A DECADE OF iPS CELLS

Induced pluripotent stem cells were supposed to herald a medical revolution. But ten years after their discovery, they are transforming biological research instead.

BY MEGAN SCUDELLARI

"We have colonies."

Shinya Yamanaka looked up in surprise at the postdoc who had spoken. "We have colonies," Kazutoshi Takahashi said again. Yamanaka jumped from his desk and followed Takahashi to their tissue-culture room, at Kyoto University in Japan. Under a microscope, they saw tiny clusters of cells — the culmination of five years of work and an achievement that Yamanaka hadn't even been sure was possible.

Two weeks earlier, Takahashi had taken skin cells from adult mice and infected them with a virus designed to introduce 24 carefully chosen genes. Now, the cells had been transformed. They looked and behaved like embryonic stem (ES) cells — 'pluripotent' cells, with the ability to develop into skin, nerve, muscle or practically any other cell type. Yamanaka gazed at the cellular alchemy before him. "At that moment, I thought, 'This must be some kind of mistake'," he recalls. He asked Takahashi to perform the experiment again — and again. Each time, it worked.

Over the next two months, Takahashi narrowed down the genes to just four that were needed to wind back the developmental clock. In June 2006, Yamanaka presented the results to a stunned room of scientists at the annual meeting of the International Society for Stem Cell Research in Toronto, Canada. He called the cells 'ES-like cells', but would later refer to them as induced pluripotent stem cells, or iPS cells. "Many people just didn't believe it," says Rudolf Jaenisch, a biologist at the Massachusetts Institute of Technology in Cambridge, who was in the room. But Jaenisch knew and trusted Yamanaka's work, and thought it was "ingenious".

The cells promised to be a boon for regenerative medicine: researchers might take a person's skin, blood or other cells, reprogram them into iPS cells, and then use those to grow liver cells, neurons or whatever was needed to treat a disease. This personalized therapy would get around the risk of immune rejection, and sidestep the ethical concerns of using cells derived from embryos.

Ten years on, the goals have shifted — in part because those therapies have proved challenging to develop. The only clinical trial using iPS cells was halted in 2015 after just one person had received a treatment.

But iPS cells have made their mark in a different way. They have become an important tool for modelling and investigating human diseases, as well as for screening drugs. Improved ways of making the cells, along with gene-editing technologies, have turned iPS cells into a lab workhorse — providing an unlimited supply of once-inaccessible human tissues for research. This has been especially valuable in the fields of human development and neurological diseases, says Guo-li Ming, a neuroscientist at Johns Hopkins University in Baltimore, Maryland, who has been using iPS cells since 2006.

The field is still experiencing growing pains. As more and more labs adopt iPS cells, researchers struggle with consistency. "The greatest challenge is to get everyone on the same page with quality control," says Jeanne Loring, a stem-cell biologist at the Scripps Research Institute in La Jolla, California. "There are still papers coming out where people have done something remarkable with one cell line, and it turns out nobody else can do it," she says. "We've got all the technology. We just need to have people use it right."

