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—November 2013

System Makes It Easy to Get Large Quantities of Stem Cells & Progeny

Two CIRM-funded researchers at the University of California, Berkeley, have developed a 3D culture system that offers a new way to increases our ability to expand stem cells in the lab as well as the numbers of their progeny cells that can be produced. The pair, Yugo Lei and David Schaffer, reported on their system that could yield clinical grade cells in the *Proceedings of the National Academy of Sciences* online November 18.

Most cell replacement therapies will require hundreds of millions and maybe a billion cells per patient and no existing cell culture system is up to the task of first expanding the stem cells to large enough quantities and then maturing those stem cells into desired cell types. Also, recent advances in chemistry and genetics make it possible to use pluripotent stem cells, either embryonic of iPS type, to screen large libraries of chemicals for desired effects. But this, too, requires cell quantities in the billions. This will be an alternative or complementary method to the large scale GMP cell suspension method published by Larry Couture and colleagues in Stem Cell Res 2012. .

The Berkeley pair followed the lead of a few other recent attempts and moved the cell culture from two dimensions to 3D. However, to avoid some of the problems with cell quality, they grew the cells in a hydrogel that was liquid at low temperatures and solid at higher temps. They introduced the stem cells and the required growth medium in the liquid state and then raised the temperature and let the cells expand with the support of the solid hydrogel. They could then return to a low-temp liquid state and replace the stem cell growth medium with one that would promote differentiation to a more mature tissue. The cells were then allowed to grow and mature when they raised the temperature of the gel again.

They got a 20-fold expansion of stem cells in five days and that rate of growth continued for up to 280 days with 95 percent purity. The most effective prior system to date resulted in a three-fold to four-fold expansion in the same five-day growth cycle. The Berkeley researchers were able to get those stem cells to grow into cells from each of the main cell types in our body, which were represented in this case by heart muscle, nerves and progenitors of endoderm. Creating the system, however, was not as simple as setting a thermostat. They tried 36 combinations of variables in the physical and chemical components of the system to arrive at this one.

3-D Renal Buds from Stem Cells Provide Model for Kidney Disease

A CIRM-funded team led by Juan Carlos Izpisua Belmonte at the Salk Institute has developed a system to coax human embryonic or iPS-type stem cells into becoming 3-D renal buds that are the precursors of developing kidney. Working with collaborators at the University of Barcelona, they published their work online November 17 in *Nature Cell Biology.*

The Salk team succeeded in producing more complex structures than a Japanese team that published related results this summer and that I wrote about in my June "President's Report." That work required a fairly complex process and resulted in some formation of rudimentary renal tubules. The current projects uses a relatively simpler two-step process and produces a more complex 3-D structure called a ureteric bud, which is the early precursor of the urinary tract in the developing embryo.

The researchers first used various chemical factors to drive the human stem cells to become intermediate, or progenitor, cells for the ureteric bud. Then to get them to further mature and assemble into the primitive kidney structures they cultured the cells with embryonic mouse kidney cells. The mouse cells seemed to provide the needed environmental cues to instruct the human progenitor cells on what to become.

The kidney is a very complex organ and the Salk team does not propose that this work is the path to replacement organs, but rather that it is a long-needed model for studying kidney development and potentially for drug screening. As proof of concept they were able to create ureteric buds from iPS cells made from a patient with polycystic kidney disease (PKD). Those cell lines can now be used to understand PKD one of the most common life-threatening genetic diseases.

Nerve Cells Show a Greater Genetic Patchwork than other Tissues

Another team at the Salk, this one led by Fred Gage, has used cutting-edge single cell genetic analysis to reveal significant variability in the genes that are in nerve cells within an individual. The work was published in *Science* November 1, Vol. 342 (632-637).

Gage's group used two relatively new techniques to analyze the genes of single cells. They looked at both nerve cells from three postmortem human brains and nerves grown from iPS type stem cells created from three individuals. For the latter they compared the genetic variation in the matured nerves and the skin cells used to create the iPS cells. They looked at genetic changes called copy number variations (CNV), which can either be added copies of genes or deletions of genes.

Decades of dogma has said that we have all the same genes in all our cells, with the difference in function resulting merely from which genes are turned on. But recent finding have started to point to some exceptions in the accepted rule, and this study shows an extreme exception in nerve cells. The team found significant variation in up to 41 percent of cadaver nerves and nearly as many in the nerves created from iPS cells, even though they found very little gene variation in the skin cells the iPS cells were derived from.

A few of the nerve cells accounted for the majority of the genetic variation, in particular the most extreme forms where whole chromosomes were added or deleted. The researchers speculated that such drastic genetic changes could be responsible for some of the differences in the function of individual nerve cells. They suggested that the new genetic analysis techniques should begin to shed light on this in the coming years. In the meantime, this study serves as a cautionary note for researchers who have considered making iPS cells from nerve cells. Since iPS cells seem to retain some memory of the cells from which they were made, researcher have hoped nerve-derived iPS cells might be easier to mature into various brain tissues that other iPS cells. However, with this degree of genetic variability you would be risking creating stem cell lines that are not truly representative of the individual.

Completely "Reset" Stem Cells Are a Step to Growing Organs

An Israeli team at the Weizmann Institute led by Jacob Hanna has succeeded in turning back the clock on pluripotent stem cells so that they are no longer primed to mature into adult tissues. Creating these "ground state" stem cells opens the door for humanized animal models of disease and potentially growing human organs in animals. They published their work in *Nature* online October 30.

Mouse embryonic stem (ES) cells are much easier to keep in a self-renewing pluripotent state than their human counterparts. Human ES cells are primed to begin to mature into various adult tissues. So, Hanna's team looked at the genetic activity of mouse ES cells that were not primed to differentiate and slowly developed a growth medium with the right chemical factors to get human ES cells to mimic the mouse cells. They also showed that their growth medium could get the same result with reprogrammed iPS type stem cells and with newly harvested cells from a blastocyst, the early stage embryo that is the source of cells that become new ES cell lines.

While this may seem esoteric at first, it has huge implications for research going forward. We can learn a great deal about human tissues if we can get them to grow in animal models. But the best way to do this is to create what is know as chimers by introducing human stem cells into the mouse blastocysts so that the two types of cells develop side by side in the mouse embryo. But this does not work with stem cells that have been primed to mature. Hanna's group showed that his new cells could succeed. This will allow researchers to grow human organs in animals and conduct tests not possible in human subjects. It is also an important step toward growing replacement donor organs in animals.

A Gene Enhances the Ability of Mice to Repair Tissues

A research team led by Harvard's George Daley along with colleagues at the University of Texas Southwest Medical Center in Dallas has shown that by reactivating a gene normally turned off at birth they can enhance the ability of mice to repair and regrow tissue. They published their results in *Cell* November 7, Vol. 155 (778-792).

The gene Lin28 worked to enhance hair growth, repair holes made in the animals' ears and to regrow the tips of their toes. However, it did not help to repair heart damage, and it also failed to enhance repairs once the animals reached the equivalent age of a young adult human. The latter is not a surprise as all our repair mechanisms are less robust in certain tissues and decrease in effectiveness with age.

The team's tracking of the cellular mechanisms behind the enhanced repair turned up the most useful information in the study. It turns out that Lin28 seems to enhance cellular metabolism by turning up the activity of the mitochondria, the cell's energy machine. This suggests that looking at this mechanism could be a fruitful approach to treating injuries and diseases resulting from tissue damage.