Induced Pluripotent Stem Cells Generated from Patients with ALS Can Be Differentiated into Motor Neurons

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Abstract

The generation of pluripotent stem cells from an individual patient would enable the large-scale production of the cell types affected by that patient's disease. These cells could in turn be used for disease modeling, drug discovery, and eventually autologous cell replacement therapies. Although recent studies have demonstrated the reprogramming of human fibroblasts to a pluripotent state, it remains unclear whether these induced pluripotent stem (iPS) cells can be produced directly from elderly patients with chronic disease. We have generated iPS cells from an 82-year-old woman diagnosed with a familial form of amyotrophic lateral sclerosis (ALS). These patient-specific iPS cells possess properties of embryonic stem cells and were successfully directed to differentiate into motor neurons, the cell type destroyed in ALS.

One of the most intriguing possibilities of iPS is the potential to create cell lines of human diseases for scientific study. Scientists are also interested in making patient specific iPS lines to be used in cell therapies. If iPS cells are generated from a patient's own cells then there will be no risk of immune system rejection. In this study Kevin Eggan and colleagues created an iPS cell line from a patient with ALS and differentiated those cells into **neurons** and **glial** cells.

ALS (Amyotrophic lateral sclerosis), also known as Lou Gehrig's disease is a neurodegenerative disease. Motor neurons, or neurons that control movement, in the brain and the spinal cord die. ALS is a mysterious illness. There appear to be many causes, some of which have not been identified. One identified cause of the disease is mutations in a gene called SOD1. How exactly mutations of this gene causes neuronal death is not well understood. Therefore scientists are very interested in being able to study motor neurons in culture to examine why they are dying.

In this study researchers isolated fibroblasts from skin samples from 82 and 89 year old sisters who each had a rare mutation in the SOD1 gene. The fibroblasts were cultured and transfected with the four Yamanaka fragments using retroviruses. After 3 weeks they selected cells that

visually appeared similar to embryonic stem cells. They were able to obtain embryonic stem cell-like cells from both sisters' skin, however after this point the only used the 82 year-old's cells as her disease was more progressed than her sister's.

They first examined the DNA of the iPS cells and confirmed that they carried the SOD1 mutation as well as DNA inserted by the retrovirus. They next examined the expression levels of several pluripotency genes to confirm the cells were in fact iPS cells. They found several markers were expressed at levels comparable to embryonic stem cells. Next the cells were allowed to differentiate into cell types from the three germ layers in culture, also indicating they were pluripotent.

They next tested the cells to see if they could be differentiated into motor neurons and glial cells. They applied two chemical signaling molecules that are used to differentiate embryonic stem cells into motor neurons. They found that the cells differentiated. They expressed TUJ1, a gene expressed specifically neurons. 20% of all cells also expressed HB9, a motor neuron specific gene. 90% of the HB9 positive cells also expressed ISLET1/2, a gene involved in motor neuron development. Cells were also found that expressed glial cell specific genes S100 and GFAP. The researches concluded that it is possible to create patient specific iPS cells, even from patients of advanced age. They also showed that iPS cells can be successfully differentiated into specific cell types in a manner similar to embryonic stem cells.

This paper is important because it shows that iPS cells can be differentiated in a controlled manner into desired cell types in a manner. Without this essential feature iPS cells would not be usable in stem cell therapies. Embryonic stem cells and iPS cells have to be differentiated into the desired cell type in therapies. Without differentiating cells the therapy would be ineffective and the cells could form tumors. It is also important because this technique allows scientists to create diseased cells in culture to examine what is happening in these cells that causes them to die.