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—August 2011

Tumor Forming Cells Removed from Stem Cell Cultures

CIRM-funded research by Micha Drukker in the lab of Irving Weissman at Stanford University outlines a method that looks like it may completely eliminate tumor-forming pluripotent cells from cell cultures that have been matured into a desired progenitor cell. The work was published online August 14 in *Nature Biotechnology.*

All pluripotent cells, whether embryonic in origin or induced by reprogramming adult cells, cannot be transplanted into patients without significant tumor formation. When translational research teams mature these cells into desired neuronal or other progenitor cells for transplantation they must try to sort out as many lingering pluripotent cells as possible. Up to now, this has been a very laborious multi-step process, and in most cases less than perfect.

The Stanford team developed a new antibody that binds to a protein highly expressed on the surface of both embryonic and induced stem cells. It alone was able remove the vast majority of pluripotent cells in a highly mixed culture of cells. By adding antibodies for just two more surface markers to their original one they were able to remove all tumor-forming cells. If replicated, this study could remove a major stumbling block to stem cell therapies that originate with pluripotent cell lines. This will greatly improve the safety of cell therapies derived from pluripotent stem cells.

Lab-Grown Sperm Results in Birth of Healthy Mice

In the August 19 issue of *Cell* Vol. 146(4) a team led by Mitinori Saitou at Kyoto University reported for the first time the ability to efficiently create primordial mouse germ cell-like cells in a dish that had the capacity to go on to form viable sperm. They started from embryonic stem cells.

While other groups have generated primordial germ cells from both embryonic and induced pluripotent (iPS) cells, their processes were very inefficient, generating few of the desired cells and those cells generally were not good at generating sperm. The Kyoto team used a three-step process. First they developed a very specific cocktail of three factors that were able to differentiate the stem cells into epiblast-like cells, a cell type that appears very transiently around day 8 of embryo formation in mice. They then used a protocol they developed a couple years ago to mature those cells into primordial germ-like cells, and then used molecular markers to select those cells in the culture that were most likely to have the ability to form sperm. Those cells were then used in artificial insemination that resulted in healthy births.

This new ability to produce large numbers of primordial germ-like cells should allow significant progress in fertility/infertility research and in the long-term may be a cure for premature sterility.

Gene Modified Stem Cells Corrects Model of Muscular Dystrophy

An Italian team headed by Giulio Gossu and Francesco Tedesco at Milan's San Raffaele Scientific Institute published results in the August 17, Vol. 3(96) *Science Translational Medicine* showing improved muscle function in a mouse model of Duchenne Muscular Dystrophe after treatment with genetically modified stem cells.

They worked with mice lacking the dystrophin gene, and corrected the defect with an artificial chromosome containing the human dystrophin gene. They inserted this chromosome into a type of progenitor stem cell associated with blood vessels called mesoangioblasts and transplanted those stem cells into immune suppressed mice.

The team had earlier isolated the mesoangioblasts and showed that they could differentiate into various mesoderm tissues including skeletal muscle. In this experiment the stem cells carrying the new gene were able to generate new muscle fiber but also improve muscle function in mice that had clear symptoms of dystrophy. The benefit remained evident for several months and with as few as one million cells injected.

More Reprogramming of Skin to Neuron, this Time in Alzheimer's

A paper in the August 5 *Cell* Vol. 146(3), reports on work at Columbia University led by Asa Abeliovich that builds on the spate of recent papers that have turned skin cells directly into functional neurons without first passing through a pluripotent state, this time using skin from Alzheimer's Disease patients.

This team used a different mix of genetic factors and culture conditions to create forebrain neurons from the skin of both normal individuals and those with familial, early onset Alzheimer's. In both cases the cells matured and behaved like neurons responding to neurostransmitters. The cells derived from patients were also clearly abnormal. They had altered ability to process and transport the amyloid precursor protein (APP) and a resulting increase in production of amyloid beta, which has long been a suspect in the disease. Depending on which year you choose to look at the literature, amyloid beta is theorized to be the causing agent, or just an artifact of the disease.

This disease in a dish model may help to finally settle this long-brewing scientific disagreement.

Another Take on Why the Heart is So Poor at Self Repair

A team led by Robb MacLellan using a CIRM funded facility at UCLA published a report in the August 8 *Journal of Cell Biology* Vol. 194(3) offering a molecular reason why the few progenitor stem cells we have in our hearts generally don't start replicating and marshal to the site of cardiac injury.

The UCLA researchers proposed that a pair of genes was responsible for keeping the cardiac myocytes in a quiescent, non-proliferating state. They knocked out those two genes in a mouse model and saw the cadiac myocytes start to divide and proliferate. The group also hypothesized as to why larger animals have heart cells locked in the mature state. They noted that cardiac cells able to divide freely are less mature and not as good at contracting and that larger animals—unlike salamanders and other small organisms that can regrow heart tissue—need the maximum muscle contracting ability of fully mature cells.

Knowing that turning off these two genes can restore the ability of heart cells to proliferate opens up the possibility of finding a way to temporarily turn them off during a period when repair is necessary and turning them back on later so that the new heart tissue matures into muscle that is good at contracting.

Parkinson's Study Evokes Discussion on Controlled Trials

A multi-center team headed by Emory's Robert Gross published a study in the June *Lancet Neurology,* Vol 10, that showed no difference between Parkinson's patients who received transplants of dopamine producing cells and those who received sham surgery. The paper also spawned think pieces on the role of sham surgery and the placebo effect in two of the *Nature* group's publications, by Angela Cenci and Hakan Widner of Sweden's Lund University in *Nature* Reviews/Neurology in August, Vol. 7, and by Alla Katsnelson a correspondent for *Nature* writing online August 10.

The clinical trial team had used a commercial product branded, Spheramine, produced by Bayer HealthCare. The cells were post-mortem derived human retinal pigment epithelial cells, which produce the levodopa that Parkinson's patients need to improve their motor function. The 71 patients enrolled in the trial all had symptoms for at least five years and their symptoms were not fully controlled with oral levodopa. It was hoped that the transplanted cells would improve the continuity of administration of the needed compound. Thirtyfive patients got the cell transplant and 36 had sham surgery. After 12months, the investigators found no significant difference in the motor scores of the two groups, with both improving approximately 21 percent.

Both *Nature* commentaries noted that the placebo effect in Parkinson's studies can be quite pronounced. Katsnelson noted that patient's expectations that they will benefit from a treatment can induce the release of dopamine so there is a physiologic basis for the placebo effect in these patients. Cenci and WIdner go on to discuss the need for all cell-based therapies to have adequate proof of mechanism of action and functional integration of the transplants in pre-clinical models, which they suggest this study did not have. Both commentaries discussed the risk of these invasive therapies as well as the somewhat lower risk of the sham surgery and the need to proceed cautiously, but to continue to move cell transplantation into clinical trials.

Katsnelson found experts on both sides of the issues regarding the value and importance of sham surgical controls for trials. The author also found a patient advocate who voiced concern that sham surgery-controlled phase 2 trials had stopped three Parkinson's therapies that had looked good in phase 1, suggesting some of these therapies may be halted prior to being optimized in their technique and the phase 2 results could be false negatives just as much as the phase 1 could be a false positive.

This is undoubtedly an issue we will need to wrestle with as we proceed forward in funding phase 2 cell-based clinical trials.