Not all embryonic stem cell lines are created equal

August 6 2007

When it comes to generating neurons, researchers have found that not all embryonic stem (ES) cell lines are equal. In comparing neurons generated from two NIH-approved embryonic stem cell lines, scientists have uncovered significant differences in the mature, functioning neurons generated from each line. The discovery implies that culture conditions during ES cell generation -- which have yet to be identified -- can influence the developmental properties of human ES cells.

The report, which was published August 6, 2007, in the early online edition of the *Proceedings of the National Academy of Sciences*, also describes a new technique for producing functioning neurons from stem cells that will be important for creating models of human neurodegenerative diseases.

The research team was led by UCLA stem cell biologist Yi Sun and Howard Hughes Medical Institute investigator Thomas Südhof at the University of Texas Southwestern Medical Center at Dallas.

Embryonic stem cells are developmentally immature cells that are capable of self-renewal and of differentiating into any type of tissue in the body. Researchers believe they hold the potential for generating neural, cardiac and other cells that can be implanted to restored damaged tissue.

“To the best of my knowledge, until now there have been few functional studies of the neurons derived from embryonic stem cells,” said Südhof.

“People in the field have traditionally been interested in whether they can make neurons and what molecular markers characterize those neurons. However, because different embryonic stem cell lines were derived under diverse conditions, the possibility existed that cell lines would produce neurons with distinct properties.”

The researchers compared mature neurons grown from two embryonic stem cell lines approved for research by the National Institute of Health. Sun and her colleagues developed procedures to differentiate the two stem cell lines first into neural progenitor cells, and then into mature neurons. They were also able to purify those neurons for study.

To probe how the neurons functioned, the researchers developed a culture technique that induced the newly produced neurons to establish synapses with one another. Synapses are the critical junctions between neurons where much of the signaling and communication between neural cells occurs.

Through functional analyses of these neurons, Sun, Südhof and their colleagues found that the two ES cell lines differentiated into two distinct types of neurons that are actually found in different parts of the brain.

The researchers next performed electrophysiological studies of the synaptic connections between the neurons. “We found that the neurons derived from the two cell lines have completely different properties in terms of what type of synapses they develop and at what time course this happens during culture,” said Südhof. Furthermore, the studies showed that the neurons derived from the two cell lines used different chemicals called neurotransmitters to communicate with one another, he said.

Sun and her colleagues compared the microRNAs produced by the two types of neurons. MicroRNAs are small snippets of genetic material that

are believed to be significant regulators of stem cell differentiation.

“It’s been proposed that microRNAs might be part of the defining signatures for human ES cells, and many are expressed in the brain,” said Sun. “It was comforting that our analysis showed that as the ES cells matured into neural progenitors and neurons, the expression of the microRNAs genes specific to ES cells dropped thousands of times, and those specific to brain cells increased thousands of times. But on the other hand, when we compared the two lines, we found differences in microRNA gene expression that might contribute to this neuronal bias in the lines,” she said. Südhof said that the differences among ES cell lines could have implications for potential treatments using the cells.

“It’s clear that if you’re going to treat a motor neuron disease, you need those types of neurons; whereas if you want to treat a forebrain disease like Huntington’s, you need ES cells that differentiate into that type of neuron,” he said.

The differences in neurons produced by cell lines may offer both advantages and disadvantages for treatment, he said. “On the one hand, it may actually be good to have ES cells with a particular propensity for differentiation, because it may make it easier to get certain types of tissue. On the other hand, it may also limit the ability of these ES cells to fully replicate those types of tissues.”

Sun said that her technique for differentiating ES cells into mature neurons is likely to have important future research applications. “This technique enables us to produce pure cultures of functioning human neurons that we can genetically manipulate to mimic human disorders,” she said. “Before, it was only possible to use mouse or other animal cells to model neurodegenerative diseases, but the genetic background is so different from that of humans that key aspects of diseases such as Alzheimer’s could not be reproduced.”

Both Sun and Südhof said that their findings have implications for the production of ES cell lines. “There is absolutely no question that these findings mean that there need to be more embryonic stem cell lines for research purposes and for use in potential treatments,” said Südhof.

Sun said that developing more ES cell lines is important “because right now we still don’t know the causes for the functional differences we found. Understanding the causes will require more cell lines for study. And once we understand the causes, we can take them into account in generating new cell lines that will be better defined and enable more reproducible applications.”

Source: Howard Hughes Medical Institute