Stem Cell Recipe for Astrocytes

26 May 2011

Researchers at the University of Wisconsin-Madison have cooked up a method to nudge pluripotent stem cells all the way to astrocytes. The new protocol, published online May 22 in Nature Biotechnology, means researchers can better model the astrocyte contributions to neurodegenerative diseases. Mimicking the natural developmental recipe for these star-shaped neuron supporters, the scientists created astrocytes that swallow glutamate, encourage synapse formation, and whip up a blood-brain barrier, just like the nature-made versions do.

Senior author Su-Chun Zhang and first author Robert Krencik, who studied in the Zhang lab but has since moved to the University of California, San Francisco, led the effort. Zhang, along with others, figured out how to turn stem cells into neurons nearly a decade ago (Zhang et al., 2001). But astrocytes have proved more elusive. With several studies pointing to astrocytes as participants in neurodegenerative diseases such as amyotrophic lateral sclerosis, researchers needed a way to grow their own.

Krencik used human starting material, either embryonic stem cell (ESC) or induced pluripotent stem cell (iPSC) lines. He applied standard protocols to shepherd them through the neuroepithelial and into the neural progenitor stage, then figured out how to make them into astrocytes. Three key factors allowed the group to succeed, Zhang told ARF: patience, chemically defined media, and regular disruption of clumped-together cells.

During human development, Zhang pointed out, astrocytes appear three months after neurons. Hence, the researchers waited out two months of culture, and at that point the progenitors started making more astrocytes than neurons.

Although serum works to feed and produce mouse astrocytes, it impedes the process in human cells, Zhang said. Again, natural biology provided the clue: “In the brain, you do not need serum,” he said, adding that because of the blood-brain barrier, the brain actually “hates” serum. If the culture is contaminated with any cells that do prefer serum, he said, those cells could take over the population. Thus, carefully defined chemical media proved to be the way to go.

The progenitors tend to make neurons when clustered together, but are more likely to switch to glia when single cells float alone in suspension. Therefore, Krencik dissociated the cells in the growing cultures every week. Finally, he plated individual cells and added growth factors to help them continue developing as astrocytes. Adding particular growth factors produced different astrocyte subtypes.

To measure his success, Krencik looked for, and found, every astrocyte marker he could think of. Further, he checked that the cells can carry a current but do not produce action potentials as neurons do. Their glutamate receptors and transporters were operable, and they propagated calcium waves like proper glia do. When co-cultured with neural progenitors, the astrocytes nursed the formation of synapses between developing neurites.

In a final experiment, Krencik implanted the immature astrocytes into the lateral ventricles of

neonatal mice. The human cells not only survived, but also reached out to encircle blood vessels, suggesting they helped form the blood-brain barrier. The implanted cells lasted for at least six months, with no evidence of overproliferation or tumor formation, Zhang said.

François Berthod of the University of Laval in Québec, Canada, wrote in an e-mail to ARF that the functional test results were convincing. However, he noted, the researchers started with standard cell lines. What many researchers would like to do, and are already doing with neurons, is to use patient-derived iPSCs to make disease-specific cell lines for drug screening and other experiments (see ARF related news story). It remains to be shown that a person's cells, turned from fibroblasts to iPSCs to astrocytes, will still exhibit pathology, he wrote.

The study also shows that making these kinds of cell lines will be a slow process, said Nicholas Maragakis, who called the timeline "sobering." Maragakis works at the Johns Hopkins School of Medicine in Baltimore, Maryland. Obtaining statistically significant results will require hundreds of patient-specific cell lines. "It takes a long time to generate a cell line; that is something as a community we are going to have to face," he said.

Some scientists are already testing transplantation of stem cell-derived lines as a therapeutic for amyotrophic lateral sclerosis (see ARF related news story). Although he was careful to call astrocyte transplants "purely speculative," Berthod noted that the protocol might, in the future, allow doctors to collect a person's cells and create customized astrocytes for grafts.
—Amber Dance

COMMENTS

MAKE A COMMENT

Comments on News and Primary Papers

François Berthod
LOEX, Université Laval

Posted: 26 May 2011

Paper: Specification of transplantable astroglial subtypes from human pluripotent stem cells.

