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 2013

Stem Cells Model a Type of Autism, Point to Target for Therapy

A CIRM-funded team at Stanford has used iPS type stem cells to model PMD syndrome, a type of Autism disorder, and found a molecular path that could be a target for treating the disorder. The team was led by Ricardo Dolmetsch who recently moved to the Novartis Institutes in Cambridge, Massachusetts. They published their results in *Nature* online October 16.

The Stanford team started with skin cells from two patients who had Phelan-McDermid (PMD) syndrome. The condition is characterized by many developmental disabilities, often including a form of autism, which was the case with these two children. Once they had reprogrammed the skin cells into iPS cells they grew them into nerve cells and compared them to nerve cells grown from iPS cells created from normal individuals and as well as nerve cells grown from normal embryonic stem cells. Through a series of tests they showed that in PMD cells the balance between signals that excite the nerves and signals that inhibit the nerves is not normal. The cells from the patients did not have enough excitatory nerve connections, or synapses.

Because PMD patients tend to have multiple genetic defects leading to the multiple disabilities, they next sought to verify that a specific defect was responsible for the neural disability linked to the autism in these children. That defect in the SHENK3 gene has been shown to have a role in the development of those excitatory synapses. When they used genetic manipulation to introduce a correct copy of the gene into the PMD nerves in a dish, they did see a return to normal electrical activity.

Five existing drugs have previously been shown to increase the level of the protein coded by the SHENK3 gene. They tested all five and one, Insulin-like Growth Factor 1 (IGF1), indeed did restore normal function to the CMP nerves in the dish. Since IGF1 has many roles in our bodies, it is not a good drug candidate, but with the molecular path of the defect so well characterized, people searching for potential therapies have a much better idea of where to look.

The Dolmetsch team had previously conducted similar work with Timothy Syndrome, another condition in the Autism Spectrum and come up with a therapy target for it as well. Perhaps with the Novartis machine now behind him, Ricardo can move these therapeutic targets forward.

Research Unlocks Pathway to Stem Cell Aging and Reduced Function

A research team led by Hartmut Geiger at Cincinnati Children's Hospital has uncovered a second molecular switch that seems to cause blood forming stem cells to age and do their jobs poorly. Working with collaborators at the University of Ulm in Germany, they published their work online October 20 in *Nature.*

The team looked at a molecular pathway, Wnt signaling, that has been implicated in aging in other tissues, notably muscle, but had not been linked to aging in the vital blood forming stem cells that become much less efficient at generating the various components of blood as we age. They did find that aging blood stem cells over produce one Wnt protein, Wnt5a. When they increased the expression of that protein in young blood stem cells they began to behave like older cells. And when they knocked out the gene for the protein in a mouse model, the animal's stem cells continued to act like young cells as they aged.

The researchers found that the Wnt signal activated another protein, Cdc42, which they had previously also linked to aging in blood stem cells in a paper published last year. So the aging appears to involve a cascade of at least two molecular switches. In the current study and in the prior work the researchers were able to reverse the aging by inhibiting the proteins for those switches, which provides an avenue into slowing the ravages of aging. One of the things they need to do next is figure out what causes the increase in the production of the first protein switch, the Wnt5. They need to know if that trigger comes from inside the stem cell or from its environment.

iPS Cell Model for Blood Diseases Overcomes Two Hurdles

A team led by George Daley at Harvard and Children's Hospital, Boston, has found a way to make large quantities of human blood stem cells and their offspring from iPS cells and get those blood system cells to engraft in animal models. The work overcoming these two long-term barriers was published in *Cell Stem Cell* October 3, Vol. 13 (459-470).

The power of disease models developed using iPS type stem cells from patients is well documented. But, for one set of diseases, those linked to our blood system, that power has been limited. While stem cells lines from many anemias and other blood diseases have been created, they generally make the desired cell-type in the lab only briefly and not in large quantities. Also, when the cells are transplanted in a mouse model they don't engraft making it difficult to manipulate the cells and detect the impact of that manipulation on the animal. This live, or in vivo, testing is a key aspect of gaining the full potential of iPS disease models.

