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—February 2012

A Practical Advance Simplifies Scale-up of Growing Cells

The first issue of *Stem Cells Translational Medicine*, the new journal supported by CIRM—published online last month and recently out in print—contains a CIRM-funded study from Michael Kahn's team at the University of Southern California. They report on a new cell culture system for growing and expanding pluripotent stem cells that makes major strides toward simplifying the process of large-scale production of cells that meet FDA requirements for Good Manufacturing Practice (GMP).

This article is a classic example of why we committed our agency to supporting this type of journal. The results are extremely practical and critical to the ultimate commercial success of the field, but are not the type of findings that would make it into a traditional research journal. Early cell cultures for pluripotent cells had the double problem of being difficult to scale up and requiring animal feeder cells, which would greatly complicate creating GMP grade cells. Numerous reports have shown various ways to eliminate the animal cells, but those systems have still been quite complex involving several human-derived proteins. The USC team was able to eliminate two of those human proteins with a single small molecule named ID-8. Maintaining cell cultures using small molecules could greatly accelerate our ability to make clinical-grade off-the-shelf cells for therapy.

In order to arrive at their pragmatic candidate molecule the USC team had to delve into a lingering unsettled question in basic research. The signaling protein dubbed Wnt has been shown to be involved in maintaining pluripotent cells in their pluripotent state, but it has also been shown to be involved in directing pluripotent cells to differentiate and mature toward adult tissue. These dual and contradictory roles are not well defined. In their current work they sought a compound that could keep Wnt signaling in the mode of maintaining pluripotency. ID-8 did the job.

iPS Cells Might Be a More Robust Source of Mesenchymal Stem Cells

The second issue of *Stem Cells Translational Medicine*, recently published online, has another practical paper on scaling up cell production, this one dealing with the relative scarcity of mesenchymal stem cells (MSC) in their traditional sources: bone marrow and fat tissue. This paper was produced by a team at the University of Queensland in Australia led by Nich Fisk.

Teams around the world have begun clinical trials using mesenchymal stem cells in attempts to treat numerous diseases and inflammatory conditions. But when those trials get into later stages enrolling more patients many may hit a ceiling in their ability to produce enough cells efficiently. And if the trials involve the elderly and their own autologous cells, MSCs decline in quality and quantity with age.

The Australian team tried their technique with both human Embryonic Stem Cells (ESC) and reprogrammed skin cells, induced Pluripotent Stem Cells (iPSC). The established methods for maturing either type of pluripotent stem cell into mesenchymal stem cells has taken 30 to 40 days, required cumbersome techniques that don't scale up easily, and tended to produce mixed cell types that must be sorted before use. Fisk's group treated their cells with an inhibitor of a growth factor known to be needed to maintain the pluripotent state. After 10 days of culture with this compound they transferred the cells to standard cell culture materials used for growing MSCs, and in just 10 more days they had uniform cells that expressed all the characteristics of MSCs, shaving off a significant amount of time and complexity from the process.

New Method Improves Practicality of By-passing Stem Cell State

A CIRM funded study by Marius Wernig's team at Stanford builds upon his earlier work that converted skin cells directly into neurons without first passing through the induced Pluripotent Stem Cell (iPSC) state. The paper reporting on mouse cells was published in the *Proceedings of the National Academy of Sciences* Vol. 109 (7), February 14.

Most important, the reprogramming this time resulted in neural precursor cells, which unlike the end product neuron, can be grown in large quantities in the lab. Once expanded, these precursors can be directed to mature into all three major nerve cells: neurons, astrocytes, and oligodendrocytes. When transplanted into a mouse, these precursors were able to integrate into the mouse brain and produce proteins used for sending signals from one cell to another. The team used a virus to carry just three growth factors into the skin cells and were able to get transformation to neural precursors in one out of 10 cells, a relatively high success rate compared to their prior work and that of others.

Many researchers in the field see a significant portion of future clinical progress coming from protocols that reprogram one adult cell directly into another. This work showing you can hit the intermediate precursor state, with its ability to grow in large numbers in the lab, makes this a much more practical possibility.

iPSCs Yield Model for Understanding the Mysteries of Hepatitis C

In the same February 14 issue of *the Proceedings of the National Academy of Sciences* Vol. 109 (7) a team from MIT and Rockefeller University take the concept of "disease-in-a-dish" to a new plane. They used induced Pluripotent Stem Cells from specific patients to model for the first time the complex interplay between an infectious agent and the host's genetic makeup.

Hepatitis C afflicts more than 170 million people worldwide, but is highly variable in its impact on those people and its response to current therapies. Scientists have previously shown that a number of genetic variations in individuals can impact their ability to clear the virus completely, the ability of the virus to enter their cells, and their response to interferon-based therapies. But the mechanisms for this variation remain unclear. The current team hopes that watching hepatitis C infection in cells in a dish will allow them to determine why various patients respond to the virus the way they do. Eventually, they suggest they will be able to develop personalized medical interventions based on how medications work on an individual's cells in the disease model in a dish.

The researchers started out by making iPSCs from patients with specific genetic variations. They then matured those cells into liver-like cells and verified that those cells still expressed the genetic variation seen before the reprogramming. They were able to show that Hep C was able to infect those cells and the cells were able to support the full life cycle of the virus. Also, they showed that the cells produced an antiviral inflammatory response. The study clearly lays the foundation for personalized disease-in-a-dish models for this vexing pathogen.

Stem Cell-derived Neurons Produce Dopamine in Monkey Parkinson's

A paper published in the *Journal of Parkinson's Disease* Vol. 1 (2011) on January 24 showed for the first time that neural precursor cells derived from induced Pluripotent Stem Cells (iPSC) can engraft in a primate model of Parkinson's disease and produce dopamine, the missing neurotransmitter in the disease. A team lead by Jun Takahashi at Japan's Kyoto University did the work.

The team showed that the engrafted neurons survived at least six months and that the dopamine they produced resulted in slight symptom improvement in the monkey. Thus they provided key proof of concept that iPSCs could be a source of therapeutic cells for transplantation in Parkinson's patients. The cell culture technique they employed did not use animal feeder cells, so it is more easily adapted to GMP conditions necessary to make clinical grade cells for trials.

The Japanese team started with human iPSCs and matured them into neural precursors in the lab using six growth factors and chemicals over the course of four weeks. When they transplanted them into the putamina region of the brain they verified survival and function using Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) scans. They verified the grafts produced dopamine, so some of the precursor cells had to mature into dopamine-producing neurons. But perhaps equally important, the graft was larger after six months so, some of the precursor cells had to maintain their ability to proliferate. Both cell outcomes would be valuable in trying to produce a long-lasting treatment for Parkinson's.