## NEWS AND VIEWS

# Complete reprogramming to all-iPS mice

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The landmark discoveries by Shinya Yamanaka in mice and later separately by James Thompson in humans that adult animal cells can be reprogrammed to a pluripotent state of embryonic stem (ES) cells by forced expression of just a few key transcription factors have permanently altered a long-held view about the stability of differentiated cells in developmental biology, and suggested a way of generating personalized replacement tissues from patients' own cells for the regenerative medical treatment. However, those initial reprogrammed cells, called induced pluripotent stem (iPS) cells, were not completely identical to ES cells because of their limited ability to contribute to every type of cells in an embryo. Although subsequent experiments with improved technique produced iPS-derived offspring in F2, thus confirming the germline transmission by iPS cells, this nevertheless led some to question the totalipotency of iPS cells. Now, two papers that appeared in *Nature* from Kristin K. Baldwin's group in the Scripps Research Institute in San Diego (Boland et al., 2009) and Qi Zhou's group in the Institute of Zoology in Beijing (Zhao et al., 2009) and a brief report in *Cell Stem Cell* from Shaorong Gao's group in the National Institute of Biological Sciences also in Beijing (Kang et al., 2009), showed that iPS cells can directly produce viable mice through tetraploid complementation, the most rigorous test of totalipotency.

Tetraploid complementation is a technique developed by Andras Nagy and Janet Rossant in the early 1990s for creating mice derived entirely from genetically engineered ES cells in F1 so that certain gene functions essential in fetal development can be directly studied (Tam and Rossant, 2003). Because ES cells are derived from the inner cell mass, which is part of the epiblast layer destined to the future embryo, they will not form the primitive endoderm and the outer trophectoderm that collectively give rise to the placenta and the Reichert's membrane enclosing the embryo. When injected into normal diploid blastocysts, the ES and the host cells will co-develop to form chimeric embryos and one must rely on cumbersome breeding in order to generate a fertile mouse colony derived from the engineered ES cells. In tetraploid complementation experiments, the recipient blastocysts are made tetraploid, which means that they contain 4 sets of chromosomes, by electrofusion of normal diploid blastomeres at the two-cell stage. These tetraploid blastomeres will continue to develop to give rise to extraembryonic tissues but rarely to the embryo proper. When aggregated together, the tetraploid blastomeres will complement the development of ES cells to generate "all ES cells" pups directly in F1, including the germ cells. This technique is useful in studying all aspects of a gene's function, particularly when it is required during embryonic development. It is also used as the ultimate test in demonstrating the totalipotency of ES cells to contribute to every cell lineage in a future embryo.

The iPS cells generated in the Yamanaka laboratory were done by forced expression of the four so-called Yamanaka factors, Oct4, Sox2, c-Myc, and Klf4, in mouse or human fibroblasts, to select G418 resistant clones with ES cell appearance and that re-express Fbx15, an ES cell-specific marker gene. After these initial showings of possibility, many laboratories have made attempts at generating iPS cells from different cell sources or with different combinations of transcription factors to widen the potential use and to improve the induction efficiency of this technique. All of these efforts have created iPS cells that meet a number of criteria of the pluripotency such as giving rise to three different germ layers in embryoid bodies differentiated in vitro or in teratomas formed subcutaneously in severe combined immunodeficient (SCID) mice, or producing live chimeric mice after injection into normal diploid blastocysts; however, none succeeded in creating an "all iPS cell" mouse through tetraploid complementation for reasons that are still not understood. This led some to speculate that the inappropriate and continued expression of reprogramming factors may hamper embryonic

and postnatal development. So the Baldwin group decided to make another attempt using a drug-inducible lentiviral reprogramming strategy to achieve tight control of transgene expression in iPS cells and their derivatives. They placed the four factors under the control of the tetO promoter, which is activated by the reverse tetracycline transactivator, rtTA, in the presence of the tetracycline analogue doxycycline. A histone deacetylase inhibitor, valproic acid, was also used to increase reprogramming efficiency by inhibiting cell division of incompletely reprogrammed cells. Infection of mouse embryonic fibroblasts isolated from fluorescence capable indicator mouse lines with these lentiviruses yielded a series of ES-cell-like colonies dubbed as iMZ iPS cell lines and one of which with a complement of euploid chromosome generated 18 live born pups after injection into 140 tetraploid blastocysts (a nominal rate of 13%). The Gao group also adopted the controlled expression strategy and was able to generate four full-term pups through tetraploid complementation with one survived into adulthood.

However, if the inappropriate expression of the four reprogramming factors had prevented the production of full-term embryos through tetraploid complementation earlier, its effect must have been stochastic. This point can be taken from the experiments reported from the Zhou group, who used the 4 original "Yamanaka" factors carried in a constitutive expressing retroviral vector. As they reported, different iPS cells were not created equal in producing live born pups autonomously, a fact also corroborated in the experiments reported by the Baldwin group. In all, the Zhou group established 37 iPS cell lines from two different genetic backgrounds at 14, 20, and 36 days post viral infection. Injection of the iPS lines established at 20 and 36 days post infection into tetraploid blastocysts resulted in early termination of fetal development, but the 20-day iPS cells showed a 5-fold increase in producing embryos that reached to mid-gestation stage. This developmental advantage is even more pronounced in iPS cells established at day 14 post infection. After injecting 624 tetraploid blastocysts with such iPS cells, they were able to obtain 22 live-born pups plus 4 embryos arrested in the late gestation stage. This rate is comparable to the animal production rate that is routinely attainable in this group with normal ES cells, suggesting that these iPS cells are after all not much different from the normal ES cells. Finally, global gene expression analysis of all 37 iPS cell lines clearly grouped them with ES cells instead of the MEFs from which they were derived.

So, we are uncannily back to the square one: what made these recent attempts at tetraploid complementation successful when all previous ones failed? Perhaps a reasonable answer to that question lies in the old saying: the devil is in the detail.

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