Large number of stem cell lines carry significant DNA damage, say researchers

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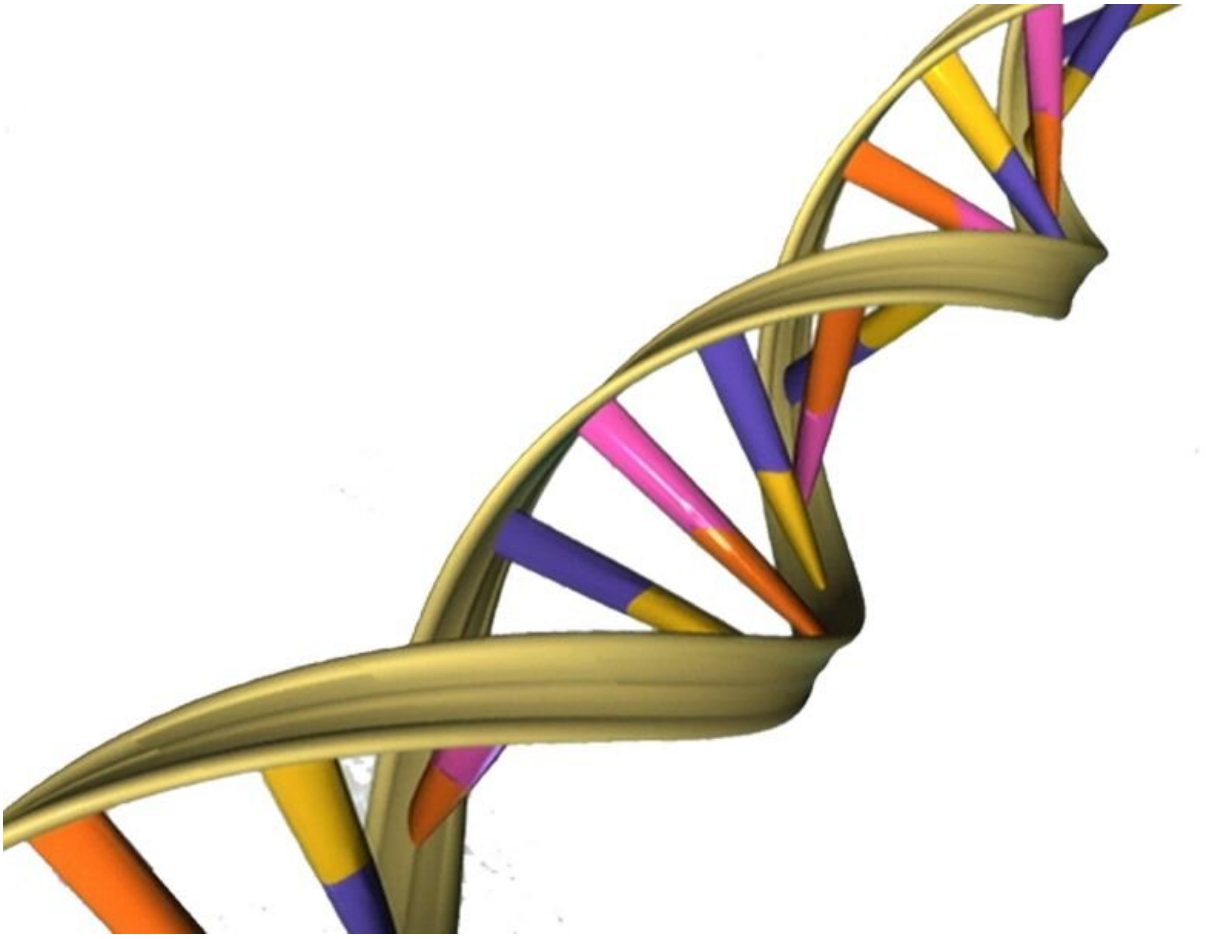

A depiction of the double helical structure of DNA. Its four coding units (A, T, C, G) are color-coded in pink, orange, purple and yellow. Credit: NHGRI

DNA damage caused by factors such as ultraviolet radiation affect nearly three-quarters of all stem cell lines derived from human skin cells, say Cambridge researchers, who argue that whole genome sequencing is essential for confirming if cell lines are usable.

Stem cells are a special type of cell that can be programmed to become almost any type of cell within the body. They are currently used for studies on the development of organs and even the early stages of the embryo.

Increasingly, researchers are turning to stem cells as ways of developing new treatments, known as cell-based therapies. Other potential applications include programming stem cells to grow into nerve cells to replace those lost to neurodegeneration in diseases such as Parkinson's.

Originally, stem cells were derived from embryos, but it is now possible to derive stem cells from adult skin cells. These so-called induced pluripotent stem cells (iPSCs) have now been generated from a range of tissues, including blood, which is increasing in popularity due to its ease of derivation.