FROM SKIN TO EYES

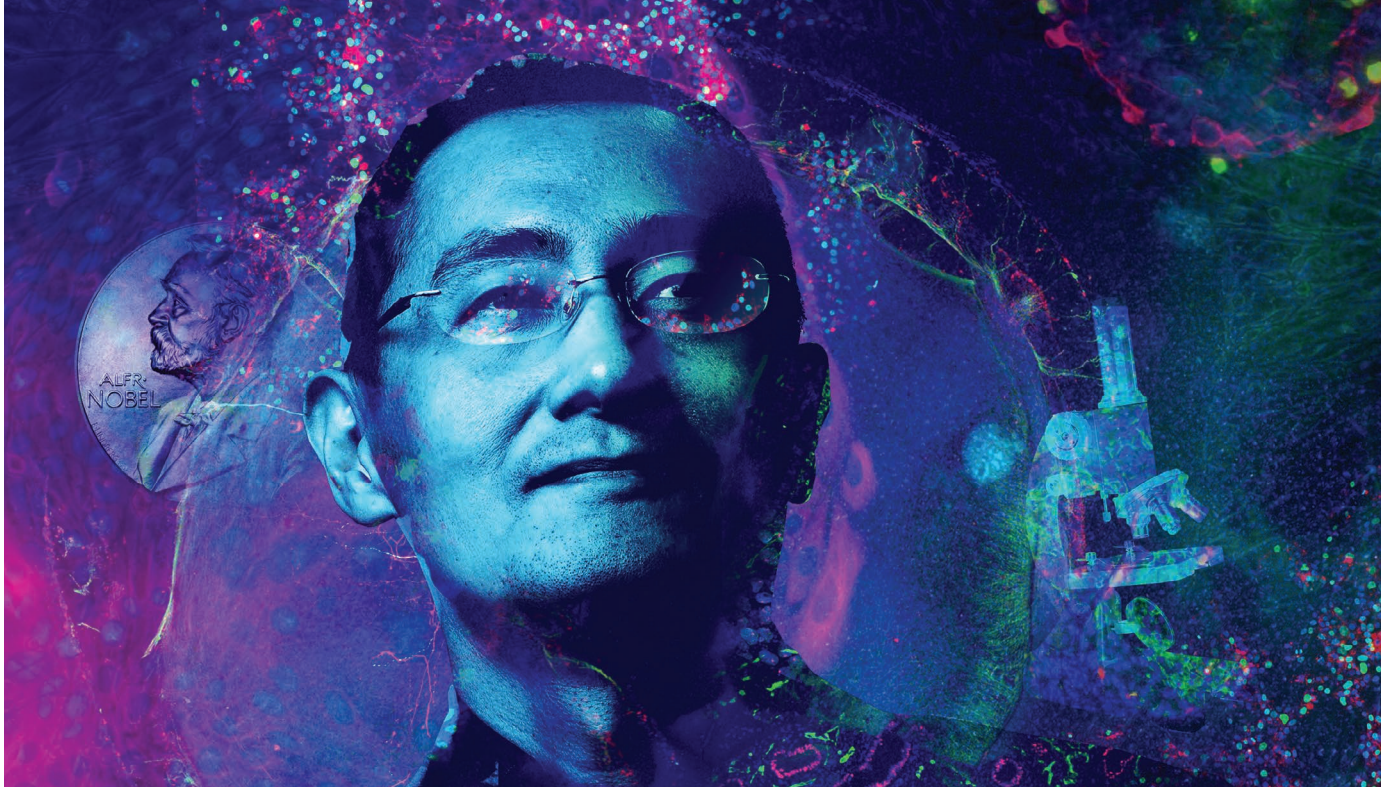
Six weeks after presenting their results, Yamanaka and Takahashi published[1] the identities of the genes responsible for reprogramming adult cells: *Oct3/4*, *Sox2*, *Klf4* and *c-Myc*. Over the next year, three laboratories, including Yamanaka's, confirmed the results and improved the reprogramming method[2–4]. Within another six months, Yamanaka and James Thomson at the University of Wisconsin–Madison managed to reprogram adult cells from humans[5,6]. Labs around the world rushed to use the technique: by late 2009, some 300 papers on iPS cells had been published.

Many labs focused on working out what types of adult cell could be reprogrammed, and what the resulting iPS cells could be transformed into. Others sought to further improve the reprogramming recipe, initially by eliminating[7] the need to use *c-Myc*, a gene with the potential to turn some cells cancerous, and later by delivering the genes without them integrating into the genome, a looming safety concern for iPS-cell-based therapies.

Another big question was how similar iPS cells really were to ES cells. Differences started to emerge. Scientists discovered[8] that iPS cells retain an 'epigenetic memory' — a pattern of chemical marks on their DNA that reflects their original cell type. But experts argue that such changes should not affect the cells' use in therapies. "There might be some differences from ES cells, but I believe they are really not relevant," says Jaenisch.

By 2012, when Yamanaka won half of the Nobel Prize in Physiology or Medicine for the work, the first human trial of an iPS-cell-based therapy was being planned. Masayo Takahashi, an ophthalmologist at the RIKEN Center for Developmental Biology (CDB) in Kobe, Japan, had been developing ES-cell-based treatments for retinal diseases when Yamanaka first published his reprogramming method. She quickly switched to iPS cells, and eventually began to collaborate with Yamanaka.

