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—June 2013

Cold War Proof that Adult Human Brains Can Produce New Neurons

A team at Sweden's Karolinska Institute led by Kirsty Spalding produced definitive evidence that humans are able to produce new neurons throughout our adult lives, in particular in the hippocampus, the part of the brain responsible for learning and memory. The long-debated finding was published in *Cell* June 6 Vol. 153 (1219-1227).

While researchers have long been able to identify neural stem cells in humans, as well as the intermediate progenitors cells they produce called neuroblasts, it has been hotly debated as to whether or not those neuroblasts can mature into functioning neurons. Another Karolinska team provided some evidence in 1998. They injected a compound that can label cells that are dividing into patients who had agreed to have their brains examined after they died. The team found evidence of new cells, but later found out the compound was toxic, so no one has been able to replicate the work.

Spalding's team took 10 years to perfect the complicated science that produced the definitive proof. They used an artifact created by the cold war nuclear bomb tests that occurred between 1955 and 1963. Those tests doubled the amount of the isotope Carbon 14 in the atmosphere. Because the plants and animals that people ate took up the Carbon 14, the carbon our cells used to make new cells would have higher than historic concentrations of the isotope. They analyzed the brains from 55 cadavers who had lived both before and during the cold war. The first trick was to be able to separate neurons from other brain tissue, which took five years to perfect. Next they had to figure out how to separate the carbon incorporated into the DNA from the rest of the carbon in the cell. They needed to do this because while a cell might renew other parts of its structure with the heavier carbon, the only reason it would make new DNA would be to divide. So any Carbon 14 in DNA would mark new cells.

The Swedes shipped the genetic material to Lawrence Livermore Laboratory in California where they used a particle accelerator to measure the ratio of routine carbon to carbon 14. They found that on average one third of the neurons in the hippocampus had been exchanged with new ones. This is an important finding as we try to generate neurons from stem cells for brain repair.

Major Step in Creating Donor Cells Tolerated by the Immune System

A team led by Laurence Cooper at MD Anderson Cancer Center in Houston used genetic engineering tools to eliminate one of the main reason donor cells are rejected by a patient's immune system. The work, in both human T cells and human embryonic stem cells, was published online June 5 in *Blood.*

Using a patient's own stem cells to get desired therapeutic cells generally requires considerable time and expensive laboratory manipulation that has to be done individually for each patient. Starting with donor cells can improve consistency and availability at the same time it lowers cost. The issue becomes how do you get the donor cells to engraft, or stick around, long-term and not be rejected as foreign by the patient's immune system. The primary way cells are recognized as foreign is their Human Leukocyte Antigens (HLAs).

The Anderson team used a type of molecular scissor to turn off the genes for one set of HLA expression. (They used the same Zinc Finger Nuclease technology being used by the CIRM-funded Disease Team at City of Hope hoping to cure HIV.) They first worked with T cells, the immune system cells that are most important therapeutically when cancer patients receive bone marrow transplants. They were able to create batches of T cells in which 95 percent of the cells expressed no genes from one subset of HLAs. They then successfully repeated the work with human embryonic stem cells (ESCs), which greatly expands the technique's potential because ESCs can generate donor cells of many types that might be tolerated by the immune system.

The immune system's complexity remains notorious. So, whether this genetic manipulation will be sufficient to create an off-the-shelf therapy cannot be answered yet. But complete immune tolerance is likely to require elimination of other subsets of HLA gene activity. Nonetheless, it a major stride towards reaching that goal.

Kidney Specific Cells, Structures Created from Embryonic Stem Cells

A team led by Hiroshi Itoh at Keio University in Tokyo has created cells that have the molecular markings of kidney cells and are able to form the tubular structures seen in kidneys when grown in laboratory cultures. The work started with mouse embryonic stem cells and was published online in *PLOS ONE* June 3.

While researchers have ben able to direct embryonic stem cells (ESCs) to become many different tissue types, creating renal tissue has proven difficult because of the complexity of structures in the kidney and the variety of cell types involved. While several teams have succeeded in driving ESCs toward kidney cells, they have not been able to get large quantities or get them to form the complex tubes of a kidney in the lab, where tissue for kidney repair would need to be created.

Itoh's team took the earlier results of other team who had tried various factors to drive ESCs toward renal cells and by trying new combinations they generated the desired cells with greater efficiency. But they were still among many other types of cells. They found that the renal cells had a protein that was only expressed in kidney tissue, so they created an antibody that could detct that protein and used it to sort and purify the cells. Even if purer cell populations the next step was not efficient, they did not get a high number of kidney tubules forming in the cell cultures. So they added a protein known to help cells mature called Wnt4. When they combined that last step with just the right three-dimensional growth medium, they saw significant numbers of tubules formed.

Chronic kidney disease is a major global health care problem, cutting short lives and costing economies tremendous sums for dialysis other end stage renal disease therapies. Much more lab work lies ahead in this field, but the current research should put potential regenerative renal therapies on a faster track.

Getting a Handle on the Chromosome Switches in Maturing Embryos

An all-star research team from around the globe collaborated on an elaborate project on the epigenetics—in essence the switching mechanism for turning genes on and off—during the early stages of embryo development. Understanding these switching mechanisms is essential to efficiently getting embryonic stem cells to mature into the type of cell needed for regenerative therapies. The 42 listed authors worked in 11 institutions in four US states and four countries. The three senior authors were James Thomson from Wisconsin and UC Santa Barbara, Joseph Ecker from the Salk Institute and Bing Ren from UC San Diego. The paper was published in *Cell* May 23, Vol. 153 (1134-1148).

This collaboration was funded in large part through the National Institutes of Health Epigenome Roadmap Project. The multi-institutional grant allowed the collaboration to pull in researchers from anywhere who were known to have proven protocols for various aspects of the project and then to use expensive high-throughput screening technologies to narrow the search for the most important gene switching mechanisms.

It would have been almost impossible to get enough human embryos at the various stages of development to accomplish this project. So, in this case embryonic stem cells (ESCs) stood in for developing embryos. Various members of the team had shown prior success in making four types of cells that play key roles in the developing embryo. Those were two cell types from the very early stage embryo: mesendoderm, which are cells that can become everything except brain and skin; and trophoblast-like cells, which can form the placenta and other support structures for the embryo. They also worked with two types of cells from later stages of embryo development, nerve progenitor cells and mesenchymal stem cells that can form bone, cartilage and other connective tissues.

After reviewing the literature to see which genes were likely to be turned on or off specifically in certain types of cells, the team measured the expression of over 19,000 genes. They found that about 17 percent of those genes were turned on only in cells directed toward one of those types of cells. They then looked at the epigenetic markers, or switches, in those four cell types, as well as embryonic stem cells and an adult tissue used as a control. They looked at nearly 104,000 so-called epigenetic marks that could enhance the activity of genes in those cells. Of those, high throughput screening found more than 32,000 that were only active in certain types of cells.

Most important, they found that the gene enhancers that were active were quite different between the two early stage cells and the two later stage cells. This provides valuable new understanding of normal embryonic development now, and could point to ways to enhance the creation of specific cell types from ESCs in the future.

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