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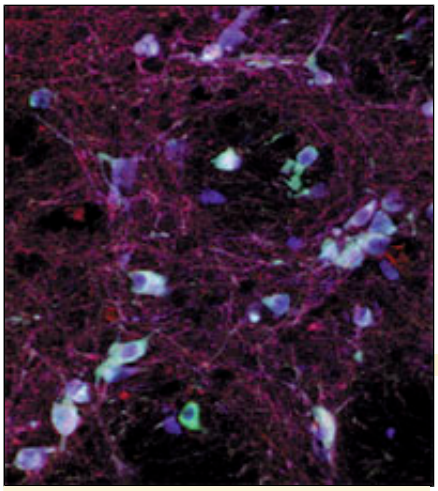
Embryonic Stem Cells Work

Dopaminergic neurons compensate for loss in Parkinson animal models | By Maria W. Anderson



HALF A MIND: Lesioned brains 16 weeks after embryonic stem cell transplant stain positive for tyrosine hydroxylase (TH: green), dopamine transporter (DAT: red), and aromatic amino acid decarboxylase (AADC: blue) in an overlaid image. Bar equals 25 µm.

Since drawing the attention of scientists, ethicists, and policymakers, stem cells have not lost their place as one of the most promising yet controversial scientific discoveries of the 20th century. Scientists admit that they still know very little about how these undifferentiated pluripotent precursors work, but they're looking to develop a more sophisticated view, one that will unlock stem cells' therapeutic potential. Many regard embryonic stem cell (ESC) transplant as the ideal therapy for treating conditions such as Parkinson disease and diabetes, since they could replace malfunctioning cells, but so far the results in animal models have been mixed. Indeed, some groups have published different interpretations of seemingly similar studies.

In this issue's Hot Papers, two research teams demonstrated that they could guide murine ESCs to become functional dopaminergic neurons in rats with an induced form of Parkinson disease. In February 2002, Ole Isacson, Lars Björklund, and colleagues at Harvard Medical School published a study in which they injected small quantities of undifferentiated mouse ESCs into the striatum of rats with Parkinson-mimicking brain damage. Of 25 animals treated, grafts did not survive in

six, and five had grafts that developed into tumors, or teratomas, due to uncontrolled growth. But in 14 rats, cell grafts survived and became dopaminergic.[1]

Five months later, Ron McKay's team at the National Institute of Neurological Disorders and Stroke (NINDS) described a method of converting ESCs to dopaminergic neurons in culture before transplanting them into a Parkinson rat model.[2] Both groups tested dopaminergic neuron regrowth by measuring recovery of amphetamine response in damaged parts of the brain.

These papers were the first to test whether ESCs could live up to the hype. "The Parkinson's model has been regarded as the number-one model system to test whether stem cells will fulfill their potential or not," says University of Wisconsin neurologist Su-Chun Zhang. Together these papers show that ESCs could differentiate into functional neurons and could be used to treat disease.

But each paper has its caveats, according to Lorenz Studer, a developmental biologist at the Sloan-Kettering Institute in New York and a former postdoc in McKay's lab. Isacson's study demonstrated that ESCs can differentiate and function in vivo, but controlling tumorigenic growth is a hurdle that has yet to be cleared. McKay's group prevented tumorigenesis by differentiating the ESCs outside of the brain, but the transgenic techniques they used might not pass muster for clinical trials in humans.

For this article

Data derived from the Science Watch/Hot Papers database and the Web of Science (Thompson ISI) show that Hot Papers are cited 50 to 100 times more often than the average paper of the same type and age.

L.M. Björklund et al., "Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model," *Proc Natl Acad Sci*, 99:2344-9, 2002. (Cited in 117 papers)

J.-H. Kim et al., "Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease," *Nature*, 418:50-5, 2002. (Cited in 134 papers)

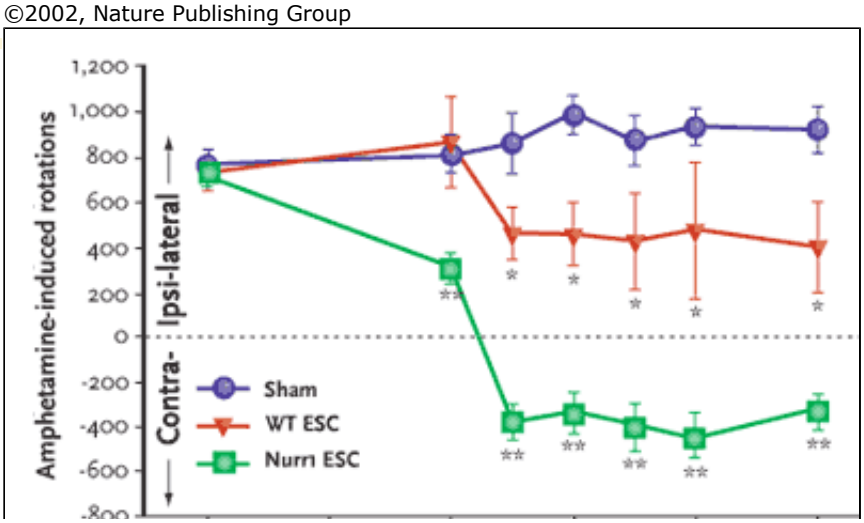
IN VIVO In 1996, Isacson's group showed that they could convert ESCs into beating heart cells and nerve cells by treating them with retinoic acid and dimethyl sulfoxide;[3] but the nerve cells produced weren't dopaminergic and couldn't be used in Parkinson studies. Three years later, he showed that ESCs have a default pathway: They become nerve cells whether implanted in the brain or under the kidney capsule.[4]

Although the exact biology behind this is unclear, he says, it may involve bone morphogenic protein signaling.

