Putting pluripotent pieces together

By Tracey Baas, Senior Editor

An international team has developed a method for genetically altering induced pluripotent stem cells that could be useful for treating inherited diseases.[1] The team now plans to scale up the method and will need to show the cells are safe for long-term use.

A potential roadmap for using induced pluripotent stem (iPS) cells as therapeutics for genetic diseases has been in place for at least five years[2] and involves first harvesting accessible cells such as fibroblasts from the patient and converting them into iPS cells. Next, the patient's genetic defect is corrected in those iPS cells. Finally, the corrected iPS cells are differentiated into the desired cell type and transplanted back into the patient, in which the cells engraft and proliferate in the local tissue to treat the disease.

A key stumbling point comes at the second step, as the targeted correction of a mutation in one gene must avoid introducing errors in surrounding genes. Many standard methods of gene targeting leave residual errors, which makes them unsafe for use in humans.

To address the issue, researchers from the **Wellcome Trust Sanger Institute** and the **University of Cambridge** and colleagues developed a gene-targeting method that combined two existing techniques—zinc finger nucleases (ZFNs) and piggyBac transposons. The former ensures gene targeting is efficient and accurate, and the latter that no residual genomic errors remain.

As proof of principle, the researchers studied α_1-antitrypsin (AAT; A_1AT; SERPINA1) deficiency, an autosomal recessive disorder that results from a mutant A_1AT protein. The deficiency leads to protein inclusion bodies in the liver and to cirrhosis. Currently, the only therapy is a liver transplant.

The researchers first harvested fibroblasts from A_1AT-deficient patients and converted them into iPS cells using standard methods. They then used the ZFN and piggyBac transposon technologies to correct the mutant *A_1AT* gene in the iPS cells.

About 4% of the iPS cells were corrected for both mutant alleles. The genomes of the corrected iPS cell lines proved to be relatively unaltered compared to the genome of the parental iPS cell line. Exome sequencing of one of the corrected cell lines detected 29 mutations in protein-coding DNA that could have occurred during reprogramming or growth in culture.

In their paper in *Nature*, the researchers said the mutations were minor and were unlikely to disrupt any biological functions of the cells.

Next, the team differentiated the corrected iPS cells into hepatocyte-like cells, which were injected into the livers of transgenic mice whose hepatocytes had been destroyed by albumin-urokinase (Alb-uPA). The injected cells engrafted throughout the liver and secreted human proteins without forming tumors (*see* **Figure 1, "From fibroblast to functional hepatocyte"**).

The entire process, from fibroblast to functional hepatocyte, took less than six months.

