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—October 2011

First Human Stem Cell Lines from Nuclear Transfer

A New York Stem Cell Foundation team lead by Rudolph Leibel and Deiter Egli reported on the first embryonic stem cell lines created through somatic Cell Nuclear Transfer (SCNT) in the October 6 *Nature*, Vol. 478 (70-75).

SCNT is reprogramming an adult cell into a pluripotent state by first creating an embryo. The nucleus of an adult cell is introduced into an egg, which reprograms the genetic activity to that of the very earliest state of development so that the new cell starts to divide and eventually reaches the blastocyst stage when embryonic stem cells can be harvested. This has worked well in many animals and to a lesser extent in lower primates, but until now in humans the early embryo stopped dividing before it reached the blastocyst stage or in several cases reached this point but the teams failed to create stem cell lines.

The New York team got around this roadblock by changing the protocol of what most researchers have called SCNT. They did not remove the nucleus from the egg, which resulted in cells with three sets of chromosomes; the usual two from the donor adult cell and one from the egg. The benefit is the egg's own nucleus is properly programmed for development and can carry the somatic cell chromosomes spontaneously through development to a blastocyst and embryonic stem cells. It was also of interest that an embryonic cell of an early embryo can be reprogrammed by insertion into the oocyte to grow to a blastocyst. This begs the question can an induced pluripotent stem cell (iPSC) do this?

Trisomy, the three sets of chromosomes, precludes the cells from ever being used clinically, and may result in atypical behavior from the cells, but it also allow researchers to begin to ask the questions: "What did the egg nucleus bring to the mix that allowed the embryo to keep progressing toward a blastocyst? And what is different about the human egg than those from lower animals." It could also provide clues for making reprogrammed stem cells through iPS technology more efficient. This may be one "first" in which follow-on research provides more usable results than the original.

Reprogramming Adult Cells Might Not Result in Gene Defects After All

In research partially funded by CIRM published in the October 7 *Cell Stem Cell*, Vol. 9 (366-373) a group lead by the Scripps Research Institute's Kristin Baldwin and the University of Virginia's Iris Hall found very few spontaneous mutation in mouse induced Pluripotent Stem Cells (iPSC).

Past studies have shown high levels of structural gene mutations in human iPSCs compared to the adult cells from which they originated. This team performed whole genome sequencing of three mouse iPSC lines and their parent skin cells with a method that was 30-fold more powerful than that of the genetic analysis used in prior studies. They found only four structural mutations total among the three lines that were likely attributable to the reprogramming.

More research is needed to determine if this difference in result is a difference between mouse and human cell lines or a difference in reprogramming technique. The Scripps/UVA team started with fetal skin cells. However, they did predict that it should be able to produce human iPSC lines lacking significant mutations.

Mutation Fixed in Reprogrammed Cells

A group led by Allan Bradley of the Sanger Institute and Ludovic Vallier of Cambridge University published work in *Nature* Vol. 478 (391-394) showing that it is possible to fix mutations in reprogrammed cells.

The British team worked with Sangamo Biosciences from Richmond California, which is also working with a CIRM disease team to create a therapeutic mutation in blood-forming stem cells from AIDS patients with lymphoma. In the current study, the Sanger/Cambridge team used the firm's zinc finger technology to remove the mutated gene from induced Pluripotent Stem Cells (iPSC) that had been made from patients with an inherited liver disease caused by a defective gene for alpha-1 antitrypsin. They then used a second new technology called piggyBac to carry the correct gene into place without leaving any residual genetic markers of the carrier, which is a major step forward from prior technologies that left unwanted gene sequence foot prints.

The authors then matured the iPS cells into liver cells and transplanted them into immune compromised mice where the cells showed signs of normal liver function. They suggested the technique is five to 10 years away from full clinical application, but the methodology points to a great potential for providing personalized cells capable of correcting diseases caused by single gene mutations.

Genes of Clinical Grade Embryonic Cell Lines Sequenced

A team at CIRM grantee Bio Time led by Walter Funk published the complete genetic sequence of five clinical grade human embryonic stem cell (hESC) lines on October 8 in *Stem Cell Research*. CIRM did not fund this particular study.

This is the first such analysis of hESC lines. The report documents the sequences of the five GMP-grade lines and analyzes key traits that can impact success of transplanting them, including blood type, telomere length and HLA tissue compatibility. It also documents the genetic integrity of the lines. Including the presence or absence of disease genes like ApoE, which is associated with Alzheimer's and cardiovascular diseases. The genomes did not differ from genomes of normal adult cells. The firms Complete Genomics and Cell Line Genetics both collaborated in the analysis.

At our recent grantee meeting several speakers made the point that they thought it was highly likely the FDA would demand a screen of the genomes of cell products as apart of quality control in the

future. So, these five BioTime cell lines that are available to CIRM grantees at a discount rate could save time in moving to the clinic.

Discovery May Yield Target for Skin Cancer Stem Cells

On November 17 *Nature* Vol. 478 (399-403) published a report by a Belgian team led by Peter Cameliet and Cedric Blanpain showing that stem cells in squamous cell skin cancer require a high level of expression of a particular growth factor, VEGF.

Angiogenesis, the growth of new blood vessels, is critical to tumor growth, so it is not surprising that VEGF (Vascular Epithelial Growth Factor) is highly expressed in cancer stem cells. But the Belgian team found evidence of an additional direct effect of VEGF on tumor cells. They showed that by blocking VEGF they not only caused tumor regression by decreasing the growth of new blood vessels but also by reducing the size of the pool of cancer stem cells and of those cancer stem cells' ability to renew. Tumors in their mouse model completely disappeared in two weeks. They also found that genetically removing a co-receptor for VEGF also resulted in tumor regression. That receptor is Neurpilin-1.

This study provides two important targets, both VEGF and the co-receptor, for targeting the cancer stem cells that can make squamous cell cancers recur as dangerous malignancies.