Grown Up Mice From Gene-Corrected iPS Cells

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In 2008, Science named induced pluripotent stem cells (iPS) the breakthrough of the year.1 In 2009, Nature Methods called the process of generating iPS cells the “Method of the Year.”2 Two years later, although the use of iPS cells remains a bright promise for both research and regenerative medicine, concerns remain about how the epigenetic and genetic changes that occur in the creation of iPS cells will affect their utility in the treatment of heritable conditions. This summer, a team led by Tobias Cantz, at the Max-Planck-Institute for Molecular Biomedicine, published a proof-of-principle paper in PLoS Biology demonstrating that iPS cells remained pluripotent after correction of genetic defects.3

The authors chose fumarylacetoacetate hydrolase (FAH) deficiency as a disease model for an iPS-mediated correction. FAH deficiency leads to the rare clinical syndrome, tyrosinemia type I, characterized by the accumulation of succinylacetone in multiple organs, including the liver and kidneys, leading to organ failure and death. In this paper, Wu et al first generated iPS cells from FAH-deficient fibroblasts and then corrected the FAH deficiency by transducing the iPS cells with recombinant lentiviruses. Subsequently they used the genetically corrected iPS cells to produce mice that expressed FAH (Figure).

“The study clearly showed that the gene correction procedures did not impair the full pluripotentiality of iPS cells,” said Shinya Yamanaka (director of the Center for iPS Cell Research and Application at Kyoto University). Yamanaka is the father of the iPS field. In 2006 his laboratory was the first to coax adult mouse fibroblasts into an undifferentiated state through the expression of 4 transcription factors: Oct4, Sox2, Klf4, and c-Myc.4

Yamanaka said that the findings of the German group “indicate a possibility that human iPS cells generated from people with genetic defects may be repaired and have the similar abilities.” The work should “encourage researchers to improve differentiation methods from iPS cells into functional cells.”

An intriguing aspect of the study was the relatively high efficiency at which the group’s approach yielded full-term surviving pups. Up to 60% of cellular aggregates from tetraploid complementation generated surviving pups, and a significant number of the pups lived to adulthood without a discernible phenotype. With success rates of 1% to 19% in other laboratories using tetraploid complementation, the authors declared their rates “astonishingly high.” None of the mice developed a tumor, a finding that questions clinical and biological significance of potential epigenetic and genetic changes in iPS cells.

“With this data we can demonstrate that, even if there are subtle changes, they don’t abrogate the pluripotent potential,” Cantz said. Neither viral integration nor the loss of some epigenetic markers interfered with the phenotype of the pups they created, he said.

The study suggests a high degree of flexibility in the ability of genome to tolerate random genetic insertions, said Ignacio Sancho-Martinez (Salk Institute for Biological Studies, San Diego, CA). “The novelty of this manuscript is basically [that] they have shown that the cells, iPS cells, that contain a specific mutation, are still able to generate adult animals,” Sancho-Martinez said. But what is particularly interesting, he said, is that the pluripotency was unchanged even after using a lentiviral vector, which inserts randomly into the genome.

“That opens a whole new way of research and thinking,” Sancho-Martinez said. “We are all aware that we have to edit the genome in a perfect way. We cannot have random integrations.” But this research shows “even when we don’t control integration, even when we use viral methods that everybody seems to be afraid of, we still keep potency of the cell.”

“We are investing a lot of time, and a lot of federal money, in the development of technologies that are hypothetically safer and better,” Sancho-Martinez said. “However, we already have technologies in hand.”

Cantz said they chose this third-generation self-inactivating lentiviral vector as the safest and best alternative in their investigation. They rejected the use of homologous recombination to fix the FAH gene because of the large amount of DNA damage—especially double-strand breaks—as well as offsite integrations of some of the transferred DNA. The 35 kb size of the gene, as well as its 14 exons, meant it was not amenable to more targeted forms of repair, such as the use of zinc-finger nucleases designed to home to specific nucleotide sequences. Lentiviral vectors are preferable to retroviral vectors, which can integrate near start sites and regulatory regions. Lentivirus inserts away from these sites, Cantz said. Finally, lentiviral vectors are already in use in some clinical trials, including interventions for advanced HIV, hereditary hematopoietic disorders, and Parkinson’s disease.

