

## Xiaoxiao: the Little Mouse, Giant Footprint

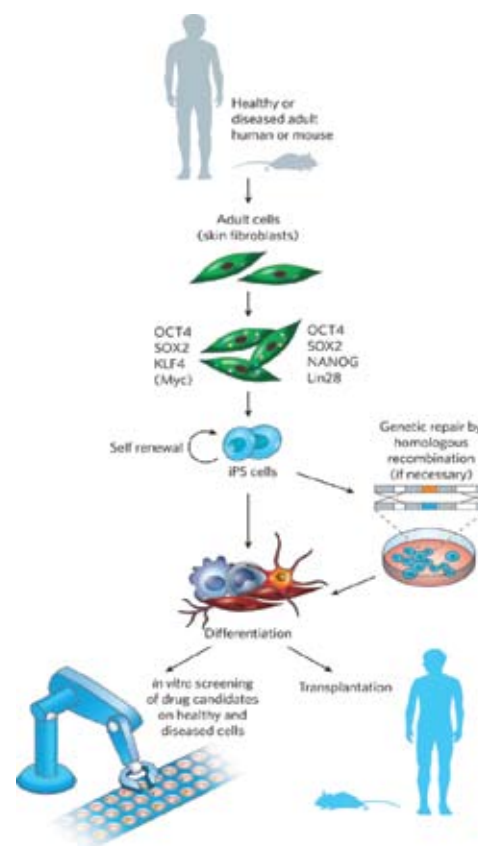
Ruby Yanru Chen-Tsai



**About Author:** Dr: Ruby Yanru Chen-Tsai graduated from Fudan University with first class honor in 1986 and received her PhD in Biochemistry, Molecular and Cell Biology from Cornell University in 1993. She is currently the Director of Transgenic Research Facility at Stanford University and a co-Founder of Applied StemCell, Inc.,

Chinese researchers recently reported a world class breakthrough in stem cell research in which they successfully created live mice from mouse fibroblast cells via induced pluripotent stem (iPS) cells, without using embryonic stem (ES) cells or cloning techniques that require eggs. This milestone opens the door to the development of exciting therapies, such as using a patient's own cells to grow replacement organs. The research is reported in the July 23, 2009, advanced online issue of *Nature*<sup>[1]</sup>.

Since Shinya Yamanaka of Kyoto University in Japan created the first iPS cells<sup>[2]</sup> (Figure 1) in 2006, researchers had not been able to generate an entire mammalian body from iPS cells, as in the case of true embryonic stem cells. Multiple groups had tried to produce mice from iPS cells but no live mice were ever born. For unknown reasons, iPS mouse embryos stopped developing about two-thirds of the way through gestation. This led to concerns that iPS cells might be inferior to embryonic stem cells and hinted that reprogramming with four factors identified by Yamanaka might not be the best method to produce pluripotent cell lines from patients. But the Chinese team led by Drs. Zhou and Zeng was up to the challenge. Their studies validated the potential application of iPS cells for cell replacement therapies.



**Figure 1:** Production of iPS cells and their applications. Taken from *Stem-cell-based therapy and lessons from the heart*, by Robert Passier, Linda W. van Laake & Christine L. Mummery. *Nature* 453, 322-329 (15 May 2008) doi:10.1038/nature07040

In this report, animal cloners Qi Zhou of the Institute of Zoology in Beijing and Fanyi Zeng of Shanghai Jiao Tong University started by

creating iPS cells the same way as Yamanaka, by using viral vectors to introduce four genes into mouse fibroblast cells. The first part of the team's new study involved gathering cells that were already "differentiated," i.e. developed into a particular cell type, such as skin, nerve, or muscle. In this case, the scientists worked with mouse embryonic fibroblast cells. Viruses were then used to insert genes coding for four proteins, called reprogramming factors, into these cells' DNA. These reprogramming factors shifted the cells out of their normal differentiated state to a "pluripotent" state resembling that of embryonic stem cells, which allows the cells to produce a wide variety of cell types. This cellular rewiring caused the cells to change their size and shape so that after only 7 to 10 days they could not be visually distinguished from embryonic stem cells.

To check whether the reprogramming had worked, Zhou and Zeng first carried out a standard set of tests, including analyzing whether their iPS cells had the same surface markers as embryonic stem cells. The ultimate test of the developmental pluripotency was to generate live mice entirely from iPS cells. To test for that, they performed tetraploid complementation experiments in

