Building the right cells

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Just after 5 p.m. doors rattle shut and feet begin to shuffle past the narrow lab where Karim Si-Tayeb sits hunched over a microscope, all but invisible to the scientists leaving the Medical College of Wisconsin. Si-Tayeb has already worked eight hours and will work five more, eyes locked on the living cells in his care. Under the microscope, their tiny colonies resemble constellations of tightly packed stars. They carry his ambition.

"A few months ago I was working and it struck me how incredibly cool this is," he said, sliding a dish of unusual cells under the microscope, cells he had scientifically altered. "This revolution is occurring, and you are part of it."

Early in 2008 the 32-year-old postdoctoral student from France joined a biomedical revolution by reprogramming human skin cells back to their embryonic origin, just as James Thomson in Madison and Shinya Yamanaka in Japan did when they made headlines in November 2007. Now Si-Tayeb and his supervisor, Stephen A. Duncan, a Medical College professor, were engaged in the next great race.

In 2008, scientists began trying to turn the new reprogrammed cells into all of the building blocks doctors might use to treat a multitude of diseases. Cardiac cells to repair a damaged heart. Insulin-producing cells to help diabetics. Photo receptor cells to restore lost vision.

The work would be crucial if stem cells were to fulfill their promise and begin a new wave of medicine.

Duncan and Si-Tayeb were trying to become the first scientists to use the new technology to make liver cells. They hoped the liver cells would someday help patients with a relatively rare form of inherited diabetes called MODY (mature onset diabetes of the young). Reprogrammed cells from MODY patients could provide a microscopic view of the disease as it progresses and give scientists a target for drug testing.

The stakes were high for Si-Tayeb, still early in his career and dreaming of a big scientific paper with his name on it.

At night, Duncan lay awake worrying. When he did drift off to sleep, sometimes he dreamed of work, the anxiety flowing through him, waking him with a jolt. What if their analysis was flawed? What if while they worried and double-checked, another scientist published the same discovery? As much as he wanted to be first, Duncan vowed no corners would be cut.

"Rigor in science is everything," he said. "Without it you have nothing."

Their dilemma was now the dilemma of many in the field, an illustration of how a major advance alters the scientific landscape.

Since the cell reprogramming discovery in November 2007, the stem cell field has been moving "with a breathtaking speed," Rudolf Jaenisch, of the Massachusetts Institute of Technology, told a packed conference hall in Philadelphia.

The sense of excitement was palpable as he addressed the International Society for Stem Cell Research in June, but so too was a nagging unease. Researchers worried that the rapid pace might have unintended consequences: flawed papers raced into publication without sufficient review; overseas clinics luring patients with unproven treatments advertised on the Internet.

Although dozens of labs were reprogramming cells, scientists had not devised a set of standards for them. They still haven't. They cannot say that the new cells made in one lab are the same as those made in another. Nor can they say whether reprogrammed cells behave the same as embryonic stem cells. Is a liver cell made through reprogramming the same as one made from an embryonic stem cell, and how do both compare to a liver cell inside the body?

"We're entering a new era here," said Lawrence Goldstein, director of the Stem Cell Program at the University of California, San Diego.

Despite the questions, researchers have embraced reprogramming for the opportunity it offers to peer into areas once inaccessible to science. Last year Goldstein began collecting skin samples from patients with Alzheimer's disease, the illness that afflicts his mother. By using the new technique, he hopes that for the first time he will be able to study and test brain cells of actual human patients.

Until now, researchers have been largely forced to simulate Alzheimer's in rats and mice, or view human neurons after a patient dies.

"You're looking at the plane crash after the plane hits the ground," Goldstein said.

By the time a person dies, he explained, cells have reached an advanced state of disease, well past subtle changes that might signal the onset of illness. Identifying those subtle signs may be crucial to developing timely treatments. By reprogramming cells back to the embryonic state and growing them into neurons, Goldstein should have the chance to watch cells change from healthy to abnormal.

The same powerful idea swept through the stem cell field in 2008. At the end of 2007, Jaenisch and his colleagues used the new technology to

rescue mice with sickle cell anemia. They reprogrammed cells, corrected a genetic defect, then matured and transplanted the corrected cells back into the mice. The Jaenisch team later performed a similar experiment that quelled symptoms of Parkinson's disease in rats.

In August a team from Harvard announced the creation of reprogrammed cell lines for 10 different conditions, including Down syndrome, diabetes, Parkinson's and muscular dystrophy. The university said it was creating a cell bank for academic researchers and would add between 50 and 200 new cell lines a year.

Late in the summer, another Harvard team turned pancreatic cells inside a mouse into the insulin-producing cells destroyed in Type 1 diabetes. The experiment was remarkable because it suggested that scientists may be able to change cells directly from one type to another without ever sending them back to the embryonic state.

Then, in December, scientists at the University of Wisconsin announced in the journal Nature that they had reprogrammed skin cells from a young boy with spinal muscular atrophy, providing the first demonstration that the technique can be used to view actual cells under attack from disease.

Most reprogramming last year was accomplished by viruses delivering genes into cells, underscoring one of the major caveats to the new research. The reprogrammed cells that have entered labs so rapidly are likely years away from entering human patients. The techniques used to create them - viruses carrying genes - could cause cancer in humans and alter the genome.

As a result, scientists have been racing to develop safer methods. Some searched for viruses that won't integrate into cells, others for ways to eliminate the viruses altogether.

Before the year was out, Yamanaka had reported a method of reprogramming cells without a virus.

