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—February 2014

New Technique Directly Reprograms Adult Cells into Liver Tissue

CIRM-funded researchers working with Sheng Ding at the Gladstone Institutes and Holger Willenbring at the University of California, San Francisco, have developed a way to reprogram adult cells into liver tissue that more closely matches natural liver than any prior research has achieved. They published their work online in *Nature* February 23.

While several teams have produced liver cells from pluripotent stem cells, either iPS or embryonic stem cells, the resulting cells have had two flaws. They don't mature fully and provide all the functions of normal liver and they don't proliferate after they are transplanted. In order to transplant cells into a severely damaged liver and have them restore its function, the cells would need to do both.

The UCSF-Gladstone team developed a method to directly reprogram human skin cells into an intermediate state, neither iPS stem cell or adult liver cell. Those cells could be expanded extensively in the lab and then matured part way toward becoming adult liver cells before transplanting into a mouse model of liver failure. About two months after transplant the cells had matured to the point the researchers could detect proteins normally produced by human liver in the mice. Up to nine months later they detected continuously increasing amounts of the protein indicating continued growth and expansion of the human liver cells.

The researchers tested several sets of genetic and chemical factors for each step of the laboratory reprogramming. They found one set of factors efficiently produced the intermediate cells, and a different set of factors could mature those cells closer to the point of being functioning liver. Both those steps required just a few weeks so the lab portion of the procedure is potentially efficient enough to create liver cells from a patient's own tissue that would be genetically matched and less likely to be rejected by the immune system. The work points to a potential path for personalized liver repair.

Old Muscle Stem Cells Rejuvenated to Act Like Young Stem Cells

A CIRM-funded team led by Helen Blau at Stanford has discovered a root cause for why older muscle stem cells don't repair damage as well as younger stem cells and they have found a possible way to reverse that decline. The researchers published their findings online in *Nature Medicine* February 16.

The Blau team's finding runs counter to conventional wisdom on why older stem cells are less effective. Researchers have generally laid fault with the environment the cells are in with older people, but the Stanford work showed the stem cells themselves had defects. They have an elevated level of activity in a cell signaling pathway that impedes the ability of stem cells to replicate.

Without that ability people don't maintain a reserve of muscle stem cells ready to repair damage. But the Stanford group found a drug capable of blocking that signaling pathway in mouse muscle stem cells growing the lab. That blocking action, however was not sufficient to get the cells to replicate like young ones. They also had to provide a growth medium, in this case a porous hydrogel, that had physical properties that matched the soft elasticity of the muscle tissue that would be their natural environment.

After taking muscle stem cells from older mice and treating them with the blocking agent in the lab for seven days the researchers report 60-fold increases in the number of stem cells. After transplanting those cells back into the animals they migrated to their natural niche and contributed to repeated demands for muscle repair. The stem cell researchers teamed up with a colleague from Stanford's engineering school to measure the impact of the healing. The engineer designed a way to measure muscle strength in animals that had a muscle injury before and after stem cell therapy. Two months after transplant the older mice exhibited muscle forces equivalent to young muscle.

While there are many steps between this research and a human therapy, the signaling pathway found by the team is now a clear target for drug development.

iPS Cells Help Link Genetic Alterations to Schizophrenia

A team led by Kazuya Iwamoto at the University of Tokyo used three different systems to show the connections between schizophrenia and a type of genetic alteration called a transposon. The team, which included researchers from four other Japanese institutions, published their work in *Neuron* January 22, Vol. 81 (306-313).

Transposons (jumping genes) amplify themselves in an individual's genome and alter the expression of neighboring genes. In this case, the researchers measured the activity of a specific type of transposon called a retrotransposon because it uses the intermediary RNA to create the extra copies of the genetic material. That type of genetic alteration has already been linked to two neuro-developmental disorders, Rett Syndrome and ataxia telangiecstasia. But both of those disorders are caused by a known single mutation. Retrotransposons had not been linked to a more complex neurologic disorder like schizophrenia that appears to have both multiple genetic and multiple environmental triggers.

The team first looked for retrotransposon amplification in brain autopsy samples from nearly 50 patients with Schizophrenia. They did find significant amplification and mapped it to areas containing genes for synapses, the area of neurons responsible for cell-to-cell communication, as well as to sites near known schizophrenia genes. They then created iPS type stem cells by reprogramming skin samples from two schizophrenic patients. In both, when they matured the stem cells into neurons, they found transposon activity similar to the autopsy sample. Last, they tested the environmental component of the disease by looking at two animals in which it is known you can trigger neurologic disturbances in the offspring by environmental manipulation in the mothers. When they induced inflammation like that occurring during infection—a suspected trigger of schizophrenia—the offspring had retrotransposon activity similar to the neurons from schizophrenics and from neurons grown from the iPS cells.

The researcher speculated that the retrotransposons impact how schizophrenia is expressed—how severe it is—and are not a direct cause of the disease. Yet, any new insights into the development of this disabling disease, provides opportunities for uncovering new therapeutic targets.

Regenerative Medicine Using Gene Therapy Creates Heart Muscle

A team lead by Hina Chaudhry has shown they can use a gene therapy strategy to gain formation of new heart muscle cells after a heart attack in a large animal, in this case pigs. Chaudhry worked with colleagues at a company he founded VentriNova and at his academic institution, the Icahn School of Medicine at Mount Sinai in New York, and they published their work in *Science Translational Medicine* February 19, Vol. 6 (224).

Stem cell therapies aimed at repairing damage following a heart attack have generally produced only modest results. They tend to show reduced scar formation, perhaps improved formation of new blood vessels at the site of injury and other subtle improvements, but not the formation of significant new heart muscle tissue. That is why Chaudhry's team tried a very different approach to heart regeneration. They sought to recreate the processes that occur in the newt and zebra fish that are able to generate a significant amount of new heart tissue after injury. These lower animals don't rely on stem cells, instead they call on cells neighboring the site of injury and get them to regress to a less mature state that allows them to multiply and become new types of tissue. In essence, a mature, non-dividing cell reenters the cell cycle that activates all the internal cellular mechanics needed to multiply.

The New York team worked with a protein involved in controlling the cell cycle in the developing heart in mammals called Cyclin A2. The gene for that protein is normally silenced at birth and that is one reason why our hearts have only a limited ability to repair damage. The team had previously used a virus to deliver that gene to areas of the heart in mice and rats that were near the site of injury from an induced heart attack. It seemed to work. In the current study, they repeated the procedure in pigs because they have hearts very similar in size and structure to humans.

They found evidence of improvements in the hearts of the pigs that were very similar to the ones they had seen in rodents. At six weeks after the injection of the virus carrying the gene they saw improved pumping ability in the hearts of the treated pigs compared to pigs injected with the virus alone. Magnetic resonance Imaging showed an 18 percent improvement in the critical heart measure, ejection fraction, while the untreated pigs had a four percent decline at six weeks. They also found direct evidence of cell division and production of new heart muscle. These improvements were accompanied by decreased scar tissue formation, as well.

One caveat on this study is key. Although the current viral vector carrying the gene is delivered directly into the heart, it has the potential to revert other tissues to the point in the cell cycle that encourages cell replication. That has the potential to cause tumors. The team is already working on a next generation of the process that would use a gene promoter that would only turn on Cyclin A2 in heart tissue.