



SCIENCE

Induced Pluripotent Stem Cells: Ready or Not for the Clinic?

Induced pluripotent stem (iPS) cells have been touted as licit replacements for embryonic stem cells, because iPS cells have virtually the same cellular characteristics as embryonic stem cells but their creation does not involve the destruction or use of embryos or eggs or the use of cloning (nuclear transfer) techniques. Moreover, because iPS cells can be made from the cells of a patient and then transplanted back into that same patient (as differentiated derivatives), there is hope that they can be used as an autologous transplant that will not elicit an immune response.

Harvard scientists have provided preclinical evidence for that concept in a standard model of Parkinson's disease in cynomolgus monkeys.¹ Monkeys exhibiting Parkinson's symptoms received injections of autologous iPS-cell-derived dopamine neurons, differentiated by two different protocols. No immunosuppression was used. Over a two-year observation period, monkeys that received iPS-cell-derived neurons showed functional motor improvements and increased numbers of dopamine nerve terminals as measured by PET scan. Controls showed no change from baseline over this time period. Postmortem analysis of injected animals showed robust re-innervation at the transplantation site and high numbers of surviving dopamine neurons with the use of iPS-cell-derived neurons from one of the more successful differentiation protocols. In this study, there was no evidence of graft overgrowth or immune reaction.

¹ Penelope J. Hallett et al., "Successful Function of Autologous iPSC-Derived Dopamine Neurons following Transplantation in a Non-human Primate Model of Parkinson's Disease," *Cell Stem Cell* 16.3 (March 5, 2015): 269–274, doi: 10.1016/j.stem.2015.01.018.

All is not roses for iPS cells, however. Japanese researchers have published evidence for long-term issues including tumor formation.² The researchers used a mouse model of spinal cord injury. Human iPS cells were produced from skin cells and injected into affected animals. The injected iPS cells differentiated into neuronal cell types, and some synapsed with spinal cord host neurons. Injected mice showed improved motor function up to forty-seven days after injection. However, motor function gradually declined after that time, and by 103 days' follow-up, tumors were growing within the injected spinal cords. The authors note that the method of iPS cell production may play a role in tumorigenic potential—they had used retroviral integration of Yamanaka factors, and they suggest that integration-free vectors may not pose the same hazard. In the meantime, however, the message seems to be that while pluripotent stem cells may provide short-term physiological improvement, there is still concern about long-term safety of such cells. The warning is all the more important now as several groups are commencing safety trials using iPS or embryonic stem cells.

Cloning Does Not Produce the Transplant Match Once Thought

For many years, proponents of cloning (somatic cell nuclear transfer) claimed that the technique had the potential to produce patient-specific embryonic stem cells that would be a tissue match for the individual who was cloned (the donor of the nuclear genetic material). This so-called therapeutic cloning actually starts with creation of cloned embryos, who are then destroyed to harvest their embryonic stem cells; the process is not therapeutic for the embryos, nor are there any existing therapies that use it, and previous evidence in animal models suggests that the claim of a transplant match is dubious. Nevertheless, claims for the possibility of producing patient-matched embryonic stem cells by cloning resurfaced after the successful cloning of human embryos by three different groups over the last two years.³

Now evidence from leading laboratories has dashed the hopes of producing immune-matched cells by cloning, showing that a mismatch in the mitochondrial DNA between the egg used to create the clone and the host can trigger an immune reaction.⁴ The team created cloned mouse embryos and derived embryonic stem cells from the cloned embryos. Cells were isolated from cloned embryos created

² Satoshi Nori et al., "Long-Term Safety Issues of iPSC-Based Cell Therapy in a Spinal Cord Injury Model: Oncogenic Transformation with Epithelial-Mesenchymal Transition," *Stem Cell Reports* 4.3 (March 10, 2015): 360–373, doi: 10.1016/j.stemcr.2015.01.006.

³ Masahito Tachibana et al., "Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer," *Cell* 153.6 (June 6, 2013): 1228–1238, doi: 10.1016/j.cell.2013.05.006; Young Gie Chung et al., "Human Somatic Cell Nuclear Transfer Using Adult Cells," *Cell Stem Cell* 14.6 (June 5, 2014): 777–780, doi: 10.1016/j.stem.2014.03.015; and Mitsutoshi Yamada et al., "Human Oocytes Reprogram Adult Somatic Nuclei of a Type 1 Diabetic to Diploid Pluripotent Stem Cells," letter, *Nature* 510.7506 (June 26, 2014): 533–536, doi: 10.1038/nature13287.