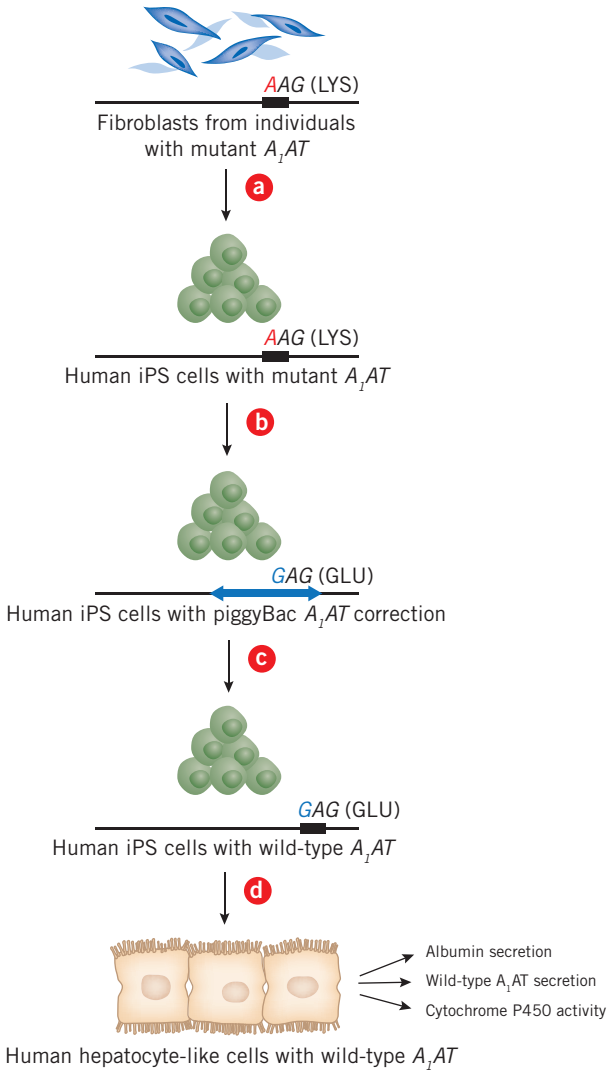

Figure 1. From fibroblast to functional hepatocyte. Fibroblasts from individuals with mutant *α_1-antitrypsin* (*AAT*; *A_1AT*; *SERPINA1*) are transformed into induced pluripotent stem (iPS) cells using reprogramming factors expressed by Sendai virus vectors, an integration-free method [**a**]. Homologous recombination is initiated with zinc finger nucleases (ZFNs) and a piggyBac transposon (blue arrow) to correct *A_1AT* [**b**]. piggyBac is removed with piggyBac transposase, leaving wild-type *A_1AT* [**c**]. iPS cells are differentiated into functional hepatocyte-like cells [**d**].

The team also included the ZFN therapeutic developer **Sangamo BioSciences Inc.**, the **Pasteur Institute**, the **Cantabria Institute of Biomedicine and Biotechnology**, **Sapienza University of Rome**, **Cambridge University Hospitals**, **DNAVEC Corp.** and the **Japan Science and Technology Agency**.

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—Sean Wu, Harvard Medical School

Philip Gregory, SVP of research and CSO at Sangamo, and Ludovic Vallier, colead author on the study, said the group's next steps are "to test the efficiency of the approach to scale up for producing the corrected iPS-derived hepatocyte-like cells and to develop a GMP protocol."

Vallier is a principle investigator and medical research council senior fellow at the University of Cambridge.

Pristine wilderness

Sean Wu, assistant professor of medicine at **Harvard Medical School** and a principal faculty member at the **Harvard Stem Cell Institute**, said using fibroblast-derived human iPS cells to correct the A_1AT genotype "was a good first target to show proof of principle. The team's idea to use zinc finger nuclease and piggyBac to enhance the homologous recombination in human iPS cells is a reasonable first choice. But the zinc finger nuclease method is not 100% site specific and may introduce additional nontargeted integrations and mutations."

Wu said he "would like to see if the corrected hepatocyte-like cells can rescue an animal model of A_1AT deficiency to see if the disease can be corrected *in vivo*."

George Daley, director of the stem cell transplantation program at **Children's Hospital Boston** and scientific cofounder of iPS cell company **iPierian Inc.**, thought the more important question is safety.

Andrey Semechkin, CEO and cochairman of **International Stem Cell Corp.**, concurred. "Genomic instability induced by reprogramming or genomic modification is a main issue for the safe use of iPS cells, and all iPS cell lines will need to be analyzed carefully before iPS technology can be successfully used in clinical application," he told *SciBX*.

"It's very important to be sure that the cells don't contain cancer-causing or other serious types of mutations," Semechkin added. "It needs to be shown that iPS cells—as well as any stem cell progeny—will function normally without tumor formation for a significant period of time, which will require extensive long-term testing in animals."

Vallier agreed and has already extended the mouse engraftment work to 12 weeks, at which time the animals still were tumor free.

He also thinks that "a deeper understanding of the biological impact of genomic changes and epigenetic instability will be crucial to achieve a high level of safety."

"Risk assessment might also be a factor in terms of what is considered safe. If an individual has a terminal or debilitating disease with no therapeutic options, well then, iPS cell therapy might become an acceptable choice," said Daley.

The patent and licensing status for the ZFN- and piggyBac-mediated gene correction of human iPS cells is undisclosed.

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REFERENCES

1. Yusa, K. *et al. Nature*; published online Oct. 12, 2011; doi:10.1038/nature10424
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2. Lou, K.-J. *SciBX* **4**(21); doi:10.1038/scibx.2011.588

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