Next steps for pluripotent iPS cells

By Tracey Baas, Associate Editor

Induced pluripotent stem cells produced by three separate groups have now passed the most stringent functional test of pluripotency—producing mice from reprogrammed fibroblasts—and thus could be viable alternatives to human embryonic stem cells.¹⁻³ The next hurdles include producing and selecting induced pluripotent stem cells with the best developmental potential and figuring out ways to coax them to differentiate into clinically useful effector cells.

In previous attempts to generate mice from induced pluripotent stem cells (iPS) cells, the embryos never got past late-stage development, perhaps because the iPS cells were not truly pluripotent.

To ramp up the capabilities of iPS cells, three separate groups—two from China and one from California—injected mouse iPS cells into tetraploid blastocysts, which are capable of producing placental and other extraembryonic tissues but not the embryo itself (see Figure 1, “Testing for pluripotency”).

The procedure, called tetraploid complementation, is the most stringent test for pluripotency. If the stem cells that are injected into the tetraploid blastocyst differentiate into embryonic tissues that produce a mouse, the stem cells are considered truly pluripotent. Tetraploid complementation had previously only worked with injection of embryonic stem (ES) cells.

All three groups used a virus to introduce the reprogramming genes *Oct4*, *Sox2*, *Klf4* and *c-Myc* into mouse fibroblasts. The California team from **The Scripps Research Institute** also introduced valproic acid to get rid of nonreprogrammed cells and used different timelines for manipulating the cells.

“Our cells were treated with valproic acid, which has been reported to inhibit division of cells that are not fully reprogrammed,” noted Kristin Baldwin, assistant professor in the Department of Cell Biology at Scripps and corresponding author on a paper in *Nature* that describes the results.¹ “The other successful

groups did not use valproic acid but also observed iPS cells that could generate mice. The valproic acid may help to enhance reprogramming or select for more fully reprogrammed cells since our method seems slightly more efficient.”

The Scripps team selected the four best iPS cell lines based on levels of the reprogramming genes and whether the cells had morphologies and proliferation rates similar to those of ES cells. The four cell lines produced live pups at rates of 0.3%, 1%, 4% and 13%.

However, the selection criteria could not be used to predict in advance which would be the most successful pluripotent cell line. The Chinese groups used similar selection criteria to identify cell lines for their respective complementation protocols, achieving success rates of about 3%.

Shaorong Gao, assistant investigator at the **National Institute of Biological Sciences, Beijing** and team leader of one of the Chinese groups, said his lab is working to identify markers unique to pluripotent mouse stem cell lines that would help researchers identify the best iPS cell lines with which to move forward. These markers include specific DNA sequences, DNA methylation, gene and protein expression patterns and histone modifications.

Gao’s team published its findings in *Cell Stem Cell*.² The third team, co-led by Qi Zhou, director of reproductive engineering at **The State**

Figure 1. Testing for pluripotency. Tetraploid complementation is the most stringent test of pluripotency.

[a] Fibroblast cells are removed from an adult mouse and reprogrammed to produce induced pluripotent stem (iPS) cells.

[b] By fusing two cells of an early-stage fertilized embryo, a tetraploid blastocyst is created. The blastocyst can produce a placenta and other cells necessary for development but cannot produce embryonic cells that would develop into a mouse.

[c] The iPS cells are injected into the tetraploid blastocyst, and the developing embryo is implanted into a surrogate mother. If a mouse is produced, the iPS cells are considered truly pluripotent.

Key Laboratory of Reproductive Biology of the **Chinese Academy of Sciences**, and Fanyi Zeng, VP of both the **Shanghai Stem Cell Institute** and the **Institute of Medical Genetics of Shanghai Jiao Tong University**, published its results in *Nature*.³

Aris Economides, senior director of the genome engineering technologies group at **Regeneron Pharmaceuticals Inc.**, and Peter Yang, a research scientist at the company, said in a joint statement that “as exciting as the new development and the conceptual breakthrough in substantiating iPS cells as truly pluripotent in the most stringent sense are, therapeutic application hinges on our ability of figuring out ways to cajole pluripotent cells, be it of embryonic or induced origin, into clinically useful cells.”