⁴ Tobias Deuse et al., "SCNT-Derived ESCs with Mismatched Mitochondria Trigger an Immune Response in Allogeneic Hosts," *Cell Stem Cell* 16.1 (January 8, 2015): 33–38, doi: 10.1016/j.stem.2014.11.003.

with strain-matched nuclear and mitochondrial DNA, and cells were also isolated from clones constructed with matched nuclear DNA but mismatched mitochondrial DNA. The cells were then transplanted into mice with nuclear DNA identical to that of the clone. Cells injected into immunodeficient controls readily formed tumors, as expected. But when injected into immune-competent hosts, the cells from cloned embryos with mismatched mitochondrial DNA caused immune responses, even when there was a match for the nuclear DNA. The mitochondrial DNA of the requisite egg used for cloning by somatic cell nuclear transfer, though it expresses only a few genes, thus looms as a significant barrier to the creation of matched transplant tissue using cloning technology.

*Improved Adult Stem Cell Transplants for Sickle Cell Anemia
Can Mean No More Anti-Rejection Drugs*

Donor-derived adult stem cell transplants from bone marrow or umbilical cord blood are curative for children with sickle cell anemia, but adults often cannot tolerate the toxicity of similar myeloablative (bone-marrow-depleting) transplants. However, development of a lower-toxicity protocol shows not only that adults can tolerate the transplant but that many patients can stop taking anti-rejection drugs as well.⁵ Thirty patients with severe sickle cell disease were treated with nonmyeloablative levels of a chemotherapeutic cocktail and total body irradiation; the lower doses left as much as half of the patient's bone marrow intact. Donor hematopoietic stem cells were then infused. Twenty-six patients showed long-term stable donor-cell engraftment and cell chimerism with no graft-versus-host disease. Half of the patients (fifteen) were able to stop immunosuppressive medication and maintain stable donor-cell chimerism and no graft-versus-host disease. This represents a significant step forward in the treatment of adults with sickle cell disease.

*Adult Stem Cells for Multiple Sclerosis:
The News Gets Better and So Do the Patients*

Two recent reports point to the use of adult stem cells to induce remissions in patients with multiple sclerosis. None of the standard interventions used to treat relapsing–remitting multiple sclerosis produces any significant reversal of disability. In one study, which is also discussed in the Medicine notes in this issue, an international team led by Richard Burt of Northwestern University Feinberg School of Medicine has published evidence that adult stem cell transplants are associated with reversal of neurological disability for patients with relapsing–remitting multiple sclerosis.⁶ A total of 145 patients with relapsing–remitting or secondary progressive multiple sclerosis were followed for a mean of 2.5 years and up to 5 years after treatment with

⁵ Matthew M. Hsieh et al., “Nonmyeloablative HLA-Matched Sibling Allogeneic Hematopoietic Stem Cell Transplantation for Severe Sickle Cell Phenotype,” *JAMA* 312.1 (July 2, 2014): 48–56, doi: 10.1001/jama.2014.7192.

⁶ Richard K. Burt et al., “Association of Nonmyeloablative Hematopoietic Stem Cell Transplantation with Neurological Disability in Patients with Relapsing-Remitting Multiple Sclerosis,” *JAMA* 313.3 (January 20, 2015): 275–284, doi: 10.1001/jama.2014.17986.

a nonmyeloablative protocol and autologous hematopoietic stem cell transplantation (that is, transplantation of blood-producing stem cells from the same patient). After transplantation, there was significant improvement in scores on the Expanded Disability Status Scale (EDSS), with 50 percent of patients showing improvement at two years after transplantation, and 64 percent showing improvement at four years after transplantation. No other intervention for multiple sclerosis has shown an improvement in neurological function for patients. Treated patients also showed significant relapse-free survival (80 percent) and decreased neurological lesions.

A separate publication from a group led by Richard Nash of the Colorado Blood Cancer Institute also provides evidence for adult stem cell transplants in the remission of relapsing–remitting multiple sclerosis.⁷ This group provided a three-year interim report on their five-year clinical study. Twenty-four patients received high-dose immunosuppressive treatment followed by autologous hematopoietic stem cell transplantation. Relapse-free survival was 86.3 percent at three years’ follow-up, and patients showed improvements in neurologic disability and functional scores.

