Improved adult-derived human stem cells have fewer genetic changes than expected

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A team of researchers from Johns Hopkins University and the National Human Genome Research Institute has evaluated the whole genomic sequence of stem cells derived from human bone marrow cells-so-called induced pluripotent stem (iPS) cells-and found that relatively few genetic changes occur during stem cell conversion by an improved method. The findings, reported in the March issue of Cell Stem Cell, the official journal of the *International Society for Stem Cell Research* (ISSCR), will be presented at the annual ISSCR meeting in June.

"Our results show that human iPS cells accrue genetic changes at about the same rate as any replicating cells, which we don't feel is a cause for concern," says Linzhao Cheng, Ph.D., a professor of medicine and oncology, and a member of the Johns Hopkins Institute for Cell Engineering.

Each time a cell divides, it has the chance to make errors and incorporate new genetic changes in its DNA, Cheng explains. Some genetic changes can be harmless, but others can lead to changes in cell behavior that may lead to disease and, in the worst case, to cancer.

In the new study, the researchers showed that iPS cells derived from adult bone marrow cells contain random genetic changes that do not specifically predispose the cells to form cancer.

"Little research was done previously to determine the number of DNA changes in stem cells, but because whole genome sequencing is getting faster and cheaper, we can now more easily assess the genetic stability of these cells derived by various methods and from different tissues," Cheng says. Last year, a study published in Nature suggested higher than expected cancer gene mutation rates in iPS cells created from skin samples, which, according to Cheng, raised great concerns to many in the field pertaining to usefulness and safety of the cells. This study analyzed both viral and the improved, nonviral methods to turn on stem cell genes making the iPS cells

To more thoroughly evaluate the number of genetic changes in iPS cells created by the improved, non-viral method, Cheng's team first converted human blood-forming cells or their support cells, so-called marrow stromal cells (MSCs) in adult bone marrow into iPS cells by turning on specific genes and giving them special nutrients. The researchers isolated DNA from--and sequenced--the genome of each type of iPS cells, in comparison with the original cells from which the iPS cells were derived.

Cheng says they then counted the number of small DNA differences in each cell line compared to the original bone marrow cells. A range of 1,000 to 1,800 changes in the nucleic acid "letters" A, C, T and G occurred across each genome, but only a few changes were found in actual genes--DNA sequences that act as blueprints for our body's proteins. Such genes make up two percent of the genome.

The blood-derived iPS cells contained six and the MSC-derived iPS cells contained 12 DNA letter changes in genes, which led the researchers to conclude that DNA changes in iPS cells are far more likely to occur in the spaces between genes, not in the genes themselves.

Next, the investigators examined the severity of the DNA changes--how likely each one would disrupt the function of each gene. They found that about half of the DNA changes were "silent," meaning these altered blueprints wouldn't change the nucleic acid building code for its corresponding protein or change its function.

For the remaining DNA changes, the researchers guessed these would, in fact, disrupt the function of the gene by either making the gene inactive or changing the way the gene works. Since each cell

contains two copies of each gene, in many cases the other, normal copy of the gene could compensate for a disrupted gene, Cheng and the team reasoned.

Cheng cautions that disrupting a single gene copy could pose a problem though, for example, by shutting down a tumor suppressor gene that prevents cells from malignant growth. However, none of the disrupted genes his team found have been implicated in cancer.

He also noted the absence of overlap in the DNA changes found among the different stem cell lines examined, implying that the changes were random and unlikely to cluster.

Based on these findings, Cheng says, iPS cells don't seem to pose a heightened cancer risk, but the risk is not zero, the researchers say.

"We need to sequence more iPS cell lines, including those derived from different cell types and ones using different methods of stem cell conversion, before we have a better picture of mutation rates and spectrums in the iPS cell lines," says Paul Liu, M.D., Ph.D., co-senior author and the deputy scientific director at the National Human Genome Research Institute.

Just because these DNA changes in the stem cells don't specifically select for cancer formation, he adds, doesn't mean that cancer mutations can't arise in other iPS cells. Liu adds that to be on the safe side "it should become a routine procedure to sequence iPS cells before they are used in the clinic."

Other researchers who contributed to the study are Chunlin Zou, Bin-Kuan Chou, Sarah Dowey and Zhaohui Ye of the Johns Hopkins University; Nancy Hansen, Ling Zhao, Frank Donovan, Settara Chandrasekharappa, James Mullikin and the NISC Comparative Sequencing Program of the National Human Genome Research Institute; and Yutao Du, Guangyu Zhou, Shijie Li and Huanming Yang of the Beijing Genomics Institute.

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