Scientists identify embryonic stem cells by appearance alone

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Some scientific results are hard to spot, especially in genetic research. Often scientists are unable to physically see if the gene they inserted into a cell has produced the desired trait. To overcome this problem researchers use various genetic markers that contain pieces of foreign DNA that cause cells to, for example, glow when exposed to ultraviolet light.

But scientists in the lab of Whitehead Member Rudolf Jaenisch didn't have to resort to these genetic markers in their latest experiment because the results were easy to see. Building on their widely publicized June Nature paper, which demonstrated that it's possible to convert specialized mouse skin cells into unspecialized stem cells, Whitehead postdoctoral researchers Alexander Meissner and Marius Wernig have now identified successfully reprogrammed cells by looks alone.

Their findings, which appear online in the journal *Nature Biotechnology* on Aug. 27, bring human stem cell therapies a step closer to reality. Before reprogramming can be applied to our own species to generate custom embryonic stem cells, scientists must be able to accomplish it without altering the DNA of the cells involved.

"This eliminates one of the major hurdles to reprogramming human cells," says Jaenisch, who is also an MIT professor of biology. "If we overcome the other obstacles, this approach could one day provide custom human embryonic stem cells for use in therapy."

Last spring, Wernig and Meissner relied on genetic markers to identify successfully reprogrammed cells. This required them to work with fibroblasts from a genetically modified mouse. The mouse was grown from embryonic stem cells that contained foreign DNA coding for antibiotic resistance. The scientists had strategically inserted these foreign DNA "markers" at particular points along the genome, next to genes expressed only in embryonic stem cells. All of the cells (including fibroblasts) in the resulting mouse contained the markers.

In the original experiment, the researchers took fibroblasts from the tail of this mouse and infected them with a special virus containing four genes (Oct4, Sox2, c-myc, and Klf4) capable of converting the cells to an embryonic state. Genes typically active in embryonic stem cells roared to life, triggering the adjacent foreign DNA to provide antibiotic resistance. Thus only fully reprogrammed cells survived exposure to an antibiotic, which allowed the scientists to isolate them.

"When we conducted the original experiment, we noticed that many of the infected cells had already started to change shape before the markers were activated," says Wernig.

So they set up a new experiment to test if visual identification alone would work. Indeed, they were able to separate the reprogrammed cells from ordinary fibroblasts under a microscope, based on several physical differences. Fibroblasts are big and flat. Embryonic stem cells are small, round and form tight colonies.

"We've shown that there's no need to use markers to isolate successfully reprogrammed cells," says Meissner. "This significantly simplifies this approach in mice, as we can now work with ordinary fibroblasts."

But another hurdle remains before the technique can be applied to human cells.

“We still used viruses containing foreign DNA to introduce the genes that induced the reprogramming,” explains Meissner.

The scientists are now working to eliminate the virus from the reprogramming process. Jaenisch believes they will eventually succeed and points out that the technique could eventually yield a bountiful supply of custom human embryonic stem cells for use in therapy.

Meissner and Wernig successfully reprogrammed about 0.5 percent of the fibroblasts. Given that there are millions of cells in a typical skin biopsy (researchers used skin from either the end of the tail or from the ear of the mouse), that translates into thousands of stem cells, each one capable of developing into any cell type of the body.

Source: Whitehead Institute for Biomedical Research