Breathing Easier with Adult Stem Cells

Researchers continue to isolate tissue-specific adult stem cells from various organs and to identify stem cells and progenitors that may facilitate tissue-specific repair. Three different groups recently reported on stem cells that participate in lung repair. Wei Zuo et al. report in *Nature* that a specific population of murine distal airway stem cells expressing p65 and Krt5 markers proliferated in response to lung damage and could form new alveoli at sites of inflammation.⁸ When transplanted into infected lungs, these cells could form type I and type II pneumocytes as well as bronchiolar secretory cells.

Andrew Vaughan et al. found that previously unrecognized distal lung stem cells could regenerate lung tissue after injury.⁹ Their paper, which appears in *Nature* alongside that of Zuo et al., notes that these regenerative cells are not the mature epithelial cells that help maintain lung tissue but are a rare population that are activated in response to lung injury and can migrate significant distances to participate in regeneration. They also found that formation of alveolar structures by these cells was dependent on blockade of Notch signaling.

A third paper on lung regenerative cells, by Ana Pardo-Saganta et al., appears in *Cell Stem Cell*.¹⁰ This group also found airway cells with specific markers that

⁷ Richard A. Nash et al., “High-Dose Immunosuppressive Therapy and Autologous Hematopoietic Cell Transplantation for Relapsing-Remitting Multiple Sclerosis (HALT-MS),” *JAMA Neurology* 72.2 (February 2015): 159–169, doi: 10.1001/jamaneurol.2014.3780.

⁸ Wei Zuo et al., “p63⁺Krt5⁺ Distal Airway Stem Cells Are Essential for Lung Regeneration,” *Nature* 517 (January 29, 2015): 616–620, doi: 10.1038/nature13903.

⁹ Andrew E. Vaughan et al., “Lineage-Negative Progenitors Mobilize to Regenerate Lung Epithelium after Major Injury,” letter, *Nature* 517.7536 (January 29, 2015): 621–625, doi: 10.1038/nature14112.

¹⁰ Ana Pardo-Saganta et al., “Injury Induces Direct Lineage Segregation of Functionally Distinct Airway Basal Stem/Progenitor Cell Subpopulations,” *Cell Stem Cell* 16.2 (February 5,

were induced to migrate and proliferate after significant lung injury. The cells showed two distinct subpopulations, but also responded to Notch signaling pathways for their activation.

A brief letter by Petrella et al. discusses the use of a different population of stem cells, mesenchymal stem cells from bone marrow, in an application to close a bronchopleural fistula (an abnormal opening between the airway and the pleural space) in a patient.¹¹ While mesenchymal stem cells are under very active investigation for numerous applications in regenerative medicine, including cardiovascular and musculoskeletal repair, these clinicians took a more direct approach, essentially plugging a hole. A patient who had undergone removal of a lung had developed a small fistula subsequent to the surgery. The authors injected 10 million autologous mesenchymal stem cells, obtained by bone marrow aspiration, close to the fistula site. Bronchoscopy at sixty days showed complete healing of the fistula.

Seeing Clearly with Adult Limbal Stem Cells—and with Dental Pulp

Adult limbal stem cells originate in the limbus of the eye, the area that appears as a dark circle around the iris. They have been used in the past to grow new corneas for patients, replacing corneas damaged by chemical burns or other trauma.¹² Limbal stem cells can be taken from the patient's own damaged eye and used to grow new tissue in vitro for transplantation, restoring sight to blind eyes. But what about direct application of limbal stem cells to damaged eyes? A recent report suggests that limbal stem cells, if applied in a timely manner, could prevent corneal damage and scarring, obviating the need to grow a completely new cornea.¹³ In the study, human limbal stem cells were obtained by biopsy and expanded in culture. When applied to corneal wounds in mice, the limbal stem cells prevented the formation of fibrotic lesions and induced the regeneration of new corneal stroma. This represents a potential autologous source of cells to treat corneal damage directly.

Limbal stem cells are not the only cells that can form corneal tissue. Opening the door to numerous jokes about “eye teeth,” a group in Pittsburgh has shown that stem cells from dental pulp can form corneal stroma.¹⁴ Dental-pulp stem cells were isolated from molars and grown in culture, where they could be induced to form stromal cells expressing typical corneal keratinocyte markers including keratocan

2015): 184–197, doi: 10.1016/j.stem.2015.01.002.

¹¹ Francesco Petrella et al., “Airway Fistula Closure after Stem-Cell Infusion,” letter, *New England Journal of Medicine* 372.1 (January 1, 2015): 96–97, doi: 10.1056/NEJMc1411374.

