CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

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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—December 2011

Autism Study Shows the Power of iPS Cells to Define the Problem

CIRM funded work in the lab of Stanford's Ricardo Dolmetsch published online November 27 in *Nature Medicine* Vol. 17 (1657-1662) found the culprit error in Timothy Syndrome using brain cells grown from reprogrammed iPS cells. Timothy Syndrome includes Autism as a symptom.

The root causes of neurological disorders have traditionally been difficult to discover. During the past year a number of labs have shown the power of patient-specific reprogrammed iPS cells to point to metabolic errors in diseases ranging from Rett Syndrome to Schizophrenia. The current study from the Stanford team unveils an elegant path from the main error to downstream changes in cell behavior and finally to a chemical that can reverse the defect "in the dish."

They found that the autism in Timothy syndrome is caused by a genetic mutation that makes the calcium channels in the membranes of neurons defective and since calcium enables neurons to fire, their communication between cells is defective. The team also found that as a result of the calcium imbalance the cells were producing too much of an enzyme needed to produce dopamine and norepinephrine, which play key roles in sensory processing and social behavior—potential links back to the outward symptoms of autism. They then showed that the chemical, roscovitine, can block the defective calcium channel and result in 70 percent reduction in the production of that enzyme needed for dopamine and norepinephrine. This particular chemical acts on too many other pathways to be a human drug, but it points to a path to find a potential drug that could be safe in humans.

The researchers manipulated the iPS cells in a way that resulted in three-dimensional, brain cell-like spheres that matured into three distinct layers that were a good approximation of living tissue in the brain. This structure makes it more likely the "disease-in-a-dish" mimics the real thing.

Disease-in-a-Dish Models from iPS and Embryonic Cells Seem Equal

In CIRM-funded work published online in the *Proceedings of the National Academy of Sciences* December 12 Michael Longaker and a team of Stanford colleagues showed that reprogrammed iPS cells and embryonic stem (ES) cells produced similar results in modeling a disease, in this case Marfan Syndrome.

With all the papers that have come out this year suggesting differences between iPS and ES cells the time was ripe to make this comparison. The opportunity came up by accident. Another Stanford faculty member found that one embryo from a couple undergoing IVF with Pre-implant Genetic Diagnosis (PGD) had the mutation that causes Marfan syndrome.

The team made stem cell lines from the IVF embryo and from a skin biopsy from a Marfan patient. When they manipulated those cells to differentiate into various cell types they found both equally showed impaired ability to form bone and a heightened propensity to form cartilage, aberrations identical to those found in the skeletal disorder.

Although several mutations can cause Marfan, they are all in the same gene, the one for FIBRILLIN, which is a protein that inhibits the cell-signaling molecule TGF-beta. These disease-in-a-dish models showed for the first time that the reason stem cells in Marfan patients produce the inappropriate ratio of bone to cartilage is because they are getting to much TGF-beta signal, which should have been turned down by FIBRILLIN, a potential clue to future therapy.

Clues Emerge to Roadblock to Creating Blood Stem Cells from ESCs

The progenitor cells that many teams have hoped would be the intermediary to creating blood stem cells from pluripotent cells, seem to come from a different lineage than blood stem cells according to a paper published in *Cell Stem Cell* December 2 Vol. 9 (541-552) by a team lead by Nancy Speck at the University of Pennsylvania.

One of the major roadblocks in stem cell research has been the general inability to get pluripotent stem cells, either embryonic or reprogrammed iPS cells, to differentiate into blood forming hematopoietic stem cells that are able to engraft and then further differentiate into all the various cells types in blood. Many teams have tried to mature pluripotent cells through the intermediate cell type known as erythroid/myeloid progenitors. Speck's team used a series of experiments to turn off or turn on expression of genes for various factors that direct cell fate at various times during the development of the fetus in mice. Their results suggest that the erythroid progenitors and the hematopoietic stem cells originate from two distinct populations of endothelial cells. That means scientists would need to use a different differentiation pathway to get functional blood forming stem cells.

