CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

ALAN O. TROUNSON
MSc, PhD, Dr. Hon Cau, FRCOG, FRCAOG
Emeritus Professor, Monash University
President, CIRM

PRESIDENT'S UPDATE ON ADVANCES IN STEM CELL SCIENCE

Highlights of recently published papers from CIRM grantees and other leading research teams around the world—July 2012

International Consortia Create Models of Huntington's & Parkinson's

Two consortia organized by the National Institutes of Health, in which CIRM participates, published papers on creating disease-in-a-dish models for Huntington's disease and Parkinson's disease. The Huntington's paper was published online June 28 and is scheduled for print August 3 in *Cell Stem Cell* Vol 11 (1-15). It includes researchers from four CIRM-funded institutions: Cedars-Sinai, UC Irvine, UC San Francisco and the Gladstone Institutes. The Parkinson's project, which did not yet have CIRM participation, was published July 4 in *Science Translational Medicine* Vol 4 (141).

The Huntington's team, lead by Leslie Thompson at UC Irvine, made 14 stem cell lines representing several variations in the Huntington's gene mutation. These iPS cells were made from skin samples of patients with early-onset and late-onset disease, which are genetically linked to a few copies of the mutation or many copies respectively. They directed these iPS cells to mature into neural stem cells, which they shared with all 10 members of the consortium. Each group matured those neural stem cells further into the type of neurons destroyed in Huntington's disease and they consistently found that variations in how the cells functioned was linked to their genetic fingerprint. The various institutions used several different tests of cell function and were able to verify the differences; a powerful testimony to the value of consortium science.

The Parkinson's team, lead by Ole Isacson of Harvard Medical School, looked at the rarer forms of Parkinson's that are linked to clear genetic mutations; the majority of cases are considered random and haven't been linked to specific mutations. They created five iPS cell lines representing two distinct mutations. After maturing those stem cells into neurons they distributed those cells to the 10 members of the consortium—most of these members are different from those in the Huntington's group. Again they undertook an independent analysis and showed that all the cells showed the same defect in their so-called energy powerhouse, the mitochondria. They found cells reacted the same way to chemicals that cause stress and sometimes reacted the same to drugs that could reverse the impact of the stress. But the cells with the two different mutations reacted differently to one rescue drug, with one cell line not responding at all. This finding points to the potential for personalizing therapy for some patients.

Both these projects point to the power of iPS cells to reveal the underlying causes of disease and to open up new avenues for potential new personalized therapies.

Huntington's Mutation Corrected, Resulting Nerve Cells Appear Normal

A team at the Buck Institute in Novato, lead by Lisa Ellerby, reported in the August 3 *Cell Stem Cell* Vol 11 (1-11) that they had used gene correction techniques to replace the mutated Huntington gene in iPS cells and matured them into nerve cells that appeared to function normally.

Ellerby's team used reprogrammed stem cells, previously created by another team, made from skin samples of Huntington's patients and unaffected individuals They then used a bacterial artificial chromosome gene replacement technique (BAC) to substitute the correct Huntington gene for the mutant one in the iPS cells from Huntington's patients. Using several different tests they showed that the corrected cells behaved more like the iPS cells from unaffected people than they did the uncorrected HD iPS cells. They found that the cell's energy organells, the mitochondria, returned to normal function and that the cells began to respond to cell signals, such as one called BDNF, like normal cells.

Most important, when they matured those stem cells into neural stems cells and then further into the type of nerve cell destroyed in HD and transplanted those into a mouse model of the disease, they saw correctly functioning nerves develop. Those new neurons persist for at least two weeks. This suggests that it could be possible to use a patient's own cells, which should be immune compatible and not rejected by the patient's immune system, to correct the fatal mutation in this devastating disease.

Steady Source of Dopamine Producing Nerve Cells from ESCs

A team led by Gary Steinberg at Stanford reported that a special line of self-renewing neural stem cells could be directed to mature into nerve cells that produce dopamine, which are lost in Parkinson's dieseae (PD). The paper was published online July 17 in *PLoS One* Vol. 7 (7).

