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—May 2013

Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer

A team at the Oregon Health & Science University led by Shoukhrat Mitalipov successfully derived six embryonic stem cell lines from embryos created by somatic cell nuclear transfer (SCNT), also called cloning. The long-anticipated feat was published online in *Cell* May 15.

Teams around the world have tried to create cell lines like these ever since Jamie Thomson first isolated human embryonic stem cells from unused IVF embryos in 1998. Starting with a donated human egg, you remove its chromosomes, and then replace this genetic material with chromosomes from an adult cell, get the egg to start to mature and on day five or six harvest stem cells. While this had been accomplished in many animal species, there was something about human eggs that did not allow them to mature to the point stem cells could be harvested.

The Oregon team used a number of tactics to keep the egg maturing to the point stem cells could be derived. Eggs are normally activated by sperm—a step this team accomplished using electrical stimulation. In previous attempts, after stimulation the eggs prematurely ended the process where chromosomes are divided up between cells. The team got around this by treating the eggs with caffeine, which prevented them from ending this critical stage too early. They then used another compound to enhance the final development into an embryo. The resulting process was much more efficient than earlier work with human eggs or non-human primates. They only used 28 eggs to get the six cell lines.

After the paper came out some concerns were raised about errors involving duplication of figures. This was unacceptable for science and needs explanation by the team. The resulting cell lines should be independently analyzed by geneticists who should be able to detect DNA from the donor egg as well as from the adult cell. Importantly, we need independent confirmation from other labs of the findings published in peer-reviewed journals. However, I do think the results will stand and will open the door to some critical comparative analysis. We can now create reprogrammed iPS cells and embryonic cells from the same donor. This gives us the opportunity to compare the genetic stability, epigenetic memory and robustness of differentiation of the two types of cells. Also, new reprogramming factors found in the eggs could help improve the efficiency of creating iPS cells. While California law prevents CIRM grantees from creating SCNT cells lines through paid egg donors, we should explore ways for our researchers to use the resulting cell lines.

Neurons Grown from iPS Cells from Down Syndrome Show Defect

A team at the University of Wisconsin, led by Anita Bhattacharyya, reprogrammed skin cells from two people with Down Syndrome to create iPS cells, grew those into neurons and detected two

distinct differences between those nerves and normal ones. They published this work online in the *Proceedings of the National Academy of Sciences* May 28.

This work shows our increasingly robust ability to gain valuable information from disease-in-a-dish models using iPS cells derived from people with genetic conditions. Other teams have created iPS cells from Down Syndrome individuals, but the Wisconsin team is the first to document the physiologic deficits associated with the extra copy of chromosome 21, which is the underlying cause of the syndrome. They were able to verify these differences in part because they were able to create a genetically matched control set of iPS cells. One of the individuals had some cells that did not have the trisomy, the third chromosome. That meant the researchers could create a cell line with the normal number of chromosomes and a cell line with three copies of chromosome 21 from the same person and compare them.

They detected two differences in the resulting neurons. The cells with trisomy had 40 percent fewer synapses, the connections between nerves that allow the cells to communicate. They also found that the cells with trisomy had a large number of active genes that respond to a chemical disturbance called oxidative stress. If those nerves live in constant chemical stress it could explain the premature aging and predisposition to Alzheimer's disease seen in Down Syndrome. Similarly the lower level of synaptic connections could account for the cognitive impairment seen. This improved understanding of what is going on in the brains of individuals with Down Syndrome could lead to potential measures to ameliorate the impact of the condition.

Functioning Human Thymus Tissue Created from Stem Cells

A CIRM funded team at UC San Francisco led by Mark Anderson and Matthias Hebrok have created thymus tissue from human embryonic stem cells that functions when transplanted into mice. The work was published online in *Cell Stem Cell* May 16 in advance of a scheduled print date July 2, Vol. 13 (1-11).

Because the thymus gland plays key roles in regulating the immune system it is of great interest to those in the field of regenerative medicine. The gland has two main activities: it helps the immune system's T cells mature into cells capable of helping us fight off infections, and it trains immune cells to know the difference between cells that are our own and those that are foreign. That detection of foreign tissue results in a huge roadblock for repairing tissue or replacing organs through stem cell-based technologies. Just by doing what it is supposed to do, the thymus could send out troops to destroy any replacement tissue. This would require using the same immune suppressive drugs currently used for organ transplants. Because of the significant side effects of those drugs, their use would generally limit stem cell-based tissue repair to acutely life-threatening situations.

For patients with less severe conditions and even with those with life threatening conditions, it would be great if we could avoid those drugs all together. The UCSF team proposes that if you start out with embryonic stem cells and direct them down two different paths to mature cells, one for the desired replacement tissue and one to become thymus tissue, and then transplanted them both you could provide the patient with the needed repair as well as an immune system that would not attack the repair.

Other groups have got embryonic stem cells part way down the path toward becoming thymus tissue, but they have not been able to get them all the way. The UCSF team used six different factors tested in more than a dozen combinations of sequencing and timing to get the cells they wanted.

When those cells were transplanted into mice, they further matured into thymus tissue that was able to help T cells develop and proliferate. Those T cells appeared to function normally in the animals. This work is many steps and years away from the goal of the dual transplant, but it is a major advance in that direction.

Inhibitory Nerves Grown from Progenitor Cells Quell Epilepsy in Mice

Two CIRM-funded teams at UC San Francisco have grown inhibitory nerves and shown those nerves can integrate into the brains of mice and, in animals that have epilepsy, they can calm the overactive nerves that cause seizures. One team, led by Scott Baraban, started with mouse embryonic progenitor cells and published their work in *Nature Neuroscience* online May 5 ahead of scheduled print publications in June, Vol. 16 (no. 6). The second team, led by Arnold Kriegstein, started with human embryonic stem cells and published their work in *Cell Stem Cell* May 2, Vol. 12 (573-586). Both teams included CIRM grantee Arturo Alvarez-Buylla.

Baraban's group started with nerve progenitor cells from donor mouse embryos that had grown to day 13. That is considerably more mature than the stage where you harvest embryonic stem cells, but the nerves are only partially mature so they are dubbed progenitor cells. The nerves they selected were medial ganglionic eminence (MGE) cell progenitors. These are the inhibitory nerves that act as checks and balances on the excitatory nerves, which tend to be over active in epilepsy. When they transplanted those cells into mice with epilepsy, they matured into functional MGE cells and eliminated seizures in half the mice and reduced seizures significantly in the other animals. The team also used two behavior tests to see if the cells reduced the cognitive deficits seen in epilepsy and they did.

Kriegstein's team developed a protocol to mature human embryonic stem cells into MGE precursor cells. They then did extensive experiments to follow the cells further maturation into functional inhibitory nerves. That maturation of the cells took several weeks, which mimicked the known development process in human embryos. When they transplanted the cells into mice they were able to detect that the cells migrated from the injection site, matured into various sub-types of nerves, and integrated into the host brain.

Together the two papers paint a nice picture of the progress of science. An animal disease model treated with animal cells verified the type of cell involved and provided valuable information about the best location to inject the cells to impact the disease. Then the second team showed the same cells could be generated with human stem cells and those cells could function in a host animal. This could be a means of providing a limitless supply of the GME progenitor cells that would not be available from human fetal donors. Perhaps the teams' next step will be to determine if these human cells can also treat the animal model of epilepsy.

.