Mice made from induced stem cells

Technical feat shows that the different route to stem cells can indeed make a full mammal body.

Two teams of Chinese researchers have created live mice from induced pluripotent stem (iPS) cells, answering a lingering question about the developmental potential of the cells.

Since Shinya Yamanaka of Kyoto University in Japan created the first iPS cells[1] in 2006, researchers have wondered whether they could generate an entire mammalian body from iPS cells, as they have from true embryonic stem cells. Experiments reported online this week in *Nature*[2] and in *Cell Stem Cell*[3] suggest that, at least for mice, the answer is yes.

For the first study, 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 researchers hoped that the introduced factors would 'reprogram' the cells so that they could differentiate into any type of cell in the body.

To check whether the reprogramming had worked, Zhou and Zeng first carried out a standard set of tests, including analysing whether their iPS cells had the same surface markers as embryonic stem cells. Going a step further, they then created a 'tetraploid' embryo by fusing two cells of an early-stage fertilized embryo (see graphic). A tetraploid embryo develops a placenta and other cells necessary for development, but not the embryonic cells that would become the body. It is, in essence, a car without a driver.

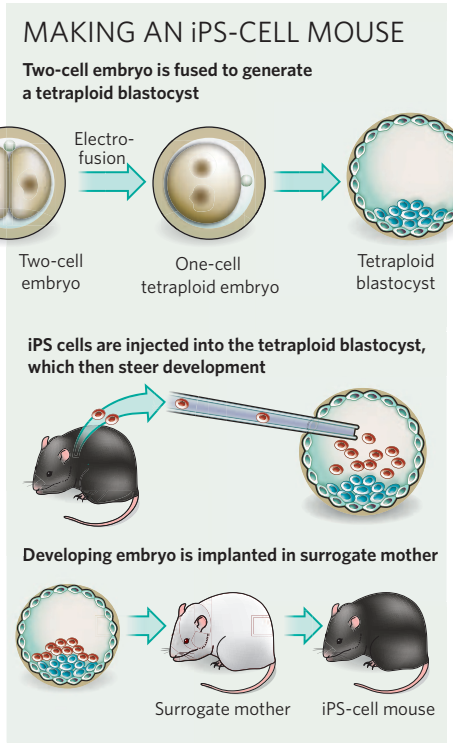
When implanted into these embryos, the iPS cells began to steer development. The developing embryo was transferred to a surrogate mother, and 20 days later a mouse was born. It was black, like the mice used to create the iPS cells and unlike the white mice used to create the tetraploid embryo. DNA tests confirmed the mouse, named Xiao Xiao or 'Tiny', had arisen from the iPS cells.

Rudolf Jaenisch, a cloning expert at the Massachusetts Institute of Technology in Cambridge, had tried to do the same experiment in 2007, but didn't succeed in getting beyond late-stage embryos[4]. "There were two possible explanations" for his team's failure, he says. "Either iPS cells aren't pluripotent so it was impossible, or we just hadn't tried hard enough. The first would have been more interesting, but I assumed it was the second explanation."

The Chinese team tried harder, tweaking the culture medium and analysing 250 developing embryos before getting their first mouse.

In the paper, the team reports 27 live births. With their best cell line and optimal recipe, they were able to get 22 live births from 624 injected embryos, a success rate of 3.5%.

Zeng says, however, that the mice seem to have a high death rate, with some dying after just two days, and others displaying physical abnormalities, details of which the team would not reveal. But some of their mice passed one of the most fundamental tests of health: all 12 mice that were mated produced offspring, and the offspring showed no abnormalities. The team says it now has hundreds of second-generation, and more than 100 third-generation, mice. The team found no tumours in the mice, although they have not systematically looked for them.



The leader of the second team, Shaorong Gao of the National Institute of Biological Sciences in Beijing, also credits persistence for success. His group, which used the same basic technique as Zeng and Zhou, transferred iPS cells to 187 tetraploid complementation embryos to get just two live births (a 1.1% efficiency rate), although one died in infancy. "The chance for generating such a cell line is rare but we tried very hard," he says. Gao's team is now trying to mate its surviving mouse.

Both groups are now trying 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. Zeng and Zhou found, for one thing, that timing was important: cells that formed iPS cell colonies quickly — after 14 days — were successful, whereas those that formed colonies after 20 or 36 days did not work. Gao suggests that "aberrant reprogramming" might be to blame, at least for the low efficiency rates.

Such mouse studies should help researchers to understand fundamental differences between human embryonic stem cells and iPS cells as well. Earlier this month, 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 cells[5]. "iPS cells might do things better or worse than embryonic stem cells," says team member Kathrin Plath. "I don't think we know the answer at this point." Because the tetraploid work cannot be done with human embryos, the Chinese studies can't say much about clinical applications of pluripotent cell lines, adds her colleague William Lowry.

Zhou and Zeng are pursuing several new avenues, including comparing the iPS mice with mice cloned with conventional techniques, and working to prove that the same experiment can be done with adult mice. (The fibroblasts used to create iPS cells in both studies came from late-stage embryos.)

This would essentially be a new way to clone adult mammals — reprogramming DNA from an adult and generating a genetically identical individual. As a potentially easier method that produces fewer abnormalities than conventional cloning, it might evoke interest among mavericks as a tool for human cloning. China recently strengthened its law prohibiting such cloning[6].

Zhou says he hopes that researchers will take advantage of the technology as "an important model for understanding reprogramming". He adds: "It is not intended to be a first step towards using iPS cells to create a human being." ■

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