Last January, a pinkie-sized vial of human skin cells arrived at the Medical College of Wisconsin, shipped in a polystyrene box cooled with dry ice. By the end of the month, Si-Tayeb had launched his first attempt at reprogramming. Just as Thomson and Yamanaka had done, he used a virus to deliver the four reprogramming genes to the cells.

In mid-March he checked the lab dishes and found a scattering of dense colonies - a hallmark of embryonic stem cells and of the reprogrammed cells that mimic them. OK, he told Duncan, I think we have it.

These were the lab's first reprogrammed human cells. One of Duncan's graduate students, Fallon Noto, had already reprogrammed mouse cells and was trying to grow an entire mouse liver from them.

Si-Tayeb checked the new cells to make sure they displayed the correct characteristics. Next he would try to turn the reprogrammed cells into liver cells called hepatocytes. He and Duncan hoped to be ready to publish their results in the fall.

In just a few months, reprogramming had assumed a major role in the lab.

"The landscape has suddenly changed," Duncan said, comparing the revolution in stem cells to an earlier age of discovery. "It's like Columbus. One person sails over the ocean, and it opens up a brand new horizon."

By June, Si-Tayeb had grown what appeared to be liver cells. Under a microscope, liver cells look different from primitive embryonic stem cells or the reprogrammed equivalent. Liver cells are bigger. They don't

form colonies like embryonic stem cells. And tiny junctions extend between the liver cells. Si-Tayeb saw these traits.

But that wasn't the end of the experiment; it was, in fact, the beginning.

"You have to make sure what you're seeing is real," Si-Tayeb said.

Even as the pace of stem cell discovery accelerated, Duncan insisted his lab not speed toward publication without performing the necessary controls and additional experiments. Through the summer the scientists continually asked themselves why they might be seeing each result. Si-Tayeb tracked the different stages of liver development and checked for the genetic markers that distinguish a liver cell from all others.

In August Si-Tayeb, Noto and Duncan flew to Snowmass Village, Colo., for a conference on liver research. Si-Tayeb and Noto both presented posters showing the data they had collected on their separate projects. Duncan received good feedback from other scientists - and a suggestion. They were presenting their data essentially as three stories: making a mouse liver with the reprogrammed cells, turning human embryonic stem cells into liver cells, and finally using reprogramming to make human liver cells.

"I have this idea," Duncan told Noto as they hiked up a mountain in Colorado. "It might be crazy, but I think we can put these stories together. It will have more impact."

The idea of being one of the principal authors of a major paper thrilled Noto, who was only in her third year of graduate studies in cell and developmental biology. The prospect of combining the work excited Si-Tayeb, too. He would be the first author on a paper that promised to make a major contribution to the study of liver development.

But the change also raised an old fear. The more research the paper reported, the more the scientists would need to verify. The more they went back and verified, the longer the whole project would take. Other scientists were bound to be doing similar work. The team at the Medical College would have to hope no one else was ready to publish.

To show that the reprogrammed liver cells would be accepted into a body just like the real thing, Si-Tayeb injected them into very young mice - injecting any reprogrammed cells into humans was likely years from receiving approval. Still, he could use the mouse as a model and see whether the human cells would engraft onto the mouse liver.

Si-Tayeb figured out a way to mark the human cells so he would see a dark red mark where they had been injected into the mouse liver. In November, when he examined the tiny liver of a baby mouse under the microscope, there it was: a deep red patch inside the pale pink liver.

Si-Tayeb was cautious. Now he had to prove his results at the cellular level. He searched for a protein distinctive to human liver cells. Once he found it, he looked for another distinctive protein.

Meanwhile Noto went through a complex, laborious procedure to show that the reprogrammed mouse cells could make an entire liver. To do this, she would actually have to produce not only a liver, but also an entire embryo made from reprogrammed cells.

By late October, Duncan knew they would be hard-pressed to publish before the end of 2008. Having reviewed research by other scientists many times, he tried now to focus the same critical eye on his own lab's work. He kept asking what was missing.

"Closing," he said, "is the worst part of it. All you're doing is dotting I's and crossing T's."

Usually such work doesn't change conclusions, it bolsters them.

The controls would provide a vital comparison, a way to show without any doubt that human liver cells were present in the mouse liver and that they could be distinguished from mouse cells.

In November, Duncan learned that other groups were at work on similar projects. He sat down with Si-Tayeb and together they outlined their paper. Duncan began writing.

But there were still final scientific loose ends to be resolved. Noto had to run a check to show that contamination had not marred her reprogrammed cells or the livers she had made from them.

Si-Tayeb had checks of his own to perform. Liver cells had engrafted onto the mouse livers, but he needed to be sure they were the reprogrammed human cells. So he set about detecting human DNA inside the mouse.

On the day before Christmas, Si-Tayeb went through one lab dish after another, searching for places where cells had grown too dense and moving some - a necessary process not so different from the way dense populations in a city migrate to the suburbs. When cells become overgrown, they leave their delicate, embryonic state.

On Christmas Day, Si-Tayeb and Noto returned to the lab to change the cells' nutrient material.

"I have to eat every day," Noto said. "My cells have to eat every day. Science doesn't take holidays."

Si-Tayeb was exhausted.

He had not taken a break in a year. No holidays away from the lab. No weekends.

After Christmas, he planned to start a vacation of sorts.

He knew that on New Year's Eve he would be back in the lab. And January would be crazy. The lab had a paper to submit. There were long days of writing ahead of him.

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