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—September 2013

Stem Cells Assembled into Multi-layered Brain Tissue in the Lab

A team lead by Juergen Knoblich at the Austrian Academy of Sciences in Vienna used iPS type stem cells to produce brain organoids in the lab to serve as a model for normal brain development and used the model to reveal a possible cause of the small-brain birth defect known as microcephaly. They published their results in *Nature* September 19. Vol. 501, 373-379.

The team built on several recent reports that have shown the strong capacity for stem cells to self organize into multi-layered tissues if they are given the right environment. The pea-sized brain tissues they created and call cerebral organoids are the most complex neural structures so far. They verified the organoids contained several types of brain cells and the different types of cells seemed to interact although the organization of the various cells did not match the human brain. Also they did not form blood vessels, which probably accounts for the limited size of the organoids.

One key to the research protocol—as with most of the groups building more complex tissues—was to grow the cells in 3-D cultures. They also used a gel that somewhat mimicked the connective tissue that would be found in the developing brain. The cells formed initial organoids quickly, within 8 to10 days, and went on to develop distinct nerve tissues within 20 to 30 days. Although their size was limited, they look like they can survive indefinitely, currently up to 10 months.

The multi-tissue structure combined with this long-term survival makes these cerebral organoids a great model for unlocking some of the mysteries of normal and abnormal brain development. The Austrians teamed up with a group at the University of Edinburgh to try to understand microcephaly. The Scots had a patient with the condition and when they created iPS cells from the patient and tried to grow organoids they ended up with smaller and less organized clumps of cells. Through some pretty elaborate tests, the researchers showed that it was highly likely the small size was because the intermediate progenitor stem cells matured too rapidly into adult nerve cells, leaving behind too few cells at the early stage of development.

The researchers have created an elegant model for studying brain development as well as understanding errors in brain development.

Method Creates Unlimited Source of Versatile Pancreas Cells

Hans Clevers and his team at the Utrecht Medical Center in the Netherlands have built on their prior work creating complex tissues to isolate progenitor-stage stem cells in the pancreas that are able to form two key tissues of the organ, beta cells and duct cells. They used mouse cells and published their work online September 17 in *EMBO.*

Clevers' prior work elucidated and then used an elegant interplay in cells' internal signaling. Very specific signals are necessary to activate adult stem cells. In proliferating adult stem cells the gene known as Wnt is turned on, and their cell surface has a receptor for proteins that promote Wnt called R-spondins (RSPO). Previously he has grown intestinal stem cells in 3-D cultures along with RSPO and created complex intestinal tissues. But the pancreas in normal situations does not have cells with active Wnt or the RSPO receptor. Thus it has been very hard to isolate pancreatic progenitor stem cells that can be expanded in the lab. Now his team has shown that if you injure the duct of the pancreas you induce the RSPO receptor and in turn Wnt, and that you can also induce the receptor by breaking up duct tissue and growing it in the lab in cultures rich in RSPO.

The cells in these cultures quickly form cyst-like organoids. Most important, they can be readily grown in the lab seemingly indefinitely. They showed five-fold expansion weekly for up to 40 weeks. The researcher were able to induce these organoids to produce both beta cells and pancreatic duct cells, two key cell types for pancreas repair or replacement. This system could eventually be an efficient source for pancreatic tissue for transplantation, although as always, there are many hurdles between mouse models and human cells.

Stem Cell Defect Linked to Down Syndrome, Points to Possible Therapy

A team partially funded by CIRM led by Michael Clarke at Stanford has found a gene that leads to defects in stem cells that could account for some of the premature aging and other symptoms seen in Down Syndrome. The work was published online in *Nature* September 11.

Clarke's team did not set out to find a Down Syndrome link. They were conducting their usual line of study related to cancer. Specifically they were looking at the genetic regulation of growth and self-renewal in normal stem cells and in cancer stem cells. They found a gene that seemed to play a role, Usp16, and noticed it was on chromosome 21, the chromosome with an abnormal third copy in people with Down Syndrome.