However, researchers at the University of Cambridge and Wellcome Sanger Institute have discovered a problem with stem cell lines derived from both skin cells and blood. When they examined the genomes of the stem cell lines in detail, they found that nearly three quarters carried substantial damage to their DNA that could compromise their use both in research and, crucially, in cell-based therapies. Their findings represent the largest genetic study to date of iPSCs and are published today in *Nature Genetics*.

DNA is made up of three billions pairs of nucleotides, molecules represented by the letters A, C, G and T. Over time, damage to our DNA, for example from ultraviolet radiation, can lead to mutations—a

letter C might change to a letter T, for example. "Fingerprints" left on our DNA can reveal what is responsible for this damage. As these mutations accumulate, they can have a profound effect on the function of cells and in some cases lead to tumors.

Dr. Foad Rouhani, who carried out the work while at the University of Cambridge and the Wellcome Sanger Institute, said: "We noticed that some of the iPS cells that we were generating looked really different from each other, even when they were derived from the same patient and derived in the same experiment. The most striking thing was that pairs of iPS cells would have a vastly different genetic landscape—one line would have minimal damage and the other would have a level of mutations more commonly seen in tumors. One possible reason for this could be that a cell on the surface of the skin is likely to have greater exposure to sunlight than a cell below the surface and therefore eventually may lead to iPS cells with greater levels of genomic damage."

The researchers used a common technique known as whole genome sequencing to inspect the entire DNA of stem cell lines in different cohorts, including the HipSci cohort at the Wellcome Sanger Institute and discovered that as many as 72% of the lines showed signs of major UV damage.

Professor Serena Nik-Zainal from the Department of Medical Genetics at the University of Cambridge said: "Almost three-quarters of the cell lines had UV damage. Some samples had an enormous amount of mutations—sometimes more than we find in tumors. We were all hugely surprised to learn this, given that most of these lines were derived from skin biopsies of healthy people."

They decided to turn their attention to cell lines not derived from skin and focused on blood derived iPSCs as these are becoming increasingly popular due to the ease of obtaining blood samples. They found that

while these blood-derived iPSCs, too, carried mutations, they had lower levels of mutations than skin-derived iPS cells and no UV damage. However, around a quarter carried mutations in a gene called BCOR, an important gene in blood cancers.

To investigate whether these BCOR mutations had any functional impact, they differentiated the iPSCs and turned them into neurons, tracking their progress along the way.

Dr. Rouhani said: "What we saw was that there were problems in generating neurons from iPSCs that have BCOR mutations—they had a tendency to favor other cell types instead. This is a significant finding, particularly if one is intending to use those lines for neurological research."

When they examined the blood samples, they discovered that the BCOR mutations were not present within the patient: instead, the process of culturing cells appears to increase the frequency of these mutations, which may have implications for other researchers working with cells in culture.

Scientists typically screen their cell lines for problems at the chromosomal level—for example by checking to see that the requisite 23 pairs of chromosomes are present. However, this would not be sufficiently detailed to pick up the potentially major problems that this new study has identified. Importantly, without looking in detail at the genomes of these stem cells, researchers and clinicians would be unaware of the underlying damage that is present with the cell lines they are working with.

"The DNA damage that we saw was at a nucleotide level," says Professor Nik-Zainal. "If you think of the human genome as like a book, most researchers would check the number of chapters and be satisfied that

there were none missing. But what we saw was that even with the correct number of chapters in place, lots of the words were garbled."

Fortunately, says Professor Nik-Zainal, there is a way round the problem: using whole genome sequencing to look in detail for the errors at the outset.

"The cost of whole genome sequencing has dropped dramatically in recent years to around £500 per sample, though it's the analysis and interpretation that's the hardest bit. If a research question involves cell lines and cellular models, and particularly if we're going to introduce these lines back into patients, we may have to consider sequencing the genomes of these lines to understand what we are dealing with and get a sense of whether they are suitable for use."

Dr. Rouhani adds: "In recent years we have been finding out more and more about how even our healthy cells carry many mutations and therefore it is not a realistic aim to produce stem cell lines with zero mutations. The goal should be to know as much as possible about the nature and extent of the DNA damage to make informed choices about the ultimate use of these stem cell lines.

"If a line is to be used for cell based therapies in patients for example, then we need to understand more about the implications of these mutations so that both clinicians and patients are better informed of the risks involved in the treatment."

More information: Serena Nik-Zainal, Substantial somatic genomic variation and selection for BCOR mutations in human induced pluripotent stem cells, *Nature Genetics* (2022). DOI: 10.1038/s41588-022-01147-3.
www.nature.com/articles/s41588-022-01147-3

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