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—March 2013

Breast Tissue Might Be Home to a Multi-purpose Replacement Cell

A CIRM-funded team at UC San Francisco led by Thea Tlsty has discovered a very unexpected cell, a rare adult cell that has the ability to become most, or perhaps all, cell types in the body. To denote the facts that the cells exist in the body, show broad plasticity and are adult cells, the team named the them endogenous pluripotent somatic (ePS) cells in a paper, published online March 19 in the *Proceedings of the National Academy of Sciences*.

The team's work built on a finding from several other groups that showed inhibiting a certain cellular pathway can allow a cell to acquire some degree of plasticity—an ability to change the type of cell it becomes when it divides. As a first step they identified cell surface markers that become present on cells when this pathway is inhibited. They then took healthy breast tissue from women who were having breast reduction surgery and separated that tissue into individual cells. Among those cells they found a few rare cells expressing the markers for inhibiting the targeted cell pathway.

What was most surprising, when they put those cells through various tests of pluripotentcy, they behaved much like embryonic stem cells (ESCs). The genes that have become classic tests for verifying the existence of ESCs were active in the cells. Both in laboratory cell cultures and in animals, when they placed the cells in the right conditions they could mature into tissues from all three of the classic "germ" lines, for example, intestine, bone and nerves. The cells were also able to make teratomas, the tumors made of many types of tissue that arise when embryonic cells are transplanted in certain areas of a laboratory animal. But unlike ESCs, these cells are mortal; they cannot divide forever. After about 60 duplications in the lab they stopped growing. This could reduce the cancer risk if these cells progress toward therapy.

Like much of science, these results will gain strength when they are replicated by other teams. But Tlsty and her team have already begun looking for similar cells in other tissues in the body.

Elusive Liver Stem Cells Isolated, Grown in Abundance in the Lab

Two teams, one led by Hans Clevers at the Hubrecht Institute in Utrecht, Netherlands, and one led by Markus Grompe at the Oregon Health Sciences University, have succeeded in isolating and growing liver stem cells in the lab. They published this first critical step toward using liver stem cells in therapy February 14 in *Nature*, Vol 494 (247-252).

The teams built on prior work done by the Dutch researchers that had shown that in tissues with constant turnover creating new cells such as the intestine and stomach, the stem cells involved have a very specific marker called LGR5. They had also shown that when those cells are grown in the laboratory with a compound that promotes a pathway that is active in rapidly dividing stem cells, they could grow intestinal cells, for example, in large quantities. The pathway that is active in these rapidly dividing cells is called Wnt and the protein that promotes Wnt in lab culture is RSPO1. This combination of marker, pathway and promoter is something that we are likely to read a great deal more about as we move toward cellular therapies that are scalable.

The big difference between liver cells and cells in the intestine is that liver cells are not normally rapidly dividing. But the Oregon team predicted that stem cells found at the site of an injury to the liver might be rapidly dividing and might have the same markers and same response to a promoter of Wnt. In a mouse model they verified this was indeed true. Once they isolate those stem cells they were able to grow them in large quantities in the lab for up to a year, and when the cells were transplanted into a mouse model of liver injury, they saw some of those cells mature into functioning adult liver cells.

For now, the conversion to functional adult liver cells was rather low but they are looking at combinations of growth factors and other conditions that could improve the yield of desired cells.

Heart Cells from iPS Cells Reveal Patient-Specific Drug Toxicity

A CIRM-funded team at Stanford led by Joseph Wu has created iPS cells from the skin of patients with three inherited heart conditions, turned them into beating heart cells in a dish and used them to verify known heart toxicities in drugs. The work was published online in *Circulation* March 21.

The team started by reprogramming skin from patients with long QT syndrome, hypertrophic cardiomyopathy, and dilated cardiomyopathy. After maturing those cells into beating heart cells in a dish they compared them to beating heart cells made from embryonic stem cells that showed no signs of the genetic mutations. They were able to verify that the iPS-derived heart cells showed the same abnormalities in cell size, gene expression and rhythms as those seen in patients with the diseases. But those abnormalities were not seen in the heart cells from the embryonic stem cells.

They then tested three drugs on these cells, one that has been shown to be safe, one that has some toxicity in certain patients and one that was pulled from the market in 2000 because it was linked to deaths. In each case, the cells in the dish verified what had been seen in patients. But in one case, with the sometimes-lethal drug, the team showed that the effect depended on dose. So this information might have allowed the company to change the dosages and salvage a potentially useful medication.

This type of modeling of genetic diseases and the accompanying ability to screen potential drugs for benefits and toxicities is a key reason CIRM recently funded an initiative to create some 9,000 iPS cell lines and bank them for use by researchers in academia and industry.

Like Wine, Heart Cells Grown in a Dish Do Better with Age

A team led by Michael Laflamme at the University of Washington has shown that heart cells matured from pluripotent stem cells, either embryonic or reprogrammed iPS cells, mature into heart cells that more closely function like adult heart cells if you grow them in the laboratory two to four times longer than what has been customary. They published their work online in *Stem Cells and Development* in March.

Up until now, teams that have grown heart cells from pluripotent stem cells have matured them in the lab for 20 to 40 days. Laflamme's team developed a protocol that allowed them to keep the cells viable and growing in lab dishes for 80 to 120 days. They found dramatic differences in the older cells. They showed a more mature structure, greater contractility and muscle fiber density. All those things would make such cells better at pumping blood and more likely to result in meaningful improvements in heart function after transplantation.

Most current stem cell clinical trials for human heart patients involve mesenchymal stem cells, the second kind of stem cell found in bone marrow. Those cells has been shown to have anti-inflammatory properties and an ability to secrete factors thought to recruit the body's own repair mechanisms, but not actually replace heart tissue themselves. Several teams are now looking at using pluripotent stem cells as a source of cells able to actually replace damaged heart tissue. The current work suggests that the resulting cells, if handled properly, could be beneficial.

Study Points to Potential to Harness Natural Killer Cells in Cancer

A team led by Dan Kaufman at the University of Minnesota has found a way to dramatically increase the efficiency of producing the immune system's Natural Killer (NK) cells in the laboratory. The team published its work online in CIRM-supported *Stem Cells Translational Medicine* March 26.

NK cells have had some success clinically when they have been used to destroy residual cancer cells that have proven resistant to traditional therapy. But these uses involve harvesting a patient's own NK cells and expanding them in the lab, which is cumbersome and inefficient. So, researchers have turned to pluripotent stem cells as a potential source of readily available NK cells. While producing these natural enemies of cancer from pluripotent stem cells has become somewhat routine, it too, has been inefficient and only able to produce small quantities of cells.

The Minnesota team devised a two-step process that eliminated the animal feeder cells used in prior methods. The animal cells make clinical use more difficult. They increased the efficiency of maturing the stem cells into NK cells using a protein known as a cytokine, in this case interleukin 21. They suggest the method can generate enough NK cells to treat a single patient starting with fewer than 250,000 embryonic or induced pluripotent stem cells, which seems like a reasonable number for clinical application.