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 2012

Genes in Reprogrammed iPS Cells More Stable than We Thought

Teams at Yale and Stanford completed a more thorough analysis comparing the genes in iPS cell lines and the genes in the skin samples that they were derived from and found that much of the genetic variation was in the original tissue and not caused by the actual reprogramming. The teams led by Alexander Urban at Stanford and Mark Gerstein and Flora Vaccarino at Yale had their work published online in *Nature* November 18.

Several previous studies have raised concerns over the genetic stability of iPS cell lines. When those earlier studies compared the genetic makeup of iPS cells and the tissue they came from using traditional methods that pool the DNA from millions of cells, they often found multiple genetic differences. The current teams noted the location of those changes in the iPS cells and went back to the original tissue and used a technique that amplified that section of the skin DNA. This technique showed that at least half the changes could be found in small subsets of those cells. These changes are called Copy Number Variations, differences in the number of copies of certain genes, which is something that occurs naturally when cells copy their DNA and divide.

The team wrote that their work suggests 30 percent of our cells have these genetic variations, and that we are really mosaics of cells with differing numbers of these genes. The work suggests that since the genetic make up of the iPS cells more accurately reflects the genetic make up of the original tissue, using them for disease-in-a-dish models may better mimic patterns of disease than some had believed. The one thing that the paper does assure is that the genetic traits of reprogrammed stem cells will be the subject of continued study.

A Roadblock to Reprogramming Adult Cells into Stem Cells Found

Researchers at the University of Pennsylvania led by Kenneth Zaret have found one cause for the extremely low efficiency of reprogramming adult cells to be iPS cells, and their initial test of knocking down that roadblock did improve the efficiency of reprogramming. Their work was published in *Cell* Vol 151 (994) November 21.

When researchers try to make iPS cells from adult tissue they often get as few as one stem cell for every 10,000 starting cells and it can take a month or more. Most adult cells are able to resist the four genetic factors that are added to the cell culture to cause the reprogramming. The four genes targeted are ones that are generally only active during early embryonic development and the cells' defense mechanism to keep them turned off in adult cells is very strong. The Penn team found that in most cells the added factors just can't get past the chromosomes' control layer and interact with the actual genes. They are essentially trapped behind a locked door.

One of the key ways cells control which genes are active on chromosomes is by adding chemical tags called histones. In those cells that refused to reprogram the team detected large blocks of chromosomes that had a specific histone. When they blocked the cellular mechanism that places that histone on the chromosome, they did see enhanced reprogramming.

The inefficiency of reprogramming greatly increases the cost of using iPS cells for disease-in-a-dish models of genetic diseases. However, the greater problem is when the field is ready to attempt personalized therapy making replacement tissues from a patient's own cells. Patients often can't wait the month or more that most techniques now require. If others verify and build on this finding so that iPS cells can be made rapidly and efficiently, it could greatly increase the probability of achieving the much-discussed goal of personalized cell therapy.

An Unexpected Route Around the Problem of Stem Cells and Tumors

CIRM-funded research at Stanford suggests that under certain circumstances it may be possible to skip the usual step of directing pluripotent stem cells to mature part way toward an adult cell in the laboratory before transplanting them—a step that has been considered necessary to avoid tumor formation. The work from the team led by Michael Longaker, published on line November 20 in *PNAS*, suggests that under certain circumstance we might be able to trust the body to direct the stem cells to become the desired cell type and not tumors.

Even considering the transplantation of pluripotent stem cells, either embryonic or reprogrammed iPS cells, has generally been considered heresy. The standard test to make sure you really have pluripotent cells is to transplant them under the kidney capsule of mice and look for the formation of tumors called teratomas. However, in recent years researcher have begun to learn about the powerful role of the stem cell niche, the immediate environment in which the cells are living. This location effect plays a major role in determining what type of mature adult cell the stem cell will become.

The Stanford team started with both human iPS cells and human embryonic stem cells and transplanted them into mice. Their goal was to get the stem cells to become bone and repair a hole they had created in the animals' skulls. So, they worked to create a niche that was conducive to bone growth. First they built an artificial scaffold the shape of the missing bone and then laced it with a protein known to foster bone growth. They loaded this structure with a million stem cells and transplanted it into the mice. In all 42 mice they transplanted they saw the stem cells develop into bone and integrate with the existing tissue. More important, they only saw two teratomas form.

The team attributed this success to both the micro-environment they created in the scaffold, but also the macro-environment of the setting in which the transplant was placed. The stem cells took cues from the surrounding bone tissue. Forty tumor-free mice is a great result, but regulators will demand all 42 be tumor-free. Longaker says the team will try to perfect the method by combining it with other techniques. One option they are considering is including other types of cells to act as "chaperones" for the stem cells.

Study Shows Why Insulin Producing Cells Mature Better in 3D

A team led by Anne Grapin-Botton at the University of Copenhagen has uncovered the molecular basis for how early-stage pancreas cells orient in three-dimensions and how that allows them to develop into insulin-producing beta cells. The paper adds to our growing respect for the importance of the stem cell's physical environment in producing the desired end-point adult cells. It was published in *Cell Reports* Vol 2 (1-14) November 29.

Over the past few years, many teams have made progress in maturing embryonic stem cells into early-stage pancreas cells in two-dimension lab plates. But they generally have had very limited success in turning these progenitor cells into insulin-producing beta cells in that flat 2-D environment. Our Disease Team led by researchers at Viacyte, do achieve this second stage of maturation into beta cells, but they place the progenitor cells into a three-dimensional teflon pouch prior to transplanting them into mice. This paper could explain why they are getting the desired results.

The Danish team went back to look at the early stages of embryo development in mice and what proteins were being produced by the cells on days 8 through 15 of development—the time when the pancreas is forming. In particular they looked at proteins that were known to impact the polarity, or spatial orientation of the cells. They conducted genetic knockout experiments that resulted in mice being born missing genes for two of these polarity proteins. At four-months of age, those mice showed a marked reduction in their ability to tolerate glucose and in the number of beta cells. The team carried out a number of additional experiments to verify that it was the progenitor cell's inability to align properly in three dimensions that resulted in the failure to mature into beta cells.

Although it is not discussed in this paper, in an interview Grapin-Botton gave to the university, she stated that they have begun the follow-on experiments to see if this works with cells grown in the lab. They have developed 3-D growth environments and claim to be getting promising results in maturation of progenitor cells into beta cells.