In his Hot Paper, Isacson transplanted low concentrations (1,000-2,000 cells) of blastocyst-derived ESCs so they wouldn't respond to cues from each other but from their surroundings, which would direct them to become dopamine cells. Three in four surviving grafts became neuronal.[1] "They have what we call a complete setup," Isacson says. "They have tyrosine hydroxylase, dopamine transporter, and aromatic amino acid decarboxylase, and that's all the enzymes you need to make a really functional dopamine neuron."

To induce Parkinson disease, Isacson's team had destroyed dopamine neurons in half of the brains in rats. An injection of amphethamine induces a response only in the intact neurons, causing the rats to run in circles. After transplantation, they observed a gradual reduction in these amphetamine-induced rotations, which indicated that the ESCs had become dopaminergic and were compensating for the disrupted nerves. "[The grafts] completely re-innervated this unilateral Parkinson model," Isacson says. "The fibers were growing into the brain, and we had behavioral recovery and also MRI scans that showed that the cells had integrated."

IN VITRO McKay, a cell biologist interested in the intricacies of the human brain, had previously worked on stem cells as well. His NINDS team developed a five-step protocol to derive a range of cells, not including dopaminergic neurons, from human ESCs in vitro in 1996.[5] In 1998, they differentiated rat neural stem cells into dopamine cells, implanted them in Parkinsonian rats, and achieved functional recovery.[6] They used their five-stage differentiation process again in 2000 to derive a range of midbrain and hindbrain neurons, including dopamine cells, from mouse ESCs.[7]



THE RUNAROUND: This figure shows the analysis of amphetamine-induced rotations in animals grafted with wild type or Nurr1 embryonic stem cells(ESCs) and sham treated animals. Rats treated with Nurr1 transfected ESCs actually reversed the direction of rotation. *= P<0.05, **= P<0.001. (from *Nature*, 418:50-5,2002)

In their Hot Paper, they used the protocol to direct ESCs to become embryoid bodies, then neural precursors, and eventually dopaminergic and serotonergic neurons in culture. McKay explains that they used "appropriate morphogenic treatments," including FGF8 (fibroblast growth factor 8) and sonic hedgehog (a signaling molecule), to control cell differentiation. To increase the percentage of dopamine cells, they inserted a transgene, the Nurr1 growth factor, previously shown to be essential for dopaminergic-neuron development. When they injected the rats' brains with amphetamine, they observed that the implanted cells not only reduced the rotations but actually overcompensated, causing the rats to start turning in the opposite direction.

While his paper provided data on behavioral recovery, McKay insists that the biology behind those data is the paper's most important contribution. "The real result is not the animals. ... That's just telling you that you got the expected outcome," he says. "The control of the differentiation, that's the real result."

According to McKay, Isacson's study focused too much on the clinical outcome and not enough on the basic science behind it, but others disagree. Studer says that although Isacson's therapeutic effect was not as robust as McKay's, his ability to get efficient dopamine neuron generation "without adding anything" was quite remarkable.

IN HOMO Despite the rats' clinical improvement in both studies, researchers find drawbacks to each approach. Zhang says that stem cell transplants to the brain shouldn't be applied in humans. Directing them to become the desired cell type is more appropriate, but there are problems with using genetics as the guiding force. Studer agrees that transgenes could disrupt the genome and cause undesirable side effects, such as oncogenesis. Preferably, scientists would use epigenetics to guide the cells, says Zhang.

But McKay doesn't see that as a problem. "The judgment is not whether it's genetically manipulated or not, but whether it works," he says. Neurobiologist Jeff Kordower at Rush University in Chicago concurs: "We are already genetically modifying things in humans. Why not genetically modify these cells?"

Researchers do agree, however, that McKay's study provided a strong proof of principle that ESCs can differentiate into functional neurons and can be used to treat disease. "I don't think anyone doubts the findings of these studies, especially McKay's," says Studer. "They really opened up the field to the medical community."

The question remains whether ESCs will ultimately work better than fetal tissue implants, says Studer. Recent trials showed that patients with Parkinson disease did not benefit from receiving fetal nerve tissue transplants; indeed, some patients experienced jerky involuntary movements known as dyskinesias.[8] These studies have cast a cloud over all cell therapies, including those involving ESCs. "Some neurologists say that cell therapy, no matter what kind of cell you're using, will never work," Studer explains.

Both Studer and McKay say that several groups have unpublished data showing that they can efficiently derive dopamine neurons from human ESCs in vitro, but so far no one has been able to get those neurons to function in vivo. Controlling cell growth is still a huge problem, too, says Kordower, but one that can be overcome. Curt Freed, director of the University of Colorado's Neural Transplant Program for Parkinson's Disease, agrees: "These papers have shown the feasibility of converting embryonic stem cells to dopamine neurons. They are far from a direct line to human therapy, but they are a start."

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