This issue has been argued for some years, and this study is likely to launch efforts to replicate or rebuff its results. But it does suggest the use of a new pathway to achieving the long sought goal of a limitless supply of blood forming stem cells that can colonize the bone marrow.

Genetically Modified Blood Stem Cells Fight Melanoma

A CIRM-funded study published online in the *Proceedings of the National Academy of Sciences* November 28 by a UCLA team lead by Jerome Zack showed that it is possible to genetically engineer blood stem cells so that they will produce T cells that can attack and eradicate melanoma.

A prior research team had isolated a T-cell receptor that seeks out a protein found on 40 percent of the melanomas that grow on Caucasians. Zack's team genetically engineered blood forming stem cells using a virus to carry the gene for this T-cell receptor into the stem cell's nucleus. These cells should then theoretically be able to generate large number of melanoma fighting T cells whenever needed. That apparently did happen. The team implanted the stem cells into human thymic tissue that had been previously transplanted into mice. Those cells matured and produced T-cells that were able to detect and kill melanoma cells. Out of nine mice, four had the melanoma completely eliminated and five had the tumor decrease in size.

In a test to make sure the T cells matured with their normal degree of precision while in the thymus, the team also implanted melanoma cells as a control that did not have the protein that is targeted by the manipulated gene. All those tumors survived in the mice, unchallenged by the T cells.

Tracheal Transplant, this Time on Artificial Scaffold, Works

In a paper published in *The Lancet* Vol. 378 (1997-2004) December 10 an international team used stem cells seeded on a synthetic scaffold to give a cancer patient a replacement for his cancer riddled trachea. The team, with members from Sweden, England, Germany and Iceland, was lead by Paolo Macchiarini currently at the Karolinska Institute in Sweden.

Macchiarini was part of the Spanish team that had previously used the collagen matrix from a cadaver's trachea as the scaffold for seeding stem cells to grow a new trachea that was successfully transplanted into a patient. Being able to use an artificial matrix could open up this procedure to many more patients as it ends the need to find a cadaver of just the right size to fit the patient. In this case, the airway was tailor-made using an artificial polymer scaffold designed from CT scan images taken prior to the surgery. The team then seeded it with stem cells from the patient's own bone marrow and grew it in a bioreactor for 36 hours.

It was functional at the time of transplant, but the team also gave the patient two cell growth factors for 14 days to enhance the maturation of the new airway. They were able to detect additional local repair from both stem cells naturally in the blood and from innate growth factors being turned on because neighboring cells sensed the not-yet-fully-mature tissue. The patient is doing well and cancer free five months after the surgery.

The Lancet titled the paper "A Proof of Concept Study," and it could indeed be a proof of concept for an entire branch of regenerative medicine that seeks to use stem cells to grow various tissues on an artificial matrix that can be built to order.

Fetal Cells Shown to Repair Mother's Heart

Hina Chaudhry and her team at Mount Sinai in New York, along with colleagues at Columbia and UCLA, published cellular level proof of a long-seen clinical phenomenon: women who suffer heart damage during or shortly after pregnancy have much higher rates of cardiac repair than other patients. Their study was published online in *Circulation Research* November 14.

In a mouse model the team found that fetal cells selectively home to injured maternal hearts and undergo differentiation into diverse cardiac lineages. The fetal cells engrafted and were clearly multipotent as they were able to form endothelial cells such as those in blood vessels, smooth muscle cells and cariomyocytes that form beating heart muscle.

The researchers verified that the new tissue that repaired the moms' hearts was fetal in origin by using green fluorescent protein (GFP) markers. They mated normal females to genetically modified males whose tissues expressed GFP. Halfway through the pregnancy they surgically caused a heart injury and they could detect fluorescent protein that would have been in the fetal cells in the tissues that made the repair. This raises interest in the use of placental stem cells for regenerative heart repair because the fetal repairing cells expressed markers that are only found in the placenta.