maintain the pluripotency of cells. These observations could allow scientists to control the behavior, and eventually the specialization, of stem cells.

Next, a research group from the Netherlands and Scotland has described the function of the important molecule called Oct4, which lies at the heart of the genetic network that is responsible for establishing and maintaining the pluripotent state of murine embryonic stem cells and induced pluripotent stem (iPS) cells ("An Oct4-Centered Protein Interaction Network in Embryonic Stem Cells," *Cell Stem Cell*, April 2, 2010). More specifically, Debbie L. C. van den Berg and her colleagues identified fifty-five molecular interactions that link Oct4 with other important molecules in the cell, including chromatin-modifying machines that change the characteristics of a cell's genes and chromosomes. This work was confirmed by a parallel but independent study published by Mercedes Pardo and colleageus, working

in Cambridge in the United Kingdom ("An Expanded Oct4 Interaction Network: Implications for Stem Cell Biology, Development, and Disease," *Cell Stem Cell*, April 2, 2010). In this second study, the British team identified ninety-three Oct4-interactions, twenty of which overlapped with the set of interactions described by their Dutch and Scottish colleagues. Together, these papers give scientists a snapshot of the molecular system that defines pluripotency. Inevitably, scientists will have to link this Oct4 network with the molecular analysis of the 385 genes important for the transformation between embryonic cells and embryonic pluripotent stem cells described above. This type of analysis, which would give us a definition of pluripotency at the molecular level, would give biologists better control over the crucial cell-to-cell transformations that will be important for the medical interventions imagined by proponents of regenerative medicine.

Comparing Pluripotent Cells: Uncovering the Genetic Signatures of Embryonic Stem Cells and Induced Pluripotent Stem Cells

How do embryonic stem cells and iPS cells compare? Both these cell types are pluripotent in that they are able to generate any of the many cell types found in the adult organism. However, as we noted in the summer issue of the *NCBQ*, there appear to be subtle but real differences between embryonic stem cells and iPS cells. Several groups had suggested that the differences in behavior of these two kinds of pluripotent stem cells could be linked to differences in the behavior of their genes. For example, two recent studies suggest that reprogrammed iPS cells retain the molecular memories of their previous cellular identities. First, George Daley and his colleagues at Harvard University report that reprogrammed iPS cells retained the chemical tags on their DNA that were reminiscent of the cell of origin (K. Kim, "Epigenetic Memory in Induced Pluripotent Stem Cells," *Nature,* September 16, 2010). They suggest that this may explain why iPS cells derived from bone marrow had an easier time becoming blood cells while iPS cells derived from generic cells called fibroblasts were better at making bone cells. Another team of researchers led by Konrad Hochedlinger at the Massachusetts General Hospital in Boston also found that the gene activity and chemical tags of reprogrammed iPS cells resembled those of the pre-reprogrammed cell of origin (Jose M. Polo, "Cell Type of Origin Influences the Molecular and Functional Properties of Mouse Induced Pluripotent Stem Cells," *Nature Biotechnology*, August 2010). However, they went on to show that the molecular memories faded over time. The cell-of-origin markers of month-long iPS cells were practically gone.

In contrast, Newman and Cooper propose, in a fascinating report, that the observed differences in the gene expression of embryonic stem cells and iPS cells are not inherent to these cells but are "lab-specific" artifacts ("Lab-Specific Gene Expression Signatures in Pluripotent Stem Cells," *Cell Stem Cell,* August 6, 2010). If this report is confirmed independently, it would suggest that embryonic stem cells and iPS cells do not represent fundamentally different pluripotent states. Strikingly, another team from the Whitehead Institute at MIT had reached a similar conclusion using an independent method that determines the expression profiles of human embryonic stem cells, iPS cells, and skin cells by looking at their chromosomal structure ("Chromatin Structure and Gene Expression Programs of Human Embryonic and Induced Pluripotent Stem Cells," *Cell Stem Cell*, August 6, 2010). Further research

will be needed to reconcile the contradictory findings described in these four scientific papers. They reveal that the questions raised by different pluripotent stem cell types remain unresolved. Nonetheless, most of the data to date suggest that embryonic stem cells and iPS cells are more similar than different, especially with regard to their therapeutic potential.