In 2013, her team made iPS cells from the skin cells of two people



Shinya Yamanaka won a Nobel prize for his work on reprogramming adult cells to an embryonic-like state.

with age-related macular degeneration, an eye condition that can lead to blindness, and used them to create sheets of retinal pigment epithelium (RPE) cells for a clinical trial. Not long after, CDB researchers working on another cell-reprogramming technique — stimulus-triggered acquisition of pluripotency, or STAP — came under investigation for misconduct (see go.nature.com/1xbnlzn). Although unconnected to the iPS-cell trial, the furore made it difficult for Takahashi to advance her study: it created a "headwind in the calm sea" in which she had been working, she says. Yet her team pushed ahead, and on 12 September 2014, doctors implanted the first RPE sheets into the right eye of a woman in her seventies. Takahashi says that the therapy halted the woman's macular degeneration and brightened her vision.

But as the lab prepared to treat the second trial participant, Yamanaka's team identified two small genetic changes in both the patient's iPS cells and the RPE cells derived from them. There was no evidence that either mutation was associated with tumour formation, yet "to be on the safe side" Yamanaka advised Takahashi to put the trial on hold. She did.

> "THE WORLD IS WATCHING TO SEE HOW IT PROGRESSES."

The suspension gave pause to other researchers interested in the field, says Paul Knoepfler, a stem-cell biologist at the University of California, Davis: "The world is watching to see how it progresses." But the difficulties iPS cells have faced getting to the clinic aren't that unusual, says David Brindley, who studies stem-cell regulation and manufacturing at the University of Oxford, UK. It generally takes about 20 years to move a scientific discovery to clinical and commercial adoption, so iPS cells "are following roughly the same trajectory", he says.

In the United States, the Astellas Institute for Regenerative Medicine in Marlborough, Massachusetts (formerly Advanced Cell Technology), has several iPS-cell-based therapies in its pipeline, including ones for macular degeneration and glaucoma, says chief scientific officer Robert Lanza. For any such therapy, it takes years to work out a suitable method for making the right cell types in large enough quantities, and with enough purity. "iPS cells are the most complex and dynamic therapies that have ever been proposed for the clinic," says Lanza. "I'm the first one who wants to see these cells in the clinic, but an abundance of caution is needed."

The other great challenge is working out what will be required to get such treatments approved. Loring hopes to start an iPS-cell-therapy trial for Parkinson's disease in the next two years. But it won't be easy: the treatment uses cells derived from individual patients, and Loring plans to do a complex series of checks and validations for each cell line to demonstrate its safety to the US Food and Drug Administration.

Developing and testing a therapy in even one person has been educational, says Yamanaka: it took one year and US$1 million. He expects future therapies to use donor-derived iPS cells from a cell bank, rather than making them for each patient.

Takahashi plans to compare banked iPS cells side-by-side with those derived from patients, to observe any differences in immune reaction. She intends to apply to the Japanese government to resume her macular-degeneration trial "very soon", but when asked, would not specify a timeline.

CELLULAR IMPROVEMENTS

Although cell therapy has suffered setbacks, other areas of research have blossomed. Methods for making iPS cells "are more refined and elegant then they were even five years ago", says Knoepfler.

But most reprogramming techniques are inefficient: only a small fraction of cells end up fully reprogrammed. And, like all cell lines, iPS cells vary from one strain to another. That has made it hard to establish controls in experiments.

Marc Tessier-Lavigne, a neuroscientist at the Rockefeller University in New York City, confronted this challenge with colleagues at the New York Stem Cell Foundation when they began to work with iPS cells made from people with early-onset Alzheimer's disease and frontotemporal dementia. They quickly realized that comparing a patient's iPS cells with those from a healthy control didn't work — the cells behaved too differently in culture, probably the result of disparities in genetic background or gene expression. "So we turned to gene editing," says Tessier-Lavigne.

The CRISPR–Cas9 gene-editing tool, which has gained huge popularity in recent years, has enabled researchers to introduce disease-associated mutations into a sample of iPS cells and then compare them with the original, unedited cell lines. Jaenisch's lab uses CRISPR–Cas9 with iPS cells daily. "We can do any manipulations we want to do," he says.

New, refined gene-editing methods are proving even more useful. In April, for example, Dominik Paquet and Dylan Kwart in Tessier-Lavigne's

ILLUSTRATION BY ANDY POTTS; PHOTO: CHRIS GOODFELLOW/GLADSTONE INST.

