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Stem Cell Technology Enables New 'Disease in a Dish' Models of Brain Disorders

For some brain diseases, the lack of good laboratory models makes it hard for researchers to understand disease-causes and develop therapies. New stem cell technology offers a powerful solution.

BY JIM SCHNABEL

Stem cell therapies for disease are still mostly over the horizon. But stem cell technology is already boosting scientists' abilities to study diseases and test drugs against them. The technology is proving particularly useful for brain disorders that are hard to model in animals.

Using an "induced pluripotent stem cell" (iPS) technique first reported for human cells only in 2007, scientists now can take skin cells from patients, convert them to iPS cells and then neurons in a lab dish, and study the patient-derived neurons to gain insights into disease mechanisms and therapies. Two recent reports show the power of the "brain-in-a-dish" technique.

"We're going to see a lot of these papers coming out," says Clive Svendsen, director of the Regenerative Medicine Institute at Cedars-Sinai Medical Center in Los Angeles.

Modeling Schizophrenia

Schizophrenia features abnormalities in some of our more evolutionarily advanced neural circuitry, including weak connectivity among the prefrontal and temporal cortices and the hippocampus. The causes of schizophrenia are not well understood: Although it is largely genetic, its known genetic risk factors are varied, and mostly subtle. The disease is often described as being caused by "many rare mutations" that somehow feed into a final common pathway of vulnerability. There is no good animal model.

In a study published online in Nature on April 13, researchers led by senior investigator Fred H. Gage at the Salk Institute took skin cells from four different people with schizophrenia. Using the iPS technique, they reprogrammed the patients' skin cells to act like fetal stem cells—then used a biochemical recipe to "differentiate" these into neural progenitor cells, which proliferated and produced mature neurons.

The technique produced a large supply of neurons per patient, and as newborn neurons will, they spontaneously formed clusters that connected to each other. Gage's team thus was able study in detail how these schizophrenia-patient-derived neurons were different from neurons that had been derived from healthy people using the same iPS technique.

Comparing gene expression patterns, for example, they found in the schizophrenia neurons 596 genes that, on average, were expressed at least 30 percent more than normal or 30 percent less than normal. "When we looked at the categories of functions for these genes, we found certain pathways that stood out quite strongly, which gives us new leads about what may be going on in schizophrenia," says Gage, who also is a member of the Dana Alliance for Brain Initiatives. The pathways disturbed in the schizophrenia neurons included those controlling neuronal growth and the maintenance of synapses—the interfaces at which neurons communicate. In one-quarter of cases, the gene expression changes in the schizophrenia neurons confirmed changes known from previous research.

Gage's team also was able to quantify how connected the neurons were to one another. On average, the schizophrenia neurons had only about half the connectivity of normal neurons, a difference that corresponded to some of the changes seen in gene expression.

When the researchers treated the cells for 20 days each with five standard schizophrenia drugs, they found that each drug improved connectivity and gene expression patterns—moving them in the direction of normality—for at least some patients' iPS-derived neurons. One drug, loxapine, significantly boosted connectivity for all four sets of neurons. "And these were all different and quote-unquote sporadic cases of schizophrenia," Gage notes. "The fact that one of the compounds could generally increase the connectivity was quite impressive to us."

Only three earlier studies of human-iPS-derived models have been published,

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and each involved relatively simple comparisons of survival/degeneration between patient-derived and control neurons. Gage's group's techniques had to be more sophisticated to detect the more subtle neuronal disturbances at the heart of schizophrenia. One such technique permitted the tracing of connections among neurons using a modified rabies virus. Rabies viruses evolved to "swim" upstream along nerve fibers and across synapses, hopping from neuron to neuron until they get to the brain. In this case, Gage's group, using a method developed elsewhere at the Salk Institute, created a rabies virus that lacks a key protein, forcing it to stop after a single hop. Because the modified rabies viruses expressed a fluorescent tracer protein, their movements—and thus the neurons' connections—could be tracked.

Gage says the set of techniques used in this study could be applied to other brain diseases in which researchers suspect abnormal connectivity. But he also wants to refine the techniques to glean more insights about schizophrenia. "We're developing protocols to differentiate these cells specifically into hippocampal neurons versus cortical neurons, so we can apply the same basic analysis using more specific neuronal types," he says. With these more sophisticated techniques and a more diverse set of patient-derived neurons, he hopes the deep biology of schizophrenia will eventually become much clearer. "We're looking for a core program that underlies the disease," he says. "Perhaps not 596 gene expression alterations, but some subset. This is not a single-gene disease."

Modeling Spinal Muscular Atrophy

One of the first clinical successes enabled by the iPS-modeling technology could be for spinal muscular atrophy (SMA). An iPS-derived model of SMA was reported in 2009 by Svendsen's lab and the lab of stem cell researcher James Thomson.

SMA is a childhood recessive genetic disease in which both parental genes for the protein SMN are defective. The deficiency of SMN—for reasons that haven't been understood—causes spinal muscle-controlling neurons to degenerate. In severe cases, SMA can proceed from the first symptoms at the age of about six months to fatal paralysis a year and a half later. "It's horrific for parents," says Svendsen. "There's nothing they can do; there's no cure for it."

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Svendsen's and Thomson's labs converted fibroblasts from a single SMA patient into motor neurons like those affected in the disease, and were able to show that the neurons underwent an SMA-like process of degeneration, compared with neurons generated from fibroblasts taken from the patient's healthy mother. "In effect we were able to replay the disease," says Svendsen. "The motor neurons were born and they were fine for a few weeks, and then they underwent degeneration, so we actually watched them die."

Since that initial demonstration, Svendsen and Thomson have developed and studied neuronal lines from other SMA patients, and Svendsen says they have found changes in the neurons that may account for their degeneration when deprived of SMN. He and Thomson are about to submit their findings for publication. "We're also getting closer to thinking about rational drug design based on the mechanism of these motor neuron deaths in the kids," he says.

Tomorrow's Must-Have Lab Tech

Svendsen expects a flood of iPS-model papers to be published in the next few years, particularly for the strongly genetic, early-onset diseases like SMA that seem best suited to the technique. The iPS induction process resets cells to something like a newborn state, and seems to erase many of the cellular changes that occur with aging and environmental stresses. "I always look at iPS models as developmental models essentially," he says. "Because you're taking the cell back in time and then making it undergo development again."

For this reason, he was surprised that Gage's group found such clear connectivity deficits in iPS-derived neurons from patients with schizophrenia—a disease that normally doesn't manifest until late childhood. "But if that [result] reproduces in more neuron lines, it'll be a very nice way of looking at the mechanism of cell connections in schizophrenia," he says.

Later-onset diseases such as Parkinson's, Alzheimer's, and Huntington's could be more difficult to model in neurons generated by the iPS technique. But researchers might still generate useful models for these disorders if they can find a way to artificially age or otherwise stress the cells, to see whether disease-derived neurons are more vulnerable than control neurons. "Many groups are looking at the possibility of adding toxins to the cell cultures, for example," Svendsen says.

He is mindful that these are only lab-dish models. "We're not going to do away with whole-animal models," he says. But the technology is potentially valuable enough that he expects it to spread widely in academic and commercial labs, replacing older cell-based disease modeling techniques. "Eventually I think if you're going to study a human disease or look at a related pathway or a system, you'll be expected to grab an iPS line from that disease model if it's available," says Svendsen.

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