This work is very interesting because it shows that astrocytes can be successfully differentiated from human iPS cells (ES cells being less relevant, in my point of view). The paper is mostly oriented toward showing that astrocytes are functional. This is convincingly shown both in vitro and after graft in mouse brain.

Generating functional astrocytes from human iPS cells will be very valuable for fundamental studies to better understand their development and function in vitro and in vivo. However, the cells in this study were obtained from cell lines. To bring this technology to the study of ALS, this work needs to be done using the patient's cells. This should be quite feasible, starting from a small skin biopsy, to generate iPS cells from fibroblasts. The next step is more challenging: to show that the ALS phenotype of astrocytes (still largely unknown) will be re-expressed in these cells even though they derived from iPS cells. In other words, it is not clear at this point if a patient-derived fibroblast transformed into an iPS cell, and then differentiated into an astrocyte, will express the same disease characteristics than a native astrocyte from the patient's spinal cord. Demonstrating this re-expression of the ALS phenotype from iPS cells would be a major breakthrough.

Another potential approach could be a cell therapy, using the patient's own cells to generate iPS cells and astrocytes in order to graft back these autologous astrocytes in the patient brain or spinal cord. However, such an approach is purely speculative, since there is no clear evidence up to now that it could be beneficial, at least for ALS.

In conclusion, this work is an encouraging step in the long process to develop in-vitro models of ALS and, perhaps one day, a cell transplantation therapy (which is pure speculation today).

View all comments by François Berthod

Make a Comment

To make a comment you must login or register.

REFERENCES

News Citations

Where in the World Are the iPS Cells? 15 Sep 2010

Honolulu: So Far, So Good in Stem Cell Safety Study for ALS 15 Apr 2011

Paper Citations

Zhang SC, Wernig M, Duncan ID, Brüstle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*. 2001 Dec;19(12):1129-33. PubMed.

FURTHER READING

Papers

Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature*. 2011 Jun 9;474(7350):212-5. PubMed.

Tomaskovic-Crook E, Crook JM. Human embryonic stem cell therapies for neurodegenerative diseases. *CNS Neurol Disord Drug Targets*. 2011 Jun;10(4):440-8. PubMed.

Abdel-Salam OM. Stem cell therapy for Alzheimer's disease. *CNS Neurol Disord Drug Targets*. 2011 Jun;10(4):459-85. PubMed.

Chen C, Xiao SF. Induced pluripotent stem cells and neurodegenerative diseases. *Neurosci Bull*. 2011 Apr;27(2):107-14. PubMed.

Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci*. 2007 May;10(5):615-22. PubMed.

Di Giorgio FP, Carrasco MA, Siao MC,

Maniatis T, Eggan K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci*. 2007 May;10(5):608-14. PubMed.

News

Where in the World Are the iPS Cells? 15 Sep 2010

Not All Stem Cells Are Created Equal: Immune Rejection of iPSCs 20 May 2011

Stem Cells Pass Initial Safety Muster in ALS, Multiple Sclerosis 29 Oct 2010

Québec: Motor Neurons from Stem Cells—Really, Truly? 5 Oct 2010

Cellular Alchemy: Turning Astroglia Into Neurons 27 May 2010

Rewriting Cellular Destiny: Science Magazine's Breakthrough of 2008 24 Dec 2008

ALS in a Dish? Studying Motor Neurons from Human Stem Cells 6 Dec 2008

Glia—Absolving Neurons of Motor Neuron Disease 23 Apr 2007

Honolulu: So Far, So Good in Stem Cell Safety Study for ALS 15 Apr 2011

PRIMARY PAPERS

Krencik R, Weick JP, Liu Y, Zhang ZJ, Zhang SC. **Specification of transplantable astroglial subtypes from human pluripotent stem cells**. *Nat Biotechnol*. 2011 Jun;29(6):528-34. PubMed.