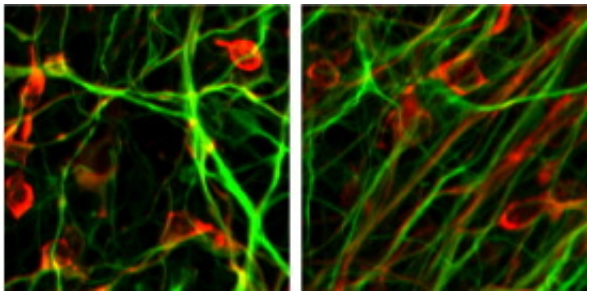
# Virus-free embryonic-like stem cells made from skin of Parkinson's disease patients

March 5 2009



Whitehead Institute researchers reprogrammed human skin cells from Parkinson's disease patients into an embryonic-stem-cell-like state. After removing the reprogramming genes, the scientists used these so-called induced pluripotent stem (iPS) cells to create dopamine-producing neurons, the cell type that degenerates in Parkinson's disease patients. To confirm that the iPS cells had become dopamine-producing neurons, the researchers stained the cells green for a neuron-specific protein (class III beta-tubulin) and red for a dopamine-producing neuron-specific enzyme (tyrosine hydroxylase). Image courtesy / Whitehead Institute

Researchers reporting in the March 6th issue of the journal *Cell*, a Cell Press publication, have developed a new way to produce human embryonic-like stem cells that are free of the viruses used to insert the key ingredients. They showed they could make those embryonic-like cells by reprogramming cells taken from people with unexplained (or idiopathic) Parkinson's disease.

"We used a modified virus you can excise," said Rudolf Jaenisch of The Whitehead Institute and Massachusetts Institute of Technology. "After they've done their job, you can get rid of them." That's important, he explained, because the use of viruses encoding the reprogramming factors represents a major limitation of the current technology. Even low activity of the virus-inserted genes may alter the potential for these human embryonic-like cells, or induced pluripotent stem (iPS) cells, to differentiate into other cell types or to cause cancer. (Pluripotent refers to the ability of these cells to differentiate into most other cell types).

In the new study, the team also converted these iPS cells into the neurons that are lost in patients with Parkinson's disease. The Parkinson's disease patient-specific iPS cells and neurons made from them offer a powerful new way to study the disease. The demonstration that this conversion from iPS cells to neurons is possible also represents another step toward the ultimate goal of using such iPS cell-derived cells in replacement therapies.

A 2006 report, also in the journal Cell, showed that the introduction of four factors could transform differentiated cells taken from adult mice into iPS cells with the physical, growth, and genetic characteristics typical of embryonic stem cells. The same recipe was later shown to also work with human skin cells. Last month, a Cell report showed for the first time that adult neural stem cells can take on the characteristics of embryonic stem cells with the addition of just one factor. In each case, the scientists used viral vectors to insert the critical genes that encode the factors.

In recent months, other methods to reprogram stem cells without the use of viruses have been developed, but those techniques have so far proved to be very inefficient, Jaenisch added. It also remains unclear whether they could be made to work in human cells.

Now, Jaenisch and his colleagues show that fibroblasts from the skin of five patients with idiopathic Parkinson's disease can be efficiently reprogrammed and subsequently differentiated into dopaminergic neurons using "Cre-recombinase excisable viruses" that could be inserted and then removed.

The resulting factor-free iPSCs maintained their ability to differentiate into other cell types, they found. The cells also showed a global gene expression profile more closely related to human embryonic stem cells than to human iPSCs still carrying the virally inserted transgenes.

"People had worried that when you have viral vectors, there can still be viral gene expression," Jaenisch said. "We didn't know if it was functionally important or not." The new findings show that indeed it is, a result that Jaenisch said he found rather surprising.

"The vector-free cells are much more closely related to embryonic stem cells than to the parental cells [they are derived from]. It argues that even low vector expression somehow changes the transcriptional profile of cells." Those differences may have real implications for the differentiation of virus-carrying iPSCs into other cell types and for their use in transplantation, he said.

Jaenisch's new method for producing virus-free cells represents an important breakthrough for scientists aiming to better understand the causes and consequences of Parkinson's and other diseases. The advance is also good news for those in search of potential new drug therapies as well as those who hope ultimately to use a patient's own cells to replace neurons lost to the degenerative disease.

"Such patient-specific cells will provide, for the first time, a system to investigate the proposed molecular and cellular mechanisms of sporadic Parkinson's disease, such as protein aggregation, mitochondrial

dysfunction, oxidative stress and altered kinase activity," the researchers noted. Due to the relatively short lifespan of cultured neurons -- which survive for a matter of weeks - studies of the typically late-onset disease may require methods to accelerate the development of symptoms, for instance by challenging the cells with oxidative stresses, neurotoxins, or by increasing the activity of known Parkinson's disease-related genes.

"Such in vitro models could be utilized for large-scale genetic or drug-based screens since large numbers of hiPSCs can be generated and robustly differentiated into dopaminergic neurons," the researchers wrote. "Furthermore our finding that dopaminergic neurons from Parkinson's disease-patients can be derived regardless of the underlying disease or the age of the donor substantiates the idea that hiPSC-based cell replacement could become a feasible therapeutic option for Parkinson's disease in the future."

More information: "Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors", *Cell*, March 5, 2009

Source: Cell Press