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—April 2014

Two Teams Reproduce Nuclear Transfer Success Creating Stem Cells

Two groups have replicated the breakthrough success of Oregon's Shoukhrat Mitalipov in creating human embryonic stem cell lines through somatic cell nuclear transfer (SCNT), also known as therapeutic cloning. One group led by Dong Ryul Lee at CHA University in Seoul with others at Texas A&M and Advanced Cell Technologies in Massachusetts published their work online April 17 in *Cell Stem Cell* prior to print publication June 5 Vol. 14 (1-4). The second team led by Dieter Egli at the New York Stem Cell Foundation with others at Columbia University and the Hebrew University in Jerusalem published their work online April 28 in *Nature*.

SCNT, which requires placing the nucleus of a mature cell into an egg that has had its own nucleus removed, has been relatively easy to accomplish in lower mammals but impossible in humans until the Oregon success. But that work got the donor nucleus from fetal tissue or a recently born baby, so it left open the question of whether the procedure would work with a less malleable cell from an older adult. The Korean team created two stem cell lines, one from a 35-year-old and one from a 75-year-old. They used largely the same procedure as the Oregon team but made one key change in the protocol. Instead of waiting just 30 minutes after fusing the donor nucleus and the egg to stimulate the egg to trigger it to divide, they waited two hours. They speculated that this longer incubation may have helped reprogram the genes of the older cells to behave like younger ones.

The New York team used a somewhat different procedure to create embryonic stem cell lines cloned from the foreskin of a newborn male and from the skin of 32-year-old with diabetes. The latter became the first disease-specific cloned stem cell line. They modified a procedure they used to create a variant of SCNT-derived stem cells in 2011. At that time they had hypothesized that there must be something in the human egg genes that is necessary to reprogram the adult nucleus. So they left in one set of the egg genes, which resulted in stem cells that were triploid; they had two sets of genes from the donor nucleus and one from the egg. Those cell lines provided a tool for some interesting research but were never really an option for therapeutic cloning where the goal is to create stem cell that match the genetics of the donor to create repair tissue that will not be rejected by the immune system. This time they changed the way they activated the egg and modified some other steps and were able to get true SCNT stem cell lines.

Replication of results remains a cornerstone of science and it is reassuring to see two teams independently replicate the results of the Oregon team in less than a year. I also enjoyed reading that the New York group had matured the stem cell lines into pancreatic tissue, but both the protocols required too many eggs to be viable for patient care procedures until they are greatly refined. However, both teams have announced that they have created iPS type stem cells from the same patients so that we can begin much needed comparison of embryonic and iPS cells.

Sloppy Data Handling Casts Shadow on Bone Marrow Studies for Hearts

A team lead by Darrel Francis at Imperial College London has completed a literature review of studies using a patient's own bone marrow stem cells to treat heart damage. Their results suggest that the likelihood that a study's authors report positive results increases with the number of discrepancies in the way they report the data. The London team published their analysis in the *British Medical Journal* Vol. 348 (g2688) on April 29.

The group reviewed 133 publications from 49 clinical trials that looked at improvement in the heart's ejections fraction, a standard measure of heart function. Although they could not say whether the conclusions of any one study were accurate the correlations with data discrepancies were consistent and linear. The five trials with no discrepancies reported no benefit from the stem cell therapy, the 24 trials with 1 to 10 discrepancies reported average improvements of 2.1 percent, the 12 trials with 11 to 20 discrepancies showed an average improvement of 3.0 percent, the three trials with 21 to 30 discrepancies showed an average improvement of 5.7 percent, and the five trials with more than 30 discrepancies showed an average improvement of 7.7 percent.

The discrepancies included inconsistencies between tables and charts in the same publication or between two publications about the same trial. They also included results that reviewers called statistically impossible as well as contradictory claims between publications about how patients were randomized in the same trial. They found 604 discrepancies in total.

This study brings to the surface a couple issues. Large studies produce vast quantities of data and discrepancies in how it is reported are not confined to the stem cell field. But with the scrutiny the field is facing right now, this report is a clarion call for due diligence by researchers, the journals and journal reviewers. Also, with two large phase 3 clinical trials of autologous bone marrow stem cells in heart damage beginning to recruit patients, 480 in one trial and 1,000 in the other, the community needs to be sure these are conducted with utmost rigor. No one expects this type of stem cell therapy to replace damaged heart muscle directly, but there is speculation that it can trigger new small blood vessel growth. The resulting improvement, if it is real, is likely to be modest but potentially significant for patients, so we need high quality, well-presented data from these new trials. The patient community has had to sort through too many contradictory reports about this work for too long.

Protein Identified that Is Key to Integrity of Reprogrammed Stem Cells

A team led by Maria Blasco at the Spanish National Cancer Center in Madrid has shown that a protein called SIRT1 is required while reprograming adult cells into iPS type stem cells in order to produce cells able to maintain healthy chromosomes. The team published their work in *Stem Cell Reports* on line April 17 ahead of print publication May 6, Vol. 2 (1-17).

As we age, the caps at the ends of our chromosomes, called telomeres, become shortened and less able to do their job of protecting the integrity of our genes as cells divide. This same research team discovered in 2009 that the reprogramming process that creates iPS cells lengthens the telomeres and returns them to a more youthful state like in embryonic stem cells, but we haven't known how this happens.

Shortly after discovering the telomere lengthening in iPS cells, Blasco's team found that SIRT1 levels are much higher in embryonic stem cells than in adult cells. So, they set out to determine if the protein was involved in reprogramming. They used a classic method of creating a mouse model in which the gene coding for SIRT1 was knocked out and looked to see how the lack of the protein impacted iPS cells made from adult cells of those mice. They found that the cells still reprogrammed but their telomeres did not lengthen properly. Over time as the cells grew and divided in culture, the cells developed extensive chromosome breaks and other chromosomal abnormalities. They also found that those iPS cells proliferated more rapidly and formed teratomas much more quickly when transplanted into mice.

Since comparing the genetic stability and integrity of iPS and embryonic stem (ES) cells remains a major area of critical research for the field, knowing the role of this key protein gives researcher another handle on how to observe and characterize those differences. This will need to be addressed as decisions are made moving forward about choosing ES or iPS cells as the starting point for therapies. Perhaps this should be looked at in the new genetically matching sets of cloned and iPS cells created by the New York foundation and the Korean team noted at the top of this report.