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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—March 2014

Technique May Make Stem Cells Created by Nuclear Transfer Practical

The team that first succeeded in making human embryonic stem (ES) cells through the nuclear transfer technique known as therapeutic cloning, has made the process much more practical by eliminating the need for oocytes, at least in mice. Shoukhrat Mitalipov and his team at the Oregon Health Sciences University published their latest work online in *Nature* March 26.

When Mitalipov reported his success in creating ES cells through nuclear transfer a year ago there was considerable excitement but also considerable caveats. While the cells immediately provided highly valuable research tools, the technique required too many donor eggs to fulfill the promise of therapeutic cloning. That technique has always offered the hope of creating replacement tissues that immunologically match the patient. Now, Mitalipov has found a way to enable nuclear transfer to work using early two-cell cleavage stage embryos instead of eggs. Many more of these early embryos are stored frozen for research at in vitro fertilization clinics than eggs.

Therapeutic cloning, or Somatic Cell Nuclear Transfer (SCNT), requires the insertion of a nucleus of an adult cell by micromanipulation into an egg cell—up until now. The method results in reprogramming the genetic instruction of the nucleus into an embryonic program capable of making ES cells. Researchers have always believed that once an egg starts to divide to form the early embryo the resulting cells lose critical reprogramming factors that are essential to redirect a donor nucleus to become a viable embryo. The Oregon team found that early two-cell embryos do retain the reprogramming factors, but are only able to use them properly if they are in the same stage of the cell cycle—the same point in the process of replicating DNA and cell division—as the cells that donate the nucleus.

This synchronization of the cell cycle between the donor and recipient cells makes for an elegant piece of science and certainly points the way forward to making SCNT and therapeutic cloning practical. But as the long period between successful SCNT with Dolly the sheep and Mitalipov's success last year, we well know how long road can be between animal work and successful application in humans.

Finger-Prick Blood Samples Yield iPS Cells, May Broaden Banking

A team lead by Yuin-Han Loh at A*STAR institute in Singapore has developed a technique that greatly reduces the amount of blood needed to create an iPS type stem cell line. This should make it easier for various stem cell banks around the world to include a broadly diverse set of cell lines that come closer to representing the population as a whole. The researchers published their findings online in the CIRM-backed journal *Stem Cells Translational Medicine* March 20.

Currently, creating iPS cells from blood samples requires about a tablespoon of blood and that means the donation needs to be drawn by a healthcare professional, which does not make it easy to expand creation of iPS cells lines to remote settings or to populations fearful of the procedure. Reducing the amount of blood required to less than a milliliter makes a simple finger prick sufficient to collect the sample. The Singapore team adjusted the standard use of a number of kits readily available from research supply companies to create this protocol that seems to be at least as efficient in creating stem cells lines as other current techniques using blood samples. They provided the donors with vials with anticoagulants so the blood could be shipped and still be viable. When the samples arrived at the lab on ice, the cells were grown with commercially available blood-cell expansion medium, and then they were reprogrammed with a kit that used a virus to provide a short term burst from the four standard reprogramming factors. They saw significant levels of reprogramming to a stem cell state in 20 days.

A single drop of blood contains about 20 microliters and the team only needed about half that to achieve the reprogramming. So, they used the remaining blood to perform the further analysis that would be needed to maximize the value of the stem cell lines. They completed some limited blood antigen typing to help in immunologic matching and they did some genetic analysis to look for disease-specific mutations. They also analyzed the stem cell lines to verify that the virus used for reprogramming had not left behind any traces of the reprogramming factors or of its own genes, and it had not. This method could be ideally used with umbilical cord blood banking where a small aliquot could be retained for iPS cell development without significantly reducing the amount of cord blood available for immune or blood disease therapies.

While this techniques yields stem cells lines fairly efficiently, the authors point to a couple ways the efficiency could be further improved. As the technique is perfected it could provide a valuable route to giving stem cell banks the breadth they need.

Stem Cells Help Find Trigger for Most Common Intellectual Disability

A team led by Samie Jaffrey at Weill Cornell Medical College used embryonic stem cell lines that carried the Fragile X mutation to uncover how the mutation causes this common form of intellectual disability. Jaffrey's team, which included researchers from Scripps Florida and Albert Einstein College of Medicine, published their work in *Science* February 28, Vol. 343 (1002-1005).

Fragile X is one of a number of so-called repeat expansion diseases. The mutation shows up as a repetition of the gene for a needed protein. The repetition can be several hundred times. For previously unknown reasons, at some point these repeats end up turning off the gene. In Fragile X this happens around day 50 of gestation.

Through an intricate series of experiments the research team found that the messenger RNA, or mRNA, that is supposed to begin the process of translating the Fragile X gene into protein seems to gum up the works. It forms RNA-DNA duplexes that eventually shut down the gene. However, they also found that in cells grown from Fragile X-derived stem cells a small molecule could rescue the

gene and prevent it from being shut down, at least in the laboratory dish. The team speculated that this molecule or a related one could be administered to a mother during pregnancy if tests had shown her fetus carried the mutation, but that intervention is many experiments away from reality.

The more immediate impact could be providing researchers with clues as to where to look to begin to unravel how other repeat expansion diseases, such as Huntington's and Friedrich's ataxia are triggered.

Directly Reprogramming Scar to Nerve Might Work for Spinal Injury

A team lead by Chun-Li Zhang at the University of Texas Southwestern Medical Center has shown they can use a single reprogramming factor to turn astrocyte scar tissue at the site of spinal cord injury into functional nerve cells in mice. They published their work in *Nature Communications* online February 25.

The UT researchers used a two-step process to create the needed new nerve cells. They used a lentivirus to carry one reprogramming factor, SOX2, into the astrocytes that had formed scar tissue at the site of spinal cord injury. That factor converted the astrocytes into intermediate or progenitor cells called neuroblasts in about four weeks. Then over the course of another eight weeks those cells matured into nerves, but to make this final process more efficient, the team injected a compound, valproic acid, known to encourage the maturation of neuroblasts.

The procedure was not sufficiently efficient in creating new nerves to be immediately a candidate for therapy. The team tested 12 reprogramming factors to arrive at the choice of SOX2, and they suggested that further studies might improve this choice or find ways to enhance the activity of SOX2. Also, and perhaps a bigger problem, the reprogramming response was much weaker in older mice. Since many people needing spinal repair are older, they need to find ways to improve the efficiency in this population.

Several teams showed this conversion of astrocytes to neurons in the brain last year, but this is the first work to make the conversion in the spinal cord, which is generally much less plastic than the brain. Getting the body to create needed new cells for the spinal cord without having to transplant donor cells that could be rejected and might not behave as desired would be a major advance for this area of medicine.