So, they worked with another group on campus that had mouse models of Down Syndrome, including one that had three copies of the Usp16 gene. They took cells from the brains of young mice and looked at the ability of intermediate neural progenitor stem cells to grow. In culture, these cells from normal mice will form clumps called neurospheres. But only one out of 958 of the cells with the extra Usp16 gene were able to mature like this. By contrast, one of every 21 of the normal cells did so. To further verify the role of the extra copy of the gene, the group bred mice in which the gene was not active on one of the three copies of that chromosome. In brain cells from those mice, the ability of the progenitor stem cells to grow returned to normal.

The defect appears to affect stem cells throughout the body. So, the team also tested the hypothesis in human skin cells. They found that in normal human tissue, when the cells are manipulated so that an extra copy of Usp16 is turned on, the cells don't proliferate normally. They also cultured skin cells from people with Down Syndrome and verified the same defect in cell growth. More important, they were able to turn off the gene on one copy of chromosome 21 and return the cells to normal growth. This suggests that finding a way to reduce the level of the protein coded by Usp16 in people with Down Syndrome could ameliorate at least some of the symptoms of the condition.

Dramatic Increase in Efficiency and Speed of Creating iPS Cells

An Israeli team led by Noa Novershtern and Jacob Hanna at the Weizmann Institute has found a way to get virtually all of the adult cells exposed to the standard reprogramming factors to convert to the iPS type of pluripotent stem cell. They published their methods in *Nature* online September 18.

The team removed a single protein to obtain the remarkable gain in efficiency, which in many standard procedures is closer to 1 percent. Not surprisingly, the gene, Mbd3, is largely depleted during fertilization and stays silent during the early days of development when embryonic stem cells form naturally.

Since you generally don't need large numbers of pluripotent cells to create a cell line for research or therapy, the aspects of efficiency that go beyond raw numbers are probably what will be most important in this work. All the cells seemed to reprogram at the same rate. In all other reprogramming techniques the cells progress to a pluripotent state at very different speeds. This results in mixed populations of cells that are difficult to sort and make it hard to produce cells that meet therapeutic guidelines. Also, the speed was much faster, 8 days instead of 30 or more. This could be important when the day comes that we reprogram a patient's own cells to create replacement tissue. If they have had a heart attack or other acute injury, speed could be critical.

For now, this system provides a much needed tool to better understand what really happens when cells are reprogrammed, something that is largely a mystery today. Perhaps blocking this protein in vivo when you are trying to reprogram cells from one type to another using transcription factors delivered by viral vectors will become much more effective.

Genetic Manipulation Gets Mouse Hearts to Repair Themselves

Kenneth Chien and colleagues at Harvard and at the Karolinska in Sweden have used genetic manipulation to get native heart stem cells to produce healthy new blood vessels at the site of an induced heart attack in mice. They published their paper online September 8 in *Nature Biotechnology*.

They targeted a protein called VEGF that had produced modest or poor results in the past. VEGF, which is Vascular Endothelial Growth Factor, is a logical choice if you want to create new blood vessels to get new heart muscle to grow rather than scar tissue. But, when the protein has been injected directly it did not seem to stick around long enough to do much good. And when the DNA for the gene was inserted, it seemed to over express and result in too many vessels that were leaky and caused edema.

Chien and his team decided to use a synthetic form of RNA, the intermediate genetic material that carries the code for the protein into the part of the cell where the protein can be assembled. The advantage of the RNA construct was that the signal to produce the protein could be pulsed resulting in short bursts of the protein being produced. In mice that had an induced heart attack, the treatment reduced the infarct size and improved survival.

In a companion paper in *Cell Research* published online September 10, the team showed that a similar process seems to work in human cells growing in the lab, a first step to transferring this process for mobilizing endogenous stem cells to humans.