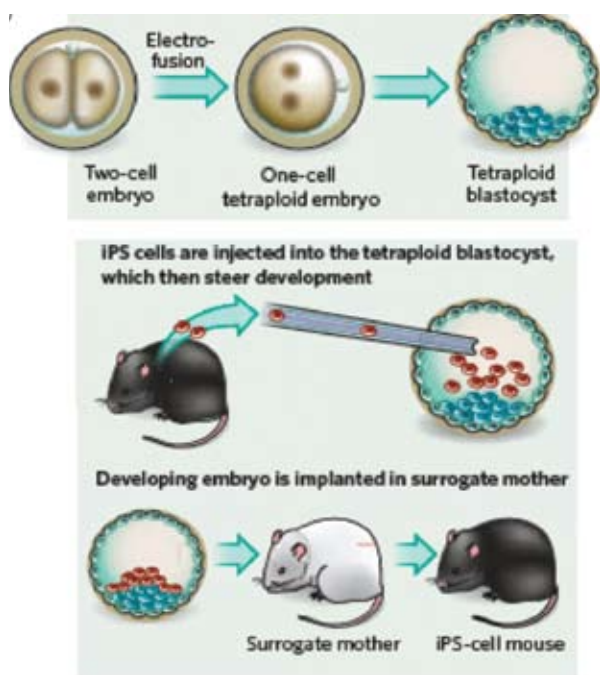
which iPS cells are microinjected into tetraploid blastocyst embryos (created by fusing two wild type embryos) (Figure 2). A tetraploid embryo develops a placenta and other cells necessary for development, but not the embryonic cells that would become the body. Any mice that are born are therefore 100% derived from the iPS cells. Drs. Zhou and Zeng's team was able to obtain live mice from iPS cells all the way to fertile adulthood, demonstrating that mouse iPS cells can, in fact, pass the most stringent test of pluripotency.

### Major Advantages

Clearly one advantage of iPS cell lines, assuming no issues with their stability emerge, is that they can be created without the need to harm the donor, meaning they offer the potential to avoid most of the ethical issues associated with embryonic stem cells. Another advantage is that iPS cells are tied to the key hopes for future work. Researchers, and perhaps eventually physicians, can take cells from mice or humans at any age to generate an iPS cell line. This opens the enticing possibility that iPS cells might be manipulated to grow replacement organs such as hearts and livers, or to provide healthy replacements for damaged cells, such as neurons needed to cure paralysis, Parkinson's, or Alzheimer's disease, all possibilities research groups around the world are vigorously pursuing. Because such cells would be derived directly from the patient, the rejection problems that plague conventional transplant therapies would be eliminated. Another hope is that iPS cells will be used to create new disease models that will foster better understanding of disease causes and more rapid identification of potential treatments.

### Future directions

In the paper, Drs. Zhou and Zeng's team reports 27 live births. Twelve of the live born mice passed one of the most fundamental tests of health: they produced offspring, and the offspring showed no abnormalities. However, the efficiency of live born mice is low. With their best cell line and optimal recipe, the Chinese group was able to get 22 live births from 624 injected embryos, a success rate of 3.5%. These mice also appear to have a high death rate, with some dying after just two days, and others displaying physical abnormalities. More studies need to be carried out to understand what differences between iPS cells and embryonic stem cells might explain the abnormalities, high death rates, low efficiency rates and the fact that most iPS cell lines don't seem to work in making mice.



**Figure 2:** Schematic graph on tetraploid complementation. Taken from "Mice made from induced stem cells, technical feat shows that the different route to stem cells can indeed make a full mammal body". By David Cyranoski doi:10.1038/460560a

Earlier this week, a group of Scripps Research scientists, led by Assistant Professor Kristin Baldwin, Ph.D., describes the creation of mice from mouse skin cells via iPS cell technology. The research is reported in the August 2, 2009, advance, online issue of *Nature*<sup>[3]</sup>. The Scripps Research team's protocols showed a higher success rate of 13% of live born mice compared to 3.5 percent reported in the Zhou and Zeng group's studies. The Scripps Research team also was able to generate live mice from four of 15 lines generated in one experiment (four of four tested) while the Zhou and Zeng's groups reported success with three of 37 (three of six tested). Now that researchers have a number of cell lines that do and do not generate live mice, comparisons among them should make it possible to zero in on exactly which parameters mark the production of successful lines. This in turn should provide invaluable information to aid in advancing iPS studies. In addition, comparison on mice raised normally with those generated using cloning, embryonic stem cells, or iPS cells will aid in better understanding on how tissues derived from iPS cells might behave in human cell transplant experiments.

It remains to be seen whether lessons obtained from these findings can be applied to human cells and thus whether human iPS cells will be a viable alternative to human ES cells in all circumstances. A few weeks ago, researchers at the University of California, Los Angeles, reported that human iPS cells that passed conventional

pluripotency tests differed in gene expression from human embryonic stem cell<sup>[4]</sup> suggesting that iPS cells might do things better or worse than embryonic stem cells. Because the tetraploid work cannot be done with human embryos, the Chinese and the Scripps' studies can't say much about clinical applications of human pluripotent cell lines at this time.

Never the less Drs. Zhou, Zeng and Baldwin's studies do provide an important model for understanding reprogramming and answer a lingering question about the development potential of mouse iPS cells.

### References

1. iPS cells produce viable mice through tetraploid complementation (2009). Xiao-yang Zhao, Wei Li, Zhuo Lv, Lei Liu, Man Tong, Tang Hai, Jie Hao, Chang-long Guo, Qing-wen Ma, Liu Wang, Fanyi Zeng & Qi Zhou. doi:10.1038/nature08267.
2. Takahashi, K. & Yamanaka, S. *Cell* 126, 663-676 (2006).
3. Adult mice generated from induced pluripotent stem cells (2009). Michael J. Boland, Jennifer L. Hazen, Kristopher L. Nazor, Alberto R. Rodriguez, Wesley Gifford, Greg Martin, Sergey Kupriyanov & Kristin K. Baldwin. doi:10.1038/nature08310
4. Chin, M. H. et al. *Stem Cell* 5, 111-123 (2009).