The research marks the next logical step in the development of the field, said Joseph C. Wu (Stanford University School of Medicine, Palo Alto, CA). “It’s common sense that this is where the field needs to go if you think about the future, 20 years from now, 30 years from now,” Wu said. He suggested that the advance paves the way for the eventual use of iPS cells by parents who want to prevent passing along gene defects to their offspring. Gene-corrected iPS cells from

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the mother or father can become sperms or eggs for use in in vitro fertilization. “Then you can implant them into the uterus and have a viable baby that no longer has the disease mutation,” he said. Today, parents can only screen for mutations causing heritable diseases via preimplantation genetic diagnosis of embryos created from in vitro fertilization.

Still to be resolved is the problem of gene silencing in pups grown from the complementation assay. Although the pups carried the corrected FAH gene, only 20% to 25% of the liver cells actually expressed the gene. However, when researchers eliminated dietary supplementation of a chemical known to partially correct FAH-deficiency—Nitisinone, (2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione), usually referred to by its chemical abbreviation, NTBC—FAH-expressing cells overtook their silenced neighbors. RT-PCR indicated a 2.7-fold increase in FAH gene expression. “This is something we are now investigating in follow-up studies,” Cantz said. “Nevertheless, the mice can survive. The few cells with the transgene, they have selective advantage. After a couple of weeks, over 70% with the transgene expressed the protein.”

But for all the technical prowess of the paper, some scientists are not certain it brings iPS research any closer to its therapeutic goals. “So, it’s a step forward to fix a gene and make a mouse, and it’s a step forward that the cells take over in the liver,” said Jonathan Seidman (Harvard Medical School). “It’s a technical tour de force, but it’s not clear how much it moves the field forward. It leaves a great many steps between this and actually repairing cells in the adult mouse.”

“It’s very nice, but from a practical point of view, it won’t lead to a cure in a patient,” said Sancho-Martinez. The tetraploid complementation technique is unlikely to have a future in human medicine, he notes. “You won’t generate a whole embryo for humans.”

But Cantz said the future of this system is rather in “demonstrating the feasibility of gene correcting and studying long-term effects.”

Even as a tool for testing, some foresee difficulties. “I think it’s a good testing bed for screening. But it’s not like it’s rapid high-throughput stuff either,” Mark Sussman (San Diego State University Heart Institute, San Diego, CA) said. “If you really wanted to go through the trouble, then this is probably the gold standard.” However, he notes that the process is technically demanding and difficult to implement. “I don’t get the sense that a lot of people are going to take this particular approach to ask questions with their own cell type.” At best, it might be developed as a service provided by a core laboratory facility with motivation to become skilled in the technique. “I don’t see this as being a conceptually big advance.”

The repair method also falls short as a model for many genetic diseases. “It won’t work for dominant gene diseases, when there there’s some poison polypeptide. It won’t work when you’re trying to block a bad gene,” Seidman said. This model of gene repair works best for secretory diseases, such as diabetes, where adding more insulin-producing cells could effectively counter the lost function of a missing gene, “but if you have to replace a whole lot of cells, or the cells need contractile function—this doesn’t address that,” he said.

And for some, including the watchdogs over clinical trials, the use of the lentiviral vector is still a problem. “My take on
it, from talking to regulatory agencies and clinicians, is that everybody is still pretty gun shy of using lentis, even if they’re the self-inactivating versions of the vector that this group described,” Sussman said.

The authors conclude, “As this approach can lead to a functional organism, we would expect that fully functional cells could be generated and used for transplantation purposes.” Nevertheless, many point out that there are a great many steps between this work and its promise.

“There are still numerous challenges. For instance, we need to establish methods to generate functional hepatocytes by in vitro-directed differentiation from gene-corrected iPS cells,” Yamanaka said. “It remains to be determined whether the conclusion of this manuscript is also valid with human iPS cells.”

References
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