Part of the problem has been that when you direct iPS stem cells to differentiate into blood system cells they rapidly progress to an intermediate cell type, a progenitor, that is already committed to a limited set of cell types and does not regenerate itself well. So, Daley's team started by reviewing four large sets of data that had looked at which genetic factors were active in blood forming stem cells compared to those factors that were active in the intermediate progenitor cells. They came up with several candidate factors and decided to test them on intermediate progenitor cells made from iPS cells. In directing their efforts at these progenitors they built on work out of Gordon Keller's lab at the McEwen Centre in Toronto published in *Cell Reports* December 2012.

The Boston team identified three factors that could redirect the progenitors to an earlier state where the cells could replicate extensively and could be directed to many different cell types. Then they identified two more factors that enabled cells created from the process to engraft in mouse models. They used a virus to carry the genetic factors into the cells, and when the virus was loaded with all five, the resulting cells could multiply readily and produce tissue that engrafted. This will allow scientists to transplant human cells with a specific disease trait into mice and measure and manipulate very specific aspects of the physiology of the disease.

Personalized Neurons from iPS Did Not Trigger Immune Response

A Japanese team led by Jun Takahashi at Kyoto University has created neurons from iPS type stem cells and transplanted them into the brains of the monkeys that donated the adult cells that became the iPS cells and into other monkeys. They found that the autologous nerve cells that came from the monkey's own tissue were much less likely to evoke an immune response than cells from a donor. They published their work in *Stem Cell Reports* October 15. Vol. 1 (282-292).

It has long been hoped that reprogramming skin or blood cells from a patient to become iPS cells and then using those cells to create reparative tissues would allow the transplantation of new tissue without immune system rejection or the need for immune suppressive drugs as is the case with donor organs. But a few studies over the past two years have raised the possibility that the reprogramming process might make tissues grown from your own iPS cells seem foreign to your immune system. None of those experiments, however, used models that simulated what would really happen in a clinical situation. So, this head-to-head comparison provides valuable reassurance, though not conclusive, that autologous iPS-derived tissue could be immunologically safe.

The Japanese team made iPS cells from four monkeys and then matured those into dopamine-producing neurons, the type of nerve cell lost in Parkinson's disease. Each of those monkeys received six injections of these cells into their brains. The cells from each monkey were also injected into one other monkey, a recipient of donor cells. They followed the eight animals for three to four months and found very little evidence of immune response caused by autologous cells, but significant immune activation by the donor cells. While they found some of the new neurons survived in all eight animals, a much higher percent survived with the autologous transplants.

iPS Cells Reveal Potential Therapy for Parkinson's with Help of Yeast

Researchers at the Whitehead Institute in Cambridge, Massachusetts have used a yeast model to define a defect in Parkinson's disease, find a molecule that can fix that defect and then verified the molecule works in human nerve cells grown from iPS cells created from patients. Susan Lindquist led the project that was published online October24 in *Science.*

For certain diseases of aging that are characterized by accumulated deficits over long periods of time, such as Parkinson's disease, it has been difficult to recreate the full sequence of events causing the disease in iPS cells created from patients. That has been the case in defining the role of the protein alpha-synuclein that builds up in the nerves of Parkinson's patients. So the Whitehead group developed a strain of yeast that over expresses alpha-synuclein and they were able to track the rapidly growing yeast's response to the toxic over production of the protein. They verified a similar pathway in the human neurons grown from patient iPS cells.

Then, they found a small molecule that reversed the buildup of the protein in the yeast, and verified it also worked in round worms and rats. Last, they tested the molecule on the human neurons in a dish and were able to correct the pathology caused by alpha-synuclein.

This work shows the power of combining an old workhorse of basic biology, yeast, with a new evolving technology like iPS. A potential clinical product remains well down the road, but researchers can now see down the road.