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—September 2012

Gel Helps Stem Cells Grow Across Spinal Injury, Restore Movement

CIRM-funded research at UC San Diego has shown that by mixing neural stem cells with a gel and various growth factors you can coax motor neurons to grow past the site of complete spinal cord separation in rats in a manner not observed previously, in sufficient numbers to restore some motor and sensory function. The team, lead by Mark Tuszynski and including collaborators at San Diego Veteran's Administration, published their research in Cell Vol 150 (1264) September 14.

A patient's own stem cells generally won't cross the scar tissue created by a spinal cord injury, and transplanted cells in animal models have generally not turned into neurons and supporting cells in sufficient numbers to be beneficial. The neurons that form from transplanted cells must create axons to reach out and make critical cell-to-cell connections, but these axons have not grown well in previous studies. In this one the researchers embedded neural stem cells in fibrin, a protein found in blood, and added several growth factors to this gel. After six weeks, the number of axons growing from the injury site exceeded what other teams had seen by 200-fold. They also grew 10 times the length of those in previous studies. They achieved these results when they transplanted rat neural stem cells as well as two human neural stem cell lines.

The team used several tests to verify their transplanted cells had grown and were functioning. The cells had been modified to carry green florescence proteins so their daughter cells could be visualized. They also used electrophysiology tests to show that the new nerve cells were sending signals to the existing host cells. If the spinal cord was then severed above the repaired section, mobility was again lost confirming the transplanted cells had provided the new function.

One of the human cell lines used is already approved for human testing and is being used in a clinical trial for ALS (Lou Gerhig's disease), which could reduce the time needed before the UCSD process can be tested in humans. This is likely a major advance in repair of spinal cord injury.

Very Basic Research Results Could Speed iPS to Clinic, Improve Safety

By looking at the genes in individual cells as they go down the path from adult cell to reprogrammed iPS cell, a team lead by the Whitehead Institute's Rudolf Jaenisch, has provided significant new insight into what really happens when reprogramming occurs, and what happens when it fails. They reported their results in the September 14 *Cell* Vol 150 (1209).

The genes in a cell that are active at any given time determine what type of cell it is. Up until now, the only analysis of cells going through reprogramming has been studies that looked at the genes active in a large population of cells. The problem with this is that when you are making

iPSCs very few cells in any population are capable of being fully reprogrammed; some don't change at all, some get only partially reprogrammed and some may reprogram into a cancer-like state. Finding out what really happen requires looking at single cells in that mix at various points in time.

That's what the Whitehead team did and they found that reprogramming happens in two phases. The first is extremely random and happens in the first six days. The second has a fairly orderly progression over the next few weeks. But in the end, only a very few cells get fully reprogrammed, generally significantly less than one percent. They also found that among the four genetic factors most teams use for reprogramming, one that was generally considered less important, Sox2, is the critical one during the early random phase.

The results hold great promise for improving the efficiency of making iPS cells and could improve the safety of eventual therapies using iPS cells. If we can figure out how make the Sox2 activity in that early phase less random, the yield of fully reprogrammed cells could rise dramatically. Also, the gene signatures found in cells that were not fully reprogrammed, could help to sort out any cells that may have taken on cancer-like properties. The work also turned up six new combinations of reprogramming factors, which should immediately become tools for further refining the field.

A Molecule-based Method Improves Efficiency of Creating iPS Cells

A team at Sanford-Burnham Medical Research Institute lead by Tariq Rana has dramatically increased the efficiency of reprogramming adult cells into iPS cells. They used molecules that block enzymes that prevent reprogramming, in essence they interfered with the blocks to creating iPSCs. The work was published September 25 in *Nature Communications* Vol 3 (1085).

The Sanford-Burnham team's work focused on a family of enzymes called kinases, which are responsible for much intra-cellular communication. When scientists start adding factors to increase expression of certain genes as they do to make iPSCs it increases the activity of many kinases. Some of those seem to inhibit the reprogramming needed to convert adult cells into iPSCs. Rana's team was fortunate in that Sanford-Burnham has a drug discovery facility that has libraries of compounds including 240 that inhibit kinases. They were able to use those to screen for kinases that were blocking iPS reprogramming. They found three kinases that when they were blocked by one of the inhibitors the result was dramatic increases in the number of fully reprogrammed iPS cells created.

When researchers try to reprogram a group of cells, many of the cells appear to get trapped in a partially reprogrammed state. Learning how to push past these barriers with simple molecular inhibitors could accelerate the field.

Human Embryonic Stem Cells Used to Restore Hearing in Gerbils

A British research team lead by Marcelo Rivolta at University of Sheffield used cells derived from human embryonic stem cells to repair damaged auditory neurons and restore hearing in gerbils. Their results were published online September 14 in *Nature*.

Much of the team's work focused on elaborate tests to determine which growth factors could efficiently turn embryonic stem cells into optic neural progenitors. Those are the cell type they planned to transplant at the site of nerve injury where it was hoped they would mature into new

auditory nerves. They found that two specific fibroblast growth factors did the trick. They were able to grow robust optic neural progenitors that when transplanted integrated into the host successfully connecting the inner ear to the central nervous system. Tests showed a 46 percent improvement in the hearing of the animals.

There are many causes of hearing loss, so no one repair is going to work for all deaf individuals. Two main causes are loss of the auditory hair cells in the inner ear and damage to the connecting auditory neuron. Cochlear implants replace the function of the hair cells and restore hearing in some patients, but if the person also has damage to the auditory nerve, the implant will not help. One day transplants similar to those reported here might be coupled with cochlear implants to broaden the impact of that device.

Leukemia's Arise from Mutations that Accumulate in Blood Stem Cells

A Stanford team has documented the domino effect of a series of mutations that lead to leukemia and they suggest the findings provide final proof that leukemia is caused by cancer stem cells. The paper, listing three principal authors; Ravi Majeti, Stephen Quake and Irving Weissman was published August 29 in *Science Translational Medicine* Vol 4(149).

The painstaking experiment required members of the team to examine anywhere from 80 to 500 individual blood stem cells from six patients. They were looking for mutations that were also among those found in those patients' leukemia cells. First they looked for cells early in the cascade with only one mutation that matched, this was the primary mutation. Then they looked for stem cells with that mutation and just one more. This continued until they found stem cells with the full set of mutations found in the leukemia cells.

Because the daughter cells of blood stem cells are relatively short lived, and the mutation process is slow, the authors suggest that ordinary blood cells could never accumulate enough mutations to become leukemic. They argued that only blood stem cells that self renew through our life could accumulate the full complement of mutations needed to become cancerous. Knowing this full set of deadly dominos provides more targets for potential therapies.