Origin of chromosomal oddity in some cancer cells identified

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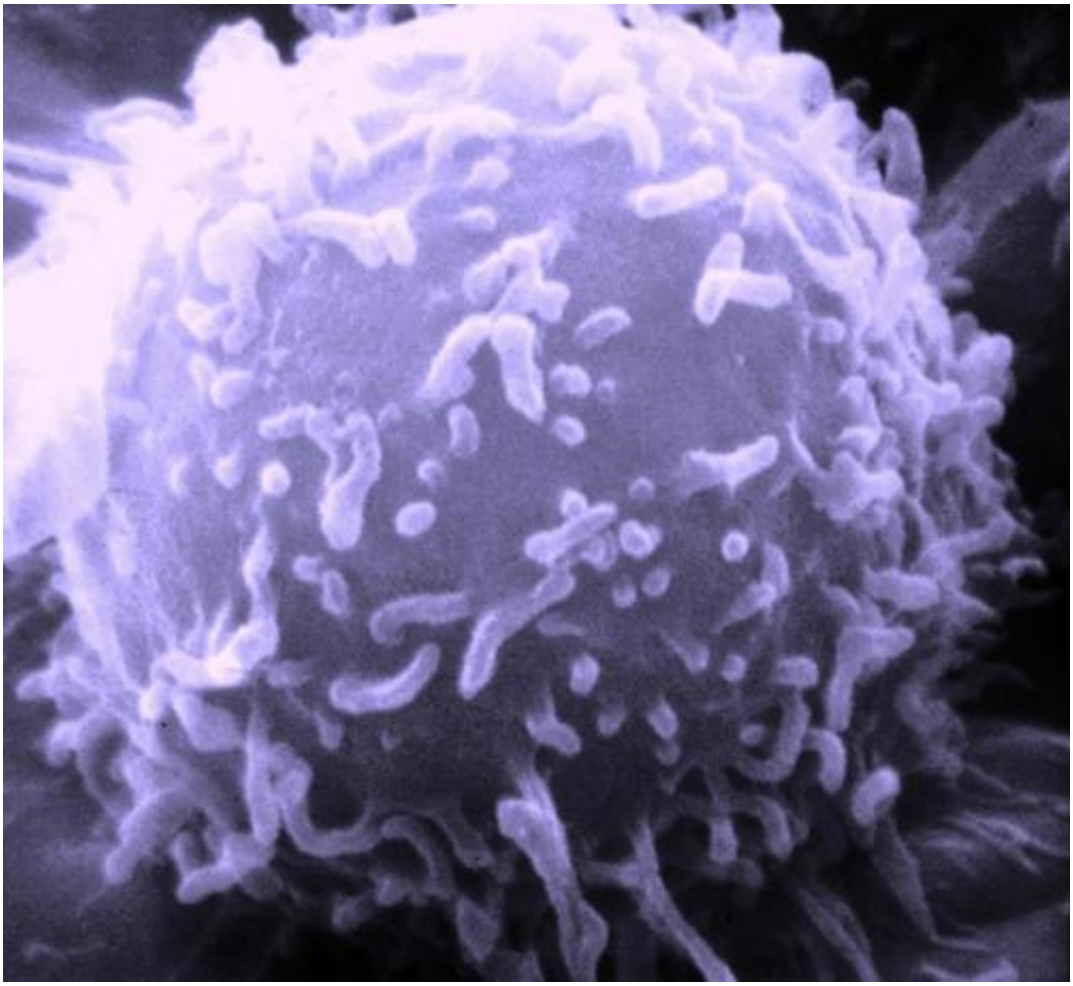

Electron microscopic image of a single human lymphocyte. Credit: Dr. Triche National Cancer Institute

Surveys of the genomic terrain of cancer have turned up a curious phenomenon in some tumor cells: a massive rearrangement of DNA in one or a few chromosomes, thought to be produced during a single cell cycle. In a new study, scientists at Dana-Farber Cancer Institute demonstrate how this sudden, isolated shuffling of genetic material - known as chromothripsis - can occur.

The discovery was made using live video images of single cells and technology for sequencing the genomes of those cells - a combination researchers dubbed Look-Seq (pronounced "look-seek"). The technique makes it possible for scientists to see how changes in specific genes or chromosomes affect cell behavior - critical information for understanding how genes function.