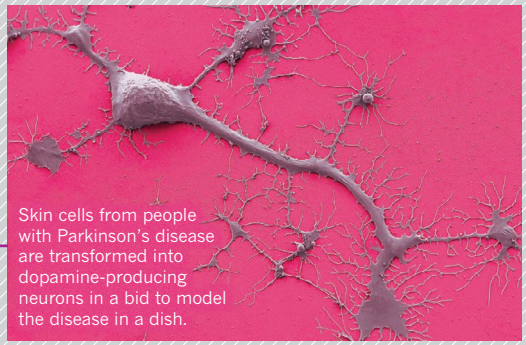
INDUCING A REVOLUTION

Shinya Yamanaka's *discovery spurred thousands of publications on the identity, characteristics and many uses of iPS cells in research.*

2006 — *Yamanaka*, of Kyoto University in Japan, reveals that just four genes can reprogram adult mouse cells into embryonic-like, 'pluripotent' iPS cells.

2007 — *Yamanaka* and *James Thomson* at the University of Wisconsin–Madison both report creation of human iPS cells.

2008



Skin cells from people with Parkinson's disease are transformed into dopamine-producing neurons in a bid to model the disease in a dish.

THOMAS DEERINCK/NCMIR/SPL

2009 — Several teams start to demonstrate that iPS cells can be created without inserting genes into the genome.

2010

2011

POOL/YOMIURI SHIMBUN/AP

2012

2013 — *Yamanaka* (pictured with King Carl XVI Gustaf of Sweden) and *John Gurdon* at the University of Cambridge, UK, receive the Nobel Prize in Physiology or Medicine for revealing that adult cells can be reprogrammed.

2014 — Researchers in Japan begin the first, and so far only, test of iPS-derived cells in people, attempting to treat a degenerative eye condition.

2015 — The Japanese trial is halted.

lab demonstrated[9] a technique for introducing specific point mutations into iPS cells using CRISPR, and editing just one copy of a gene, rather than both. This allowed them to generate cells with precise combinations of Alzheimer's-associated mutations, and to study the effects.

But because iPS cells resemble embryonic cells, they are not always ideal for studying late-onset diseases such as dementia. So researchers are exploring ways to stress cells or introduce proteins that age them prematurely. "It's a valid concern that hasn't been resolved, but there are a number of approaches to really try to tackle it," says Tessier-Lavigne.

The fact that iPS cells mimic early human development has proved useful in another field — the sprint to discover whether and how infection with the Zika virus in pregnant women might lead to microcephaly, a condition in which a baby's head is smaller than expected. Ming and her colleagues have used iPS cells to create brain organoids — 3D bits of tissue that resemble developing organs. When they exposed these to Zika, they found[10] that the pathogen preferentially infects neural stem cells over newly formed neurons, leading to increased death of the neural stem cells and a decrease in the volume of a layer of neurons in the cortex, resembling microcephaly.

Other groups have used iPS cells to create organoids such as mini-guts and mini-livers, and the list of disease-related discoveries using iPS cells is growing. It includes showing how a gene duplication in glaucoma causes the death of nerve-cell clusters[11], and recapitulating genetic and cellular alterations associated with Huntington's disease[12].

iPS cells have also been used with some success in drug discovery: they provide a plentiful source of patient-derived cells to screen or test experimental drugs. In 2012, for example, neural stem cells made from people with a nerve-cell-development disease were used to screen nearly 7,000 small molecules and identify a potential drug for the condition[13]. And this year, a team reported[14] generating sensory neurons from iPS cells made from people with an inherited pain disorder. The researchers showed that a sodium-blocking compound reduced the excitability of neurons and decreased pain in the patients. It would be great to use iPS cells to predict whether people will respond to a particular drug, says Edward Stevens, a research fellow at the Pfizer Neuroscience and Pain Research Unit in Cambridge, UK, who led the work, but there will need to be much more evidence that such a strategy works.

Even after a decade of reprogramming cells (see 'Inducing a revolution'), researchers don't know in detail how the process actually occurs. For now, the field is focused on systematically verifying cell lines' identity and safety, by checking their genomes, gene-expression patterns and more. One such effort, the European Bank for Induced Pluripotent Stem Cells, centred in Cambridge, UK, publicly launched its catalogue of standardized iPS cells for use in disease modelling this March. Yamanaka is also involved in banking iPS cells for future therapies, collecting varieties that would be immunologically compatible across a broad population.

The greatest future challenges, he says, are not scientific. Researchers are going to need strong support from the pharmaceutical industry and governments to move forward with cell therapies; for drug discovery and disease modelling, researchers must be persistent and patient. iPS cells can only shorten the discovery process, not skip it, he says. "There's no magic. With iPS cells or any new technology, it still takes a long time." ■

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