¹² Paolo Rama et al., “Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration,” *New England Journal of Medicine* 363.2 (July 8, 2010): 147–155, doi: 10.1056/NEJMoa0905955.

¹³ Sayan Basu et al., “Human Limbal Biopsy-Derived Stromal Stem Cells Prevent Corneal Scarring,” *Science Translational Medicine* 6.266 (December 10, 2014): 266ra172, doi: 10.1126/scitranslmed.3009644.

¹⁴ Fatima N. Syed-Picard et al., “Dental Pulp Stem Cells: A New Cellular Resource for Corneal Stromal Regeneration,” *Stem Cells Translational Medicine* 4.3 (March 2015): 276–285, doi: 10.5966/sctm.2014–0115.

and keratin sulfate. When injected into mouse corneas, the human dental-pulp stem cells formed typical corneal stromal matrix. These cells could represent an additional source of autologous stem cells for repair of corneal damage.

Direct Conversion of Cells and Tissues Becomes More Common

For years a major research goal has been to isolate or create stem cells that, because of their flexibility, could potentially be channeled into the formation of new, diverse types of tissue for the regeneration and repair of damaged or diseased tissues and organs. One of the ultimate expressions of this search was the production of induced pluripotent stem cells—cells with one of the most diverse potentials for their differentiation, made from normal differentiated cells. The discovery by Shinya Yamanaka won him the Nobel Prize. But for applications both basic and clinical, these stem cells must still be induced back into a differentiated or at least semi-differentiated form. Direct conversion of cells bypasses this pluripotent stem cell intermediate, going straight from one cell type to another—sometimes a targeted differentiated cell, sometimes a specific tissue-specific stem cell type that is more constrained in its eventual differentiation.

At first it was thought that this cellular conversion process was impossible, that a dedifferentiated stem cell middle-man was needed, but in the last few years there has been a watershed of discoveries for turning one cell type directly into another, akin to cell alchemy. Here are several recent examples.

John Cassady et al. report the direct conversion of mouse fibroblasts, as well as adult liver cells and B lymphocytes, to neural stem cells.¹⁵ Their technique involved transduction and transient overexpression of eight neural-specific transcription factors. These induced neural stem cells were indistinguishable from primary isolated brain neural stem cells in their genetic expression, and retained the ability to form the three primary neural cell types—neurons, astrocytes, and oligodendrocytes—even after extended passage in culture.

Matheus Victor et al. used a different strategy—microRNA expression in concert with transcription factors—to produce direct conversion of fibroblasts specifically to striatal medium spiny neurons.¹⁶ Their previous work had shown that expression of certain brain-enriched microRNAs could convert fibroblasts into neuronal cells. When they co-expressed the microRNAs with transcription factors that had been identified as enriched in the developing brain striatum, they were able to convert human fibroblasts into striatal medium spiny neurons. They further showed that when the cells were transplanted into the brains of mice, the human neuronal cells persisted for over six months and formed axonal projections typical for this cell type.

¹⁵ John P. Cassady et al., “Direct Lineage Conversion of Adult Mouse Liver Cells and B Lymphocytes to Neural Stem Cells,” *Stem Cell Reports* 3.6 (December 9, 2014): 948–956, doi: 10.1016/j.stemcr.2014.10.001.

¹⁶ Matheus B. Victor et al., “Generation of Human Striatal Neurons by MicroRNA-Dependent Direct Conversion of Fibroblasts,” *Neuron* 84.2 (October 22, 2014): 311–323, doi: 10.1016/j.neuron.2014.10.016.

One other recent success at direct conversion involved changing mouse pancreatic acinar cells (which synthesize and store a variety of digestive enzymes) into cells resembling pancreatic beta cells (which produce only insulin), directly in the pancreas.¹⁷ Weida Li et al. improved on a method they had used previously, increasing conversion of acinar cells into what they termed “induced beta cells” by polycistronic expression of three beta-cell factors—*Ngn3*, *Pdx1*, and *Mafa*. The resultant induced beta cells formed islet-like clusters and persisted for up to thirteen months in the mouse pancreas. The converted cells gradually acquired beta cell functionality and could maintain glucose homeostasis in mice.

DAVID A. PRENTICE

¹⁷ Weida Li et al., “Long-Term Persistence and Development of Induced Pancreatic Beta Cells Generated by Lineage Conversion of Acinar Cells,” *Nature Biotechnology* 32.12 (December 2014): 1223–1230, doi: 10.1038/nbt.3082.