The team had previously developed a technique that starts with embryonic stem cells and coaxes them into maturing into the type of neural stem cell found in the mid-brain region where dopamine-producing neurons are destroyed in PD. They can successfully grow these cells in the lab over time and can expand their numbers sufficiently to be useful clinically. Now they have developed a technique using various growth factors and growth conditions that can mature significant portions of these cells into dopamine-producing neurons.

When the team transplanted these neurons into non-human primates they could detect the new cells had incorporated into their brains two months later. They were also able to detect that the cells were able to produce dopamine and had extended into neighboring tissue. This ability to produce these cells in large quantities addresses one barrier to eventual cell therapy in PD.

Muscular Dystrophy Reversed with Gene-corrected Cells in Mice

A team working jointly at University College London and the San Raffaele Institute in Milan has developed a technique to grow large quantities of muscle progenitor cells from iPS cells, correct the genetic mutation for a type of muscular dystrophy, have them engraft in the muscles of a mouse model of the disease, and produce normal functioning muscle cells. Their results were published in the June 27 *Science Translational Medicine* Vol 4 (140).

The team, lead by Giulio Cossu, worked with a type of dystrophy called limb-girdle muscular dystrophy (LGMD). They started with the fact that a type of cell found in blood vessel walls called mesoangioblasts can function as progenitor cells able to create muscle tissue. But they discovered that in LGMD patients their muscles have too few of these progenitors to be a viable therapy. So they created iPS cells from LGMD patients and developed a method to drive those stem cells into the desired progenitor cells. Then they corrected the gene that is mutated in LGMD using a viral vector to carry the new gene into the cells.

When the team injected these corrected cells, either intravenously or directly into the muscle of mice with a form of the disease, the cells homed to the damage muscle, engrafted there, and formed muscle fibers. It should be noted that in order to carryout this project the team had to develop a new mouse model that carried the specific mutation for LGMD as well as an immune deficiency that would allow the animals to accept human cell transplants.

The scalable nature of generating muscle progenitor cells through iPS cells suggests this could be a model for eventual cell therapies for LGMD. The authors also suggest the technique might work for some other forms of muscular dystrophy.

Antibody Targets Cancer Stem Cells for a Pediatric Leukemia

CIRM-funded research in Catriona Jamieson's lab at UC San Diego has verified the cellular pathway that allows a type of cancer stem cell to multiply, in this case, cells that initiate acute lymphoblastic leukemia (ALL). They also found that a protein, a specific antibody that targets this cellular pathway, can help eliminate those cancer stem cells. The UCSD team, along with collaborators at Boston's Dana Farber Cancer Institute, published their findings in *PLos One* Vol 7 (6) on July 3.

Numerous researchers have produced evidence that leukemia patients relapse because chemotherapy fails to eradicate the leukemia initiating cells, now commonly called cancer stem cells. The current work verified that a particular cell signal is key to their ability to self-renew and multiply. That cell signal is called NOTCH1. Earlier studies had shown that as many as half of ALL patients have mutations in NOTCH1 and Jamieson's team found the mutation in tissue samples from seven of 12 patients studied. They found that when T cells from these patients were transplanted into mice, they resulted in much more leukemia than cells from the patients without the mutation.

The mutation that activates NOTCH1 had been targeted by earlier potential therapies using traditional drugs. But they were not sufficiently specific to NOTCH1 and had unwanted effects. The UCSD/Boston team tried an antibody that selectively blocked the activation of the NOTCH1 gene. They found that mice transplanted with leukemic cells had greatly reduced numbers of cancer stem cells if they were treated with this antibody.

This work has the beauty of showing both the rational of why a therapy might work as well as an early indication that it could work. Jamieson suggests that the antibody might also be effective in some other cancers where the cancer stem cells require NOTCH activation, including breast cancer.