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STEM CELLS

Intrinsically different

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Generating functional neurons from human embryonic stem cells (hESCs) with the aim of treating neurodegenerative diseases is the subject of intensive investigation. In a new study, Sun and colleagues describe a method to produce homogeneous cultures of neurons from hESCs, and demonstrate that different hESC cell lines have distinct differentiation properties.

hESCs are self-renewing and pluripotent — that is, they are capable of unlimited proliferation and can differentiate into any type of cell in the body — and they thus hold tremendous potential for regenerative medicine. Most published protocols for guiding the differentiation of these cells result in heterogeneous cultures that comprise neurons, glia and progenitor cells, which makes the assessment of neuronal function problematic. However, the authors of this study were able to purify enough hESC-derived neurons to carry out gene expression and protein analyses and examine whether they can form functional networks in culture.

First, the authors induced the differentiation of hESCs into human neural progenitor cells (hNPCs) using embryoid body formation or matrigel-supported monolayer cultures. They then used basic fibroblast growth factor (bFGF) to expand the hESC-derived hNPCs in an undifferentiated state. Next they removed the bFGF and supplemented the medium with brain-derived neurotrophic factor and neurotrophin 3, in order to induce neuronal differentiation. Finally, by using the cystein protease papain and a cell drainer, the authors were able to dissociate differentiated neurons into single cells and separate them from hNPCs, which remained aggregated. Immunocytochemical analyses indicated that typically 70–80% of the cells in these cultures were neurons.

Interestingly, when two different United States National Institutes of Health-registered hESC cell lines were differentiated using this protocol, the resultant neurons expressed distinct markers. HSF1-derived neurons expressed the forebrain marker FOXG1B, whereas a notable proportion of HSF6-derived neurons expressed the non-forebrain marker PAX2 and the γ-aminobutyric acid signalling marker GAD67. Morever, when the cells were cultured with astrocytes to induce the formation of synapses, HSF1-derived neurons formed more inhibitory than excitatory synapses, whereas for HSF6-derived neurons the opposite was true.

As microRNA (miRNA)-mediated regulation of gene expression has been implicated in stem cell differentiation, Sun and colleagues compared the miRNA profiles of the two hESC cell lines. They found that, for 40% of miRNAs, there was a greater than twofold difference in the expression levels between the two cell lines. However, during the conversion of hNPCs to neurons, only four miRNAs exhibited a marked difference in levels. One of these miRNAs, has-miR-10a, which is expressed in the brain stem and the cervical spinal cord, was highly expressed in HSF6-derived neurons, consistent with their more posterior identity.

Why different stem cell lines are biased to become a particular type of neuron is unclear. The culture conditions during the generation of hESC cell lines might have effects on their developmental potential that have thus far gone unrecognized. However, these findings suggest that stem cell lines might be preprogrammed — probably as a result of epigenetic phenomena — at the hESC stage, and that HSF1-derived neurons might be better suited to the treatment of diseases that affect the forebrain, such as Huntington's disease, than HSF6-derived neurons. The authors advocate the generation of more hESC lines, to improve our understanding of how best to use them therapeutically.

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