"Only three to five percent of cancer cells show signs of chromothripsis, but the mechanism by which it occurs has been a puzzle," said David Pellman, MD, of Dana-Farber, the Broad Institute of Harvard and MIT, and the Howard Hughes Medical Institute, the senior author of the new paper, published in *Nature*. "The cells have two distinctive features: a broad disarray in the genetic material of a single chromosome or a few chromosomes, as if the DNA was shredded and haphazardly stitched together; and an odd 'notched' pattern of the amount of DNA in the affected chromosome - where sections containing a full complement of DNA alternate with sections where DNA copies have been lost.

"The question has been whether this could happen in a Darwinian way, with a slow acquisition of changes over a number of cell divisions," Pellman continued. "The evidence, however, points to an all-at-once event."

In the study Pellman and his colleagues trace chromothripsis to a glitch in cell division that can cause one of the newly formed daughter cells to be short one chromosome, while the other daughter cell inherits an extra

one. The surplus chromosome doesn't always join the other chromosomes in the cell nucleus; often, it's stranded elsewhere in the cell and acquires its own tiny membrane, forming a "micronucleus." After cell division, the micronucleus can rupture, potentially exposing the chromosome inside to damage. The chromosome is then absorbed into the nucleus, knitting its imperfect DNA into the cell's genetic programming.

In theory, a micronucleus would make a perfect setting for the process of chromothripsis to unfold. The release of a single chromosome from a shattered micronucleus could cause that chromosome - and only that chromosome - to undergo DNA damage. The chromosome might lose some bits of DNA, while the remaining bits are stitched together haphazardly. Such random reassembly could account for the unusual fluctuation in the number of copies of DNA in a single chromosome in the affected cell.

"The formation and breakdown of a micronucleus would seem to satisfy all the conditions by which chromothripsis could occur," Pellman said. To see if it in fact explains what happens in cancer cells, he and his colleagues set out to recreate chromothripsis in the lab.

The researchers treated cells with a drug that can spur the formation of micronuclei. They collected the ones that actually developed a micronucleus and viewed live images of them under a microscope - the "Look" part of Look-Seq. This enabled them to identify cells where the micronucleus ruptured at a key stage of the cell life cycle.

After these cells had divided one time, researchers sorted through the pairs of daughter cells to find ones that didn't have a micronucleus. In such cells, the chromosome that had once been quarantined within the micronucleus had presumably escaped as the micronucleus burst, and rejoined its fellow chromosomes in the cell nucleus. The micronucleus-

less cells were then genetically sequenced - their strings of DNA read letter by letter. This constitutes the "Seq" part of Look-Seq, which was developed in collaboration with Matthew Meyerson, MD, PhD, of Dana-Farber and the Broad Institute.

If chromothripsis does arise as a result of the formation and destruction of a micronucleus, as researchers theorized, it should occur in only one of a cell's two daughters - the one that inherits a chromosome formerly wrapped in a micronucleus. The affected daughter cell, therefore, would have more copies of certain DNA sections than its sister cell does.

When Pellman's team genetically sequenced pairs of daughter cells from nine cells with micronuclei, they found precisely this imbalance: one member of each pair had at least one chromosome with extra copies of DNA at some locations, while the other member did not.

The researchers then tested their prediction that DNA rearrangements would be confined to the chromosome that was once AWOL and had later reunited with its mates. They found that, in eight of the nine pairs of daughter cells whose DNA had been sequenced, such rearrangements were indeed concentrated in that particular chromosome.

"By forcing cells to develop micronuclei, we derived daughter cells that recapitulate all the features seen in chromothripsis," said Pellman. "This is compelling evidence that this process is a major mechanism by which chromothripsis occurs in some cancers.

"The findings support our lab's earlier research showing that the incorrect segregation of intact chromosomes prior to cell division can produce rearrangements of DNA that result in genetic mutations - potentially playing a role in cancer development," he continued. "We also show that the Look-Seq technique is a powerful way of exploring the connection between genetic and molecular change and cell behavior."

More information: Chromothripsis from DNA damage in micronuclei, *Nature*, DOI: 10.1038/nature14493