Stem cells made by reprogramming hold onto their past

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Adult cells that have been reprogrammed into induced pluripotent stem cells (iPS cells) do not completely let go of their past, perhaps limiting their ability to function as a less controversial alternative to embryonic stem cells for basic research and cell replacement therapies, according to researchers at Children's Hospital Boston, John Hopkins University and their colleagues.

The findings, published online July 19 in *Nature*, highlight a major challenge in developing clinical and scientific applications for the powerful new technique of making iPS cells, which, like embryonic stem cells, have the capacity to differentiate into any type of cell in the body. Similar findings were published simultaneously online in Nature Biotechnology by other Boston researchers.

"iPS cells retain a 'memory' of their tissue of origin," said senior author George Daley, MD, PhD, a Howard Hughes Medical Institute investigator and Director of the Stem Cell Transplantation Program at Children's. "iPS cells made from blood are easier to turn back into blood than, say, iPS cells made from skin cells or brain cells."

In contrast, another technique known as nuclear transfer creates pluripotent stem cells without apparent memory and equally adept at transforming into several tissue types, the paper reports. In iPS cells, the memory of the original donor tissue can be more fully erased with additional steps or drugs, the researchers found, which made those iPS cells as good as the nuclear-transfer stem cells at generating different

types of early tissue cells in lab dishes.

The residual cellular memory comes in part from lingering genome-wide epigenetic modifications to the DNA that gives each cell a distinctive identity, such as skin or blood, despite otherwise identical genomes. In the study, the persistent bits of a certain type of epigenetic modification called methylation were so distinctive in iPS cells that their tissues of origin could be identified by their methylation signatures alone.

"We found the iPS cells were not as completely reprogrammed as the nuclear transfer stem cells," said co-senior author Andrew Feinberg, MD, MPH, director of the Center for Epigenetics at Johns Hopkins, whose group did systematic epigenomic analyses of the cells. "Namely, DNA methylation was incompletely reset in iPS cells compared to nuclear transfer stem cells. Further, the residual epigenetic marks in the iPS cells helped to explain the lineage restriction, by leaving an epigenetic memory of the tissue of origin after reprogramming."

Epigenetic memory may be helpful for some applications, such as generating blood cells from iPS cells originally derived from a person's own blood, the researchers said. But the memory may interfere with efforts to engineer other tissues for treatment in diseases such as Parkinson's or diabetes or to use the cells to study the same disease processes in laboratory dishes and test drugs for potential treatments and toxicities.

"These findings cut across all clinical applications people are pursuing and whatever disease they are modeling," said Daley, also a member of the Harvard Stem Cell Institute and professor of biological chemistry and molecular pharmacology at Harvard Medical School. "Our data provide a deeper understanding of the iPS platform. Everyone working with these cells has to think about the tissues of origin and how that affects reprogramming."

iPS cells became a focal point of stem cell biology four years ago when a Japanese team led by Shinya Yamanaka created the functional equivalent of embryonic stem cells from adult mouse skin cells with a cocktail of four molecular factors. A year later, Yamanaka's team, Daley's team and a University of Wisconsin group all independently reported creating human iPS cells from adult skin cells, raising hopes for future clinical and research applications. Earlier this month, Daley's team and two other groups reported making human iPS cells from adult blood cells, a faster and easier source. In that study, iPS cells from blood were also better at differentiating back into blood cells than into other tissue types.

In the current study, first author Kitai Kim, PhD, postdoctoral fellow in the Daley lab, tested mice iPS cells head-to-head with pluripotent cells made through somatic cell nuclear transfer. Best known as the cloning method that created the sheep Dolly fourteen years ago, nuclear transfer reprograms an adult cell by transferring its nucleus into an unfertilized egg cell, or oocyte, whose nucleus has been removed. The process of transferring the nucleus immediately reprograms it epigenetically, replicating the same process that happens to sperm upon fertilization, Kim said.

"Stem cells generated by somatic cell nuclear transfer are on average, closer to bona fide embryonic stem cells than are iPS cells," Daley said. "This has an important political message--we still need to study the mechanisms by which nuclear transfer reprograms cells, because the process seems to work more efficiently and faithfully. Learning the secrets of nuclear transfer may help us make better iPS cells."

Kim began the study with older mice (ages 1 to 2), aiming to emulate the future human clinical scenario, which is likely to involve older people. Older cells are set in their ways and harder to reprogram, Kim said. Kim originally wanted to compare the transplantation success of blood cells made from three different pluripotent sources: iPS cells, embryonic stem

cells (the gold standard), and nuclear transfer stem cells.

He did not get as far as transplantation. "Even in vitro we observed strikingly different blood-forming potential," he and his co-authors wrote in the paper. "We focused instead on understanding this phenomenon."

iPS cells from blood were best at making blood, and fibroblasts were best at differentiating into bone, a closely related tissue, Kim and his colleagues found. The researchers could reset the iPS cells more fully by differentiating them first into blood cells and then reprogramming them again, or by treating them with drugs that change their epigenetic profile.

In contrast, nuclear transfer stem cells from the same sources -- blood cells and skin - were equally able to differentiate into blood and bone, Kim and his colleagues found. Like iPS cells, the nuclear transfer technique also creates patient-specific cells, but has not yet proven successful with human cells.

"This paper opens our eyes to the restricted lineage of iPS cells," said Feinberg "The lineage restriction by tissue of origin is both a blessing and a curse. You might want lineage restriction in some cases, but you may also have to do more work to make the iPS cells more totally pluripotent."

Another study published online simultaneously in the journal Nature Biotechnology reports similar findings. "Our paper comes to a similar conclusion that a retention of memory reflects the cell of origin and affects the capacity of the iPS cell to differentiate into other cell types," said senior author Konrad Hochedlinger, PhD, a stem cell biologist at the Massachusetts General Hospital Center for Regenerative Medicine and, like Daley, a member of the Harvard Stem Cell Institute, who demonstrated another method to more fully reprogram iPS cells. "When

we let the cells go through a lot of cell divisions, they lose the memory," he said.

Provided by Children's Hospital Boston