The Transformation of Differentiated Cells into Pluripotent Stem Cells

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The McNair Scholars Program at USC is a prestigious federal initiative that has two main goals. Firstly, students are continually mentored throughout their undergraduate careers and eventually aided through the process of applying to graduate programs. Secondly, students are also granted funding for research, internships, and academic counseling. Baotran Vo is a 2011-2012 McNair Scholar from the Viterbi School of Engineering. A junior reigning from Huntington Beach, CA. Vo is studying biomedical engineering and bioinformatics. Her research concerns a hot topic in today's science – stem cells.

The special feature of stem cells that come from an embryo is that they can turn into almost any various cell type you want them to. Previous studies have successfully taken regular cells and turned them into stem cells (called induced pluripotent stem cells, or iPSCs) by reprogramming them. Scientists now know that there are four transcription factors that help make a cell pluripotent. By forcing these transcription factors into regular cells, researchers can potentially make them more malleable. Vo took an alternative approach. She focused on a gene that is found in regular, differentiated cells that seems to stop regular cells from being pluripotent. She decided to focus on silencing that gene in such cells while also transferring the four pluripotency promoters into those regular cells in order to see if the regular cells could take on pluripotent properties. Specifically, her studies were done on human skin cells.

Background

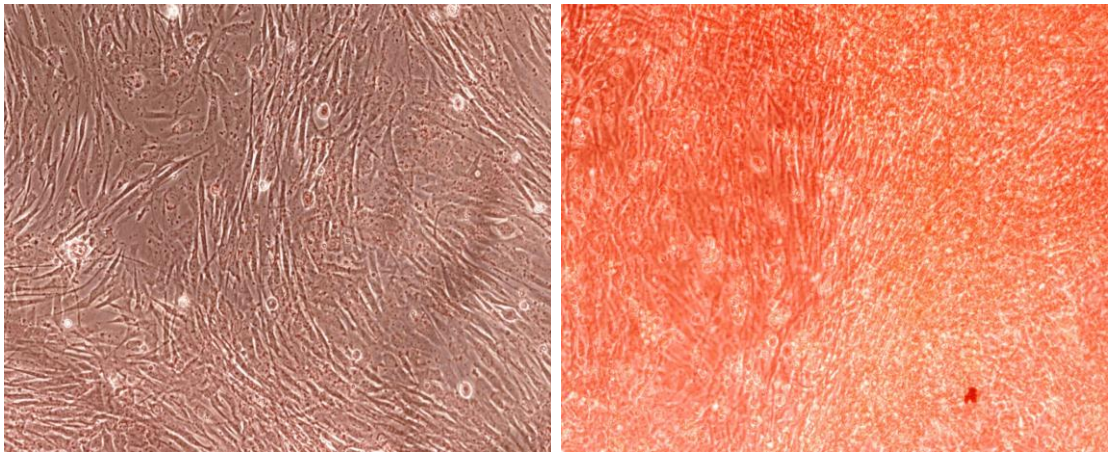
Vo was no stranger to her lab before she began her independent work. She was a student worker there for two semesters before she officially began her research project in May 2012, using McNair funding for summer research. She took a break from her work once the school year started again but plans to return to the lab soon

After three weeks of training in which she familiarized herself with the lab environment and learned lab techniques to utilize in her project, Vo worked on her experiments over the course of thirty days. Most of her work was carried out in the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at our Health Sciences Campus in Dr. Wange Lu's Lab. Her mentors, Wen-Hsuan Chang and Letisha Wyatt, were instrumental in guiding her through every part of the project. Furthermore, through the McNair program, Dr. Aaron Walker and Dr. Richard Andalon helped arrange conferences for her and her fellow scholars to present their work.

Methods

The official title of Vo's work is "Pitx2 Transcription Factor Inhibits Reprogramming of Human Dermal Fibroblast Cells." She modeled her work after a previous research study titled "FOXO1 is an essential regulator of pluripotency in human embryonic stem cells" by X. Zhang et al. Fortunately, Vo was able to report favorable results. She found that after infection, long and thin differentiated cells transformed into round embryonic stem cell-like colonies and were more transparent 11 days after reprogramming. Her study demonstrated that it is possible to reprogram human dermal fibroblast cells to a pluripotent state.

In the lab, she used Western blotting techniques and alkaline phosphatase staining to detect what factors and genes were being expressed or not expressed in the cells. Western blotting separates proteins from different samples based on their size. Knowing what proteins are in a sample can help deduce what genes are being expressed. Alkaline phosphatase is normally present on the membranes of stem cells. If a stain is observed it can be confirmed that pluripotency is present. The major focus of her analysis was centered on counting the number of colonies that displayed positive alkaline phosphatase staining. She also accounted for the change in cell shape from long, thin skin cells into circular cell-like colonies. Such a change would point to the idea that the cells had transformed from differentiated cells to colonies with properties of embryonic stem-cells.



Reprogrammed cells with OSKM Factors and Pitx2 knockdown show round ES-cell morphology.

Left: 8 days post infection.
Right: 15 days post infection.

Future Implications

As with any well-performed research study, there is room for improvement in Vo's work. She claims that further studies are needed using different reprogramming methods and other pluripotency genes. Furthermore, time was the major limitation for her work. Though she observed the successful reprogramming of cells in her work, after 30 days, she did not get as many reprogrammed colonies as expected.

Worthy of note is the controversy regarding research with stem cells. For example, stem cells taken straight from embryos may harm the developing fetus. Stem cells taken in such a manner are usually the central argument of those in opposition of stem cell research. However, most human embryonic stem cells and other cells that were used for Vo's research were derived from the surplus embryos from fertility clinics. Vo believes that using induced pluripotent stem cells (iPSCs) like she did can solve the ethical issue of taking cells from embryos because the iPSCs are derived from regular body cells and they can also be patient-specific (using a patient's own body cells to generate stem cells for his or her own use).

Her next step is to study another transcription factor – FOXI1 – that is hypothesized to enhance cell reprogramming. In the long run, the goal is to find out the most efficient methods for reprogramming cells. Vo's thought process now is focused on the long term benefits of her research: "iPS cells can also help patients with disorders that arise from cellular loss-of-function such as diabetes, Parkinson's disease and other neurodegenerative disease. Stem cell research enlightens us a little bit about the magic of life and paves a new and promising path for drug discovery and treatment in regenerative medicine."