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—July 2013

Skin Reprogrammed to Stem Cells with Chemicals Instead of Genes

A team at Peking University led by Hongkui Deng produced pluripotent stem cells by reprogramming adult cells using chemicals instead of genetic factors. They called the cells Chemically induced Pluripotent Stem Cells (CiPS cells) and published the results online in *Sciencexpresss* July 18.

Reprogramming with small molecules could provide numerous advantages over using genetic factors. The chemicals are easier to get across the cell membrane and into the cell, they are less likely to cause immune system rejection, and can be more cost efficient. It is too early to confirm these advantages with the cells Deng's group created, but they did achieve efficiency equivalent to most other reprogramming techniques. About 0.2 percent of the starting cells successfully became CiPS cells.

A few teams have reported success of substituting chemicals for some of the four traditional reprogramming transcription factor genes, but not all four genes. Deng had earlier reported his team could replace all the factors but one—Oct4—seemed to be mandatory for reprogramming pluripotency. So in the current work they focused on Oct4. They used the other three traditional genetic factors paired with combinations of 10,000 small molecules and eventually found four that together could replace the role of Oct4. But when they added those four chemicals to the two they used previously to replace the other three genetic factors they did not achieve complete reprogramming to pluripotency. They had to add a seventh chemical. Even then the reprogramming efficiency was low. It required more fiddling with the dosages and timing of the chemicals to match the efficiency of more traditional methods.

The team then performed extensive tests to prove that the cell lines were pluripotent and were able to make tissues from all three major layers of the embryo. More work with these seven chemicals and similar combinations needs to take place, but this method has the potential to be a game changer for large scale production with iPS technology.

Functional Liver Tissue, First Organ Formed from iPS Cells

A team led by Yasuhisa Adachi and Hideki Taniguchi at Yokohama City University in Japan became the first team to create complex functional organ tissue starting from pluripotent stem cells. The work, using human iPS cells turned into liver cells in the lab and then transplanted into mice, was published online July 3 in *Nature.*

While several teams have directed iPS and embryonic stem cells into liver cells, none have got those cells to go on to develop functional liver tissue including the blood vessels that are essential for liver. The team in Japan succeeded partly by accident. They were following a recent trend in stem cell science and were trying to create an environment more like the developing embryo than a traditional single layer of one type of cells in a dish. So they added two cell types that would be found alongside early liver cells in the embryo. Those were cells of the endothelial layer, the one that makes blood vessels, and mesenchymal stem cells that can make stromal or connective tissues. The endothelial cells came from umbilical cord blood. They thought that they would then need to do additional manipulation to get the cells to mature into functional liver, but they found the cells self-organized into groups they called "liver buds."

When the team transplanted the liver buds into mice they continued to mature and within 48 hours had formed blood vessels that connected to the host's blood stream. The team demonstrated that the liver tissue could produce proteins specific to the human liver and could metabolize drugs that human livers can metabolize but mouse livers cannot.

The team achieved these results by transplanting 12 liver buds in each of two locations in the abdomen. But each bud was less than a sixth of an inch in diameter, so the work is a long way from producing sufficient tissue for human patients. However, it is a significant milestone toward that goal.

Embryonic Stem Cells Used to Create Light Receptors in Eyes of Mice

A team led by Jane Snowden and Robin Ali at University Hospital London has created precursors of photoreceptors from mouse embryonic stem cells and have shown they can integrate into the host retina when transplanted. The work was published online in *Nature Biotechnology* July 21.

The team had previously shown that they could harvest precursors of photoreceptors from mice right after they were born, transplant them into adult blind mice, and restore vision. Because this source of cells would never work in humans, they wanted to create the precursor cells from embryonic stem cells (ESCs) so that they could have a readily replenishable supply. But no one has been able to show that ESCs can give rise to mature photoreceptors with their layered structure that includes an outer segment packed with visual pigment needed to convert light into electrical signals. The London group used a technique developed by a team in Japan last year that was able to result in a complex optical cup grown in the lab. Instead of growing the cells on a flat dish, they embedded them in a gel—again coming closer to mimicking the conditions of the developing embryo.

The resulting precursor cells, when transplanted into a blind mouse, were able to integrate into the host's retina and produce the critical outer segment and its visual pigment. However, the integration happened at a very low rate, with less than one percent of the transplanted cells making the needed connections. Since they only transplanted 200,000 cells and had calculated that they would need to replace 150,000 cells to detect any change in vision, they were not able to replicate the restoration of vision they had accomplished with the postnatal cells.

The team did extensive work to determine the best timing of when to transplant the precursor cells and other parameters of how to get the best result. They clearly have more work to do before this procedure moves into clinical trials, but this is an important milestone along that path.

Gene Therapy Shown to Correct Sickle Cell Defect in Lab, Mice

A CIRM-funded team at UC Los Angeles and Children's Hospital Los Angeles led by Donald Kohn has shown their tool for correcting the defective hemoglobin gene in sickle cell disease works in human cells in the lab. They also showed that those cells continue to produce the correct hemoglobin when transplanted in mice in a paper published online in *The Journal of Clinical Investigation* July 1.

The CIRM Disease Team took blood-forming hematopoietic stem cells from the bone marrow of children with sickle cell disease and used a lentivirus to carry the correct hemoglobin gene into the cells in the lab. It is a defect in this gene that leads to the sickling of these patients' red blood cells and the painful clogging of small blood vessels that results. For some years doctors have given sickle cell patients blood-forming stem cells with the correct gene from donors. But donor stem cell transplants carry much more risk than using cells from the patient themselves, making the donor procedure relatively rare and reserved for the sickest patients. The new technique should make this life-changing procedure available for many more patients.

Kohn's team expects to use this proof-of-principal data to gain permission from the Food and Drug Administration to begin a clinical trial early in 2014.

Alternative to Embryonic Stem Cells May not be Stem Cells at All

A CIRM-funded team at Stanford led by Irving Weissman has cast significant doubt on the existence of a type of stem cell that has been called Very Small Embryonic-Like stem cells (VSELsc). The company that holds the license to use the cells has garnered financial support from the Vatican touting them as an ethical alternative to embryonic stem cells (ESCs). The Stanford team published its negative findings online in *Stem Cell Reports* July 24 in advance of print publication scheduled for August 6, Vol.1.

Mariusz Ratajczak, currently at the University of Louisville first reported isolating the VSEL stem cells from mouse bone marrow in 2006 when he was in Poland. He has since reported finding the human counterpart and noted that they are rare. But he maintains that they are highly flexible and unlike other stem cells found in adults, VSEL stem cells can form tissues from many different parts of the body, not just a narrow subset of tissues. But in the past year at least three teams have reported failure to replicate some aspects of his work. The Stanford report is the fourth and the most thorough.

Weissman's team took multiple approaches to isolating cells like those reported by Ratajczak, but found very few whole cells in the fraction of cells matching the small size of VSEL stem cells. Working with those few cells, they did not detect any of three normal indicators of pluripotent stem cells. They could not find the universal genetic marker Oct4; they could not get the cells to from characteristic spheres in culture; and they saw no evidence of ability to regenerate adult tissue.