Scientists find stem cell reprogramming technique is safer than previously thought

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Stem cells made by reprogramming patients' own cells might one day be used as therapies for a host of diseases, but scientists have feared that dangerous mutations within these cells might be caused by current reprogramming techniques. A sophisticated new analysis of stem cells' DNA finds that such fears may be unwarranted.

"We've shown that the standard reprogramming method can generate induced pluripotent stem cells that have very few DNA structural mutations, which are often linked to dangerous cell changes such as tumorigenesis," said Kristin Baldwin, associate professor at The Scripps Research Institute's Dorris Neuroscience Center and a senior author of the report, which appears in the October 7, 2011 issue of the journal *Cell Stem Cell*. For this study the Baldwin lab collaborated with a genomics and bioinformatics expert, Ira M. Hall, an assistant professor of biochemistry and molecular genetics at the University of Virginia who is co-senior author.

The induced pluripotent stem cell (iPSC) technique was first described in 2006. It requires the insertion into an ordinary non-stem cell of four special genes, whose activities cause the cell to revert to a state like that of embryonic stem cell. In principle, iPSCs may be used to repair diseased or damaged tissues, and because they are made from a patient's own cells, they shouldn't provoke an immune reaction. But recent studies have found unacceptably high levels of mutations in iPSCs derived from adult human cells. That has led to widespread suspicion that the reprogramming process is largely to blame.

In the new study, the Scripps Research and University of Virginia researchers set out to investigate this issue using the latest chromosomal error-mapping methods. "The techniques that our University of Virginia colleagues brought to this study are much more sensitive than anything else that's available right now," said Michael J. Boland, a research associate in the Scripps Research Baldwin lab and co-first author of the paper with Aaron R. Quinlan, a postdoctoral researcher in Hall's lab. The new methods included a high-resolution version of a DNA-error-finding technique known as paired-end mapping, and an advanced algorithm, "HYDRA," for handling the voluminous mapping data.

To generate the iPSCs, the Scripps Research team followed the standard, four-gene reprogramming procedure, but sought to minimize other potential sources of DNA mutations that might have influenced some previously reported results. The donor cells they selected were not decades-old human skin cells, but relatively error-free fibroblast cells from fetal mice. The researchers also kept these fibroblast cells only briefly in lab dishes before reprogramming them.

When the team members analyzed these iPSCs they used two strategies to distinguish which mutations were present in rare donor fibroblast cells and which were newly acquired during reprogramming. Their advanced techniques also allowed them to find more kinds of mutations, across a wider range of the genome, than ever before. Yet instead of finding more mutations, they found almost none. "We sequenced three iPSC lines at very high resolution, and were surprised to find that very few changes to the chromosomal sequence had appeared during reprogramming," said Boland.

Each of the iPSC lines contained only a single mutation that probably originated from the reprogramming process; two affected genes while the other appeared not to. Mutations inherited from the donor fibroblast cell were present in one pair of lines, while a second line "inherited"

none. The researchers were particularly cheered by the complete absence of new "retroelement transpositions"—mutations caused by retrovirus-like sequences that burrowed into the mammalian genome long ago that can become active again in certain cell types. All cells have ways to suppress these retroelements, but the suppression mechanisms in normal cells are different from those in stem cells, so the researchers had worried that retroelements would be allowed to escape suppression during the transition to a stem cell state. While no previous surveys of iPSCs could detect these mutations, this study showed that despite very sensitive detection of controls, no retroelements had become active during reprogramming. "That was is very encouraging, because retroelement mutations can be very damaging to the genome," Boland said.

Some of the mutations seen in human iPSCs in previous studies might have been due to incomplete reprogramming that impaired the cells' DNA-maintenance mechanisms. In this study using mouse iPSCs, however, there was no doubt that a complete reprogramming to an embryonic state had occurred: all three iPSC lines were used to produce live, fertile mice, in work that Boland, Baldwin, and their colleagues described in Nature in 2009. "The mice generated from these cells have survived to a normal lab-mouse lifespan without obvious diseases that might arise from new DNA mutations," said Baldwin.

Her lab now is trying to determine whether a reprogramming method similar to the one used with mouse iPSCs in this study could also yield relatively error-free human iPSCs. "If our results with these mouse cells are applicable to human cells, then selecting better donor cells and using more sensitive genome-survey techniques should allow us to identify reprogramming methods that can produce human iPSCs that will be safer or more useful for therapies than current lines," she said.

More information: "Genome Sequencing of Mouse Induced

Pluripotent Stem Cells Reveals Retroelement Stability and Infrequent DNA Rearrangement during Reprogramming," *Cell Stem Cell*.

Provided by The Scripps Research Institute