Advances in Nuclear Reprogramming Technology

Three advances in nuclear reprogramming technology, or iPS technology, were described this past quarter. First, in a paper that made news headlines, Luigi Warren and his colleagues report that they have not only developed a safe and efficient technology to create human iPS cells, but have also discovered a way to steer these stem cells to form useful cells like blood cells, neurons, and muscle cells ("Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA," *Cell Stem Cell*, November 5, 2010). The team at the Children's Hospital in Boston developed a novel method of creating mRNA molecules that could reprogram adult cells without the cancer-causing viral technology that had been used in the past. This technology was also able to reprogram cells—which the authors call RiPS cells for RNA induced pluripotent stem cells—in half the time required by the original virus-based reprogramming techniques and was up to one hundred times more efficient. Finally, the protocol was also used to efficiently generate function muscle and other cell types. This technology should accelerate the developments in regenerative medicine. Significantly, the Harvard team has patented its findings and has recently formed a company called ModeRNA Therapeutics, which is dedicated to translating this basic research discovery into clinical practice.

Next, a series of papers published in the July 2 issue of *Cell Stem Cell* demonstrate that iPS cells can be generated from blood cells (Tomohisa Seki et al., "Generation of Induced Pluripotent Stem Cells from Human Terminally Differentiated Circulating T Cells"; Yuin-Han et al., "Reprogramming of T Cells from Human Peripheral Blood"; and Judith Staerk, "Reprogramming of Human Peripheral Blood Cells to Induced Pluripotent Stem Cells"). These protocols should make it easier and faster for stem cell biologists to generate patient-specific pluripotent stem cells. Until now, cells to be reprogrammed had to be obtained from a patient through invasive skin biopsy. Now, these cells can be obtained from a patient during a routine blood draw.

Finally, in a truly groundbreaking paper, a team from the University of California in San Francisco has been able to take connective cells from the adult mouse heart and *directly* reprogram them into heart muscle cells using three genes, Gata4, Mef2c, and Tbx5 (Masaki Ieda et al., "Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors," *Cell*, August 6, 2010). These induced cardiomyocytes behaved like cardiomyocytes—heart muscle cells—derived from the heart. Notice that in accomplishing this molecular feat of cellular reprogramming, these biologists have shown that pluripotent stem cells are *not* necessary for regenerative medicine. If this protocol becomes paradigmatic, then scientists will simply take adult cells of one type, say a blood or a skin cell, and transform them directly into another adult cell of choice, say a muscle cell or a nerve cell, that could then be used to treat the patient. Pluripotent stem cells would become superfluous. The reprogramming of adult cells to adult cells would become the norm.

Human Gene Therapy, Human Embryos, and
Genetically Altered Plants for Environmental Control

On September 16, 2010, a paper from bluebird bio (formerly Genetix Pharmaceuticals, Inc.) described the company's successful attempt to use gene therapy to treat a young adult with severe beta-thalassemia, a commonly inherited blood disorder (Marina Cavazzana-Calvo, "Transfusion Independence and HMGA2 Activation after Gene Therapy of Human Beta-Thalassaemia," *Nature*, September 16, 2010). The patient, who had needed blood transfusions since early childhood, had become transfusion independent for at least twenty-one months after treatment with the a genetically engineered gene delivery system called LentiGlobin. When LentiGlobin is introduced into blood cells, the gene delivery system is able to replace the diseased blood molecule, hemoglobin, with normal molecules that improved the patient's clinical condition. This paper represents one of the few success stories in the field of human gene therapy.

Next, a research team at Stanford University has developed a technology that is able to predict the future viability of human embryos when they are still at the four-cell stage of development ("Non-invasive Imaging of Human Embryos before Embryonic Genome Activation Predicts Development to the Blastocyst Stage," *Nature Biotechnology,* October 2010). Connie Wong and her colleagues propose that measuring a unique set of noninvasive imaging parameters associated with two-day old embryos may allow embryologists to predict if the embryos will reach the five-day old blastocyst stage with a high degree of accuracy. This may allow infertility specialists to improve their ability to pick healthier IVF embryos that would be more likely to survive a full-term pregnancy.

Finally, in his encyclical *Caritas in veritate*, the Holy Father, Benedict XVI, linked bioethics and environmental ethics by pointing out that there is a link between human ecology and environmental ecology (n. 51). The Pope has challenged Catholics to become better stewards of the Lord's creation. Genetic engineering could be used to do this. For example, researchers at both the Lawrence Berkeley National Laboratory and the Oak Ridge National Laboratory proposed that genetically altered trees and plants could help counter climate change (Christer Janssen et al., "Phytosequestration: Carbon Biosequestration by Plants and the Prospects of Genetic Engineering," *BioScience,* October 2010). Forests of genetically engineered trees could sequester billions of tons of carbon from the atmosphere each year and thus decrease global warming. This paper also raises the interesting question, Can environmental conservation benefits justify the genetic engineering of that environment?

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