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—April 2013

Hormone Increases Production of Beta Cells, and In Turn, Insulin

A team at the Harvard Stem Cell Institute led by Doug Melton discovered a hormone that increases the body's ability to produce insulin-secreting Beta cells by 30 fold. The study in mice was published online April 23 ahead of scheduled print publication in *Cell* May 9.

The technique does not require activating existing stem cells to make more Beta cells. Instead it ramps up the Beta cells' natural ability to proliferate, something known to happen during pregnancy when mothers need to produce more insulin to correspond with the sudden weight gain of the fetus. However, the work is a testament to the power of the knowledge being generated by stem cell research. Melton's team had gathered so much knowledge about the growth of Beta cells that they could develop scans to search for genes and compounds that cause Beta cell replication.

The work is most relevant in Type 2 diabetes where the problem is not the total destruction of Beta cells by an over active immune system, but rather Beta cells that just can't keep up, often because of weight gain. Melton does suggest that the hormone, which he called betatrophen, might be useful in the early stages of Type 1 diabetes. He predicts the hormone could be in human clinical trials in three to five years. It is already licensed to Janssen Pharmaceuticals.

Kidney Created in Lab Produces Urine When Transplanted in Rats

Another Harvard team, this one led by Harald Ott at Massachusetts General Hospital, created a kidney in the lab, transplanted it into a rat and the new organ produced urine. They published this step toward lab-built donor organs online in *Nature Medicine* April 14.

The team started with kidneys that they had removed from rats and used detergents to remove all the soft tissues so that what was left was just the collagen matrix. They examined this scaffold to make sure it had the framework for all the blood vessels and filtration structures the final kidney would need. They then seeded this matrix with two types of cells, one from human umbilical cord and one from the kidneys of recently born rats. By manipulating the vacuum pressure in the growing chamber they were able to get the cells to populate the entire matrix. They first tested the new kidney in the lab to see if it could produce urine and it did. They then transplanted it to replace a left kidney they had removed from a rat, and there too, it successfully produced urine.

There are many remaining advances that need to be made before this method can result in replacement kidneys for the 100,000 people in the U.S. on transplant waiting lists. First, the new

kidneys were not very efficient. The authors attributed that, in part, to the fact that they had not matured and were producing urine on a scale more in line with a newborn, which is where most the cells had originated. Since, neonatal human cells will not be an option, the team will need to perfect ways to get stem cells to generate the various tissue types needed for a functional organ. For the scaffold, they have suggested that they could use the matrix left after washing cells away from a pig kidney. The size is similar to humans and some studies suggest that this collagen alone would not cause an immune response. They plan to repeat the current experiment in pigs this month.

Skin Cells Converted Directly to Pancreatic Cells

An Italian team led by Tiziana Brevini at the University of Milan has used a chemical tool instead of genetic factors to directly reprogram human skin cells to become pancreatic cells that are capable of producing insulin when it is needed. The work was published online in the *Proceedings of the National Academy of Sciences* in advance of a scheduled print date June 18.

The team did not use any of the genetic factors normally used for reprogramming, so there is no chance extra copies of potentially dangerous genes linger in the end cell product. This meant they also did not need to use viral vectors or other tools to carry the genes into the cells. Instead, they manipulated the cells' own switches that turn genes on and off. Much of this switching occurs through what is known as epigenetics, and in this case the most common on-off signal, the placement of a chemical known as a methyl group.

By exposing the cells briefly to a single chemical, a demethylating agent, the researchers were able to reset to the cells' internal working so that it was no longer an adult skin cell. They then followed a three-step process that directed the cells down the path to becoming pancreatic cells. After 35 days about nine percent of the skin cells had become pancreatic cells that were able to secrete insulin and restore normal processing of glucose in a mouse that had its own insulin-producing cells destroyed.

This work provides a significant advance toward being able to provide diabetics with pancreatic cells that match their own genetics. It avoids passing the cells through a pluripotent state that could leave behind cells capable of causing cancer. Also, it avoids adding genetic factors that could cause lingering changes in the cells.

Skin Cells Converted Directly to Brain Cells that Produce Myelin

Two teams, one at Stanford and one at Case Western Reserve in Cleveland, have used genetic factors to directly reprogram rodent skin cells into the early brain cells that can produce the myelin protective sheath that is lost on the nerves of patients with multiple sclerosis. The work producing these oligodendrocyte progenitor cells was published online in two separate papers in *Nature Biotechnology* April 14.

Both teams ended up using three reprogramming factors, but started with larger sets that had been chosen because of their known activity in this lineage of brain cells. The Stanford team, led by Marius Wernig, started with a library of 10 factors, and the Case Western team, led by Paul Tesar, started with a set of eight. They both settled on two of the same factors but chose different ones for the third.

The two teams showed that the resulting cells were able to generate functional myelin both on nerves in the lab, and in mice. While this is a significant achievement, direct reprogramming human tissue has generally been more difficult than mouse and rat tissue. Wernig's team wrote that while human reprogramming might require the use of additional genetic factors, it should be doable. In any case, they will need to improve the efficiency of the current procedure. For both teams, it only yielded a small percent of the desired cells and those percentages will need to be higher in order for these cells to become a potential therapy.

Cells Directly Converted to Nerve Cells in Living Mice

A team led by Malin Parmar at Lund University in Lund Sweden showed that human skin and other tissue implanted in mouse brains can be reprogrammed to become neurons directly in the brain. They also reprogrammed support cells in mice brains to become neurons in the work published in the *Proceedings of the National Academy of Sciences* April 23, Vol. 110 (no. 17).

Direct reprogramming to replace lost or damaged tissue in vivo, that is in living organs, has many potential benefits. It avoids the dangers of causing tumors that lingers when you start with pluripotent cells, either embryonic or reprogrammed iPS cells. Direct reprogramming to the desired tissue in the lab is generally inefficient and still leaves the problem of getting the desired cells to the location where they are needed.

The Lund team got around these issues by transplanting various human cells into the desired location after they had been genetically altered to express three genes known to be associated with the development of neurons. They were inserted into the cells using a viral vector, but one that would only activate the genes when they came in contact with the drug doxycycline. Once the altered cells were in place, the team fed the mice the drug in their water. They were able to verify that the cells converted to functional neurons.

They then took the next step and tried a similar process in the mouse's own cells. They targeted support cells called glial cells that are often still in abundance even when a degenerative disease has destroyed the neurons. After injecting the viral vectors into a glial-cell rich area of the brain, they were able to activate the genes and detect direct conversion to neurons.

Before this could ever be done in humans the process will need to evolve so the gene delivery system delivers the genes in a way that they can be turned off as well as turned on, but this paper is a significant first step.