A potential danger of CRISPR gene editing—and why base editing may be safer

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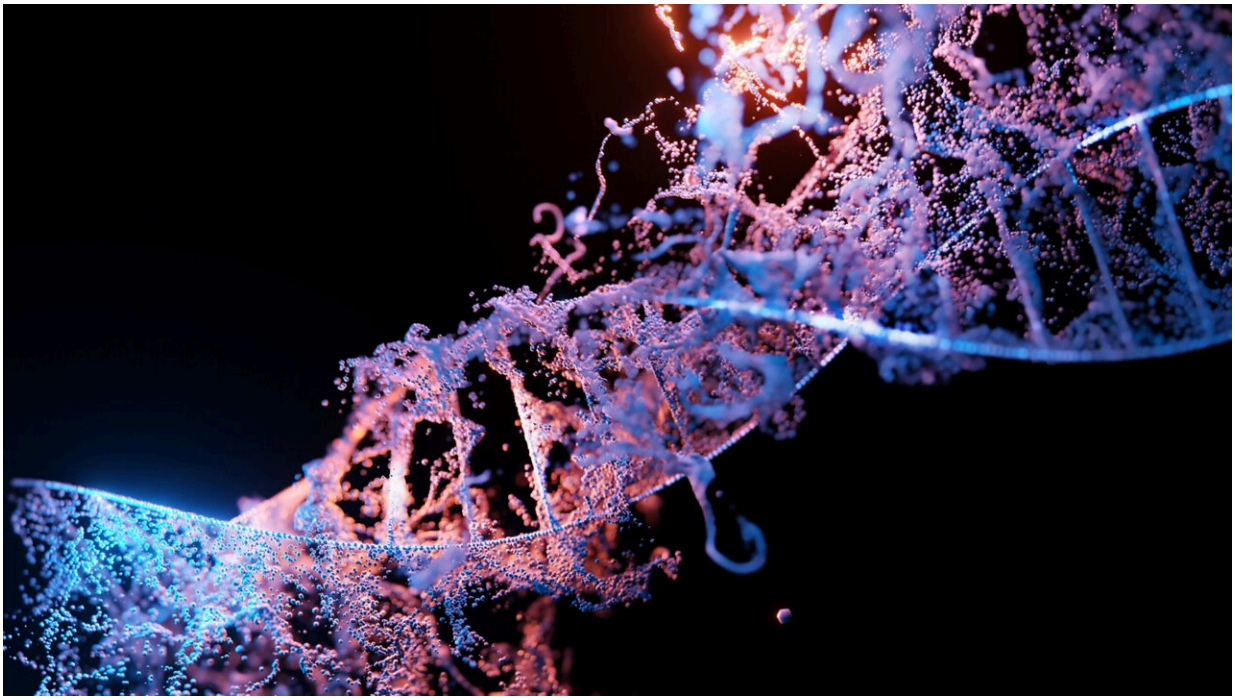

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Gene therapy using CRISPR/Cas9 gene editing is currently in clinical trials around the world for a variety of diseases. A report from Boston Children's Hospital, published June 27 in *Nature Communications*, warns of a potential, previously undiscovered danger of CRISPR editing.

Studying classical CRISPR/Cas9 in multiple human cell lines, a team led

by Roberto Chiarle, MD, and Jianli Tao, Ph.D., in the Department of Pathology at Boston