Regeneron has a stem cell research division that is exploring ways of manipulating iPS cells based on the company’s VelociGene high throughput genetic engineering technology.

Getting off to a good start

Two big hurdles remain on the path from iPS cells to useful effector cells: generating iPS cells without virus-based gene integration and determining whether molecular markers of enhanced pluripotency in mouse cells are applicable to human cells.

Any iPS cell line produced by viral delivery of reprogramming genes will not be useful in humans because of genome-integration concerns. “Even in the case of mouse cells, it would be necessary to generate integration-free iPS cells to have a safer source of cells. These animals are very likely still highly tumor prone,” noted Lorenz Studer, associate member in the Department of Developmental Biology at **Sloan-Kettering Institute**.

“Small molecule, protein transduction and transposon-based methods all aim to leave the genome intact while attaining pluripotency,” and there have been partial successes using these methods,⁴ said Economides and Yang. “The ultimate goal would be a highly efficient, robust way without any long-lasting changes to the target cells’ genetic makeup. In other words, no harm first.”

According to Scripps’ Baldwin, “we will need to determine whether cells produced using nonviral reprogramming are functionally similar to the best virally reprogrammed iPS cells or to ES cells in terms of genomic stability and their ability to generate all necessary cell types. Regardless of how the iPS cells are produced, we would like to develop methods that allow us to select cells with the best pluripotent potential.”

Finding molecular markers of pluripotency that are useful in both humans and mice could be a larger challenge than removing viral vectors from iPS procedures. “Assessing such competency in human cells will be a challenge, as the same functional assay—tetraploid complementation—is not applicable,” said Studer, because it would result in a cloned human embryo.

Following a different model

Instead of therapeutic uses, a more near-term application of the truly pluripotent iPS cells may lie with generation of mouse lines that have knocked in or knocked out genes. Although mice can be created using traditional ES cell lines, iPS cell lines would be better suited for establishing colonies of animals carrying multiple mutations or having a genetic background that, for unknown reasons, has been difficult to produce using ES cells.

“Producing specific mouse colonies should be possible once you know you have an efficient and stable iPS line,” said Ann Tsukamoto, executive VP of R&D at **StemCells Inc.**

StemCells has completed its first Phase I trial of expanded and banked human CNS stem cells to treat infantile and late infantile neuronal ceroid lipofuscinosis, a neurodegenerative disease characterized by a progressive loss of motor and psychological ability caused by high intracellular levels of lipopigments called lipofuscins.

The advantage of using tetraploid blastocyst injection for producing identical mice is the animals will be able to pass their genes to their progeny because all cells of the mouse, including gametes, would be derived from the original mouse fibroblasts.

In conventional blastocyst injection with iPS cells, Tsukamoto noted, each mouse needs to be screened to determine whether it is capable of germline transmission, because some cells of these chimeric mice will be derived from the original mouse blastocyst whereas other cells will be derived from the injected iPS cell-derived cells.

Studer also thinks tetraploid complementation could be a powerful method to generate mouse disease models more rapidly than with current cloning techniques, especially for specific phenotypes that are difficult to obtain with ES cells.

“For example,” Studer said, “the use of iPS cell technology, in conjunction with tetraploid complementation, should enable the generation of monoclonal mice where all cells have the identical immunoglobulin rearrangement, which would be a valuable tool for immunologists.”

He added that “monoclonal mice can be generated from engineered ES cells after sequential rounds of gene targeting; however, the iPS cell route is an elegant and likely faster alternative because it wouldn’t require such an iterative process.”

Baldwin did not disclose the patenting and licensing status of the findings reported in *Nature*.

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