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## Stem Cells: Turning Back the Molecular Clock to Reverse Rapid Aging

Howard Hughes Medical Institute researchers have created a specialized group of stem cells from patients who have dyskeratosis congenita, a disorder that causes accelerated aging and results in bone marrow failure. In new research reported in

*Nature*

, the scientists show that using a genetic reprogramming technique to “turn back the molecular clock” in these cells appears to reset the cells and reverses rapid aging.

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- **George Q. Daley**

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In 2006, researchers in Japan discovered that they could create induced pluripotent stem cells (iPS) by adding four genes to skin cells. The Japanese scientists showed that adding the four genes rewound the cells' developmental clocks and turned them into embryonic-like stem cells. The reprogramming strategy is an important tool because it can be used to create disease-specific stem cell lines that, like embryonic stem cells, can develop into many cell types. Daley's group, which has been actively deriving new stem cell lines from patients with a variety of diseases, wondered whether reprogramming could slow or stop the rapid aging seen in the cells of people with dyskeratosis congenita.

Such experiments were also of wider scientific interest to Daley and his colleagues because they hoped the outcome might lead to new insight into how broken telomeres are “fixed” in iPS cells. And that’s just what happened. “We ended up learning something fundamental about how telomeres are regulated in induced pluripotent cells,” said George Q. Daley, the Howard Hughes Medical Institute (HHMI) investigator who led the work, which is published online February 17, 2010 in the journal *Nature*. “We found increased activity of all of the cellular machinery required for telomere maintenance.”

During normal DNA replication, the very ends of a DNA molecule are worn down. In order to prevent erosion, chromosomes are capped with a specialized region of DNA known as a telomere — a short, repetitious DNA sequence that does not code for any protein. The telomeres of normal cells gradually become shorter and shorter with each cell division, a characteristic sign of cellular aging.

During early development and in some adult stem cells, cell division is rapid and ongoing. In such situations, an enzyme complex called telomerase kicks into overdrive and works to rebuild the ever-shortening telomeres. Patients with dyskeratosis congenita, however, have inherited defective telomerase genes. As a result, the ends of their chromosomes wear down more rapidly because telomerase cannot make the necessary repairs. The first signs of problems usually turn up in patients’ blood cells, which are normally prone to rapid proliferation. These cells end up with shorter telomeres and age more quickly than other cells in their body. The resulting decline in blood stem cells often gives rise to bone marrow failure – and people with dyskeratosis congenita typically die in their teens.

In the study reported in *Nature*, Daley and colleague Suneet Agarwal, both from the Children’s Hospital Boston and Harvard Stem Cell Institute, collected skin cells from three patients with dyskeratosis congenita. Then, as the Japanese researchers had done in 2006, Daley’s group added the same four genes to the patients’ cells, turning them into iPS cells.

Earlier work from other labs had suggested that telomerase is a key to iPS cells’ ability to proliferate forever. Agarwal said he expected the iPS cells derived from dyskeratosis congenita cells to “peter out and stop dividing,” because the cells still harbored inherited genetic defects that prevented telomerase from working normally. To the researchers’ surprise, though, the cells kept dividing and acted like normal, healthy iPS cells.

Closer inspection of the cells’ telomeres revealed that the structures grew with each successive cell division – the opposite of what happens to telomeres in the skin cells of patients with the disease.

The researchers then examined one component of the cellular machinery that lengthens telomeres, a molecule called TERC, or telomerase RNA

component. This molecule acts as a template for the short segments of DNA comprising telomeres. The cells of patients with dyskeratosis congenita normally make about 90 percent less TERC than normal cells. However, in the reprogrammed cells, TERC levels shot up and approached normal levels.

“It’s clear now that TERC activity gets reset by the iPS reprogramming technique,” said Daley. “This work suggests that if we can somehow boost the production of TERC – perhaps with a drug – we might be able to ameliorate the effects of this disease.”

However, when the researchers let the reprogrammed cells exit their pluripotent state and grow into various types of tissue, the TERC levels decreased again and the telomeres shortened. In other words, the defect is only corrected in cells that remain in the pluripotent, stem cell-like state. “The pluripotent state itself is what seems to fix the defect and lengthen telomeres,” said Daley.

Further experiments explained why this is so. When reprogramming cells to become iPS cells, researchers add four genes that act as master switches that turn on other genes. Daley and colleagues found that the proteins made by the reprogramming gene *Oct4* and Nanog, another critical protein for reprogramming, actually bind to the stretches of cellular DNA that produce TERC and the other two components of the telomerase machinery. This binding triggers the telomerase-associated genes to begin production, increasing levels of TERC and boosting overall telomerase activity. “The reprogramming factors themselves activate these genes,” said Daley. “They work on the components of the telomerase enzyme.”

Additional lab work showed that normal, healthy cells reprogrammed into iPS cells also display elongated telomeres. That is, the mechanism that produces telomere elongation in the dyskeratosis congenita cells is also at play in healthy cells that are reprogrammed into iPS cells.

Daley said this finding helps explain how iPS reprogramming works to keep cells ‘immortal’ -- able to divide and maintain themselves indefinitely. “This research really highlights how the basic technique of reprogramming can teach us about a disease process, while also teaching us about this very fascinating biology of resetting a specialized cell back to its embryonic state,” he said. “We’ve learned something fundamental here about iPS reprogramming and pluripotent stem cells in general.”