Mutations found in human induced pluripotent stem cells

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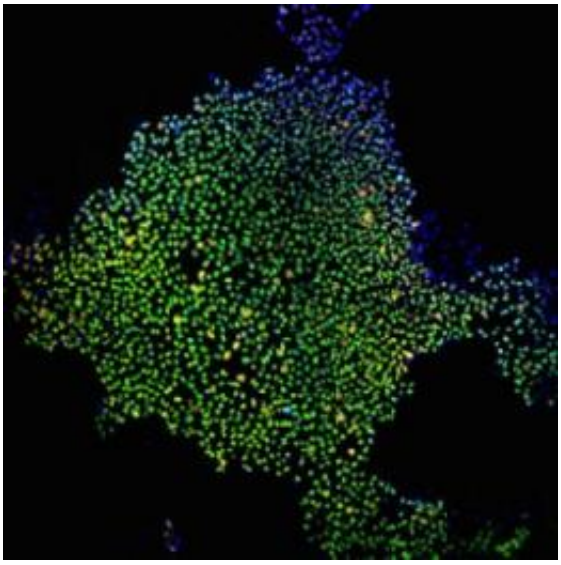

This is an image of induced stem cells, courtesy of James Thomson. Credit: University of Wisconsin-Madison

Ordinary human cells reprogrammed as induced pluripotent stem cells (hiPSCs) may ultimately revolutionize personalized medicine by creating new and diverse therapies unique to individual patients. But important and unanswered questions have persisted about the safety of these cells, in particular whether their genetic material is altered during the reprogramming process.

A new study – published in the March 3 issue of the journal *Nature* and

led by scientists at the University of California, San Diego in collaboration with other leading stem cell research groups – finds that the genetic material of reprogrammed cells may in fact be compromised, and suggests that extensive genetic screening of hiPSCs become standard practice before these stem cells are used clinically.

A national team of researchers, co-directed by Kun Zhang, PhD, an assistant professor of bioengineering in the UC San Diego Jacobs School of Engineering, examined 22 different hiPSC lines obtained from seven research groups that employed different methods to reprogram skin cells into pluripotent stem cells. In all of these cell lines, the researchers found protein-coding point mutations, an estimated six mutations per exome. The exome is the part of the genome that contains the genetic instructions for making proteins and other gene products.

"Every single stem cell line we looked at had mutations. Based on our best knowledge, we expected to see 10 times fewer mutations than we actually observed," said Zhang, a faculty member of the Institute for Genomic Medicine and the Institute of Engineering in Medicine, both at UC San Diego.

The findings help answer the question of whether reprogramming adult mammalian cells into hiPSCs affects the overall genome at the fundamental level of single nucleotides. They do. Zhang called the mutations "permanent genome scars."

The scientists said while some of the mutations appeared to be silent, the majority did change specific protein functions, including those in genes associated with causative effects in cancers.

"Reprogrammed stem cells provide an important new tool in the fight against human disease, but to use these cells directly in the clinic, we must ensure that they are safe and that we are able to define their

structure and behavior in the most precise terms," said Lawrence S.B. Goldstein, PhD, professor in the Department of Cellular and Molecular Medicine at the UCSD School of Medicine and co-director of the study with Zhang. Goldstein is also director of the UC San Diego Stem Cell Program.

"Our studies open a new window into the genetic behavior of these important types of stem cells and begin to define some new and straightforward safety standards that may help accelerate their use in clinical settings," Goldstein added.

The study examined stem cell lines from many of the leading stem cell research groups in the United States, including lines from the laboratories of James Thomson at the University of Wisconsin-Madison and George Daley at the Children's Hospital Boston, the first U.S.-based labs to reprogram human cells.

"We covered cell lines derived from seven different labs because we wanted to make sure our conclusions are general enough to make realistic extrapolations," said Zhang.

The interdisciplinary team at UC San Diego developed a new, highly sensitive assay to identify mutations that occur at very low frequencies in the starting cells of cell lines. They discovered that roughly half of the mutations found in stem cell lines were present in starting cells at very low levels. That is, they occurred in a few cells sometime during the person's life or during cell culture in the lab, and were somatic or not inherited. The other half of the mutations were too rare to detect in starting cells, meaning they could have occurred during or after reprogramming.

The mutations, which the scientists dubbed "reprogramming-associated mutations," came from three different sources: a first group that

included mutations already present in skin cells before reprogramming; a second group of mutations that occurred during reprogramming; and a third group of mutations that occurred after reprogramming, when pluripotent cells began proliferating.

The work is complementary to research published in Cell Stem Cell in January 2011 by another team of scientists at UC San Diego and elsewhere that documented other types of genetic abnormalities in both human embryonic and hiPSC lines after reprogramming and extended culture. That paper reported that human pluripotent and induced pluripotent cells had higher frequencies of genomic aberrations than other cell types. The latest work presents new findings about a different type of important genetic damage: changes occurring during reprogramming in single nucleotides or base pairs that alter the crucial protein building blocks of cells.

"These studies look at two different aspects of stem cell mutations," said Zhang, "but their take-home message is the same – things can go wrong at the genome level when reprogramming and growing reprogrammed cells. So, to maximize safety, before we put these cells back in the human body for therapeutic purposes, we must be sure that the cells contain the same genome as the recipient, with no cancer-causing or other serious types of mutations."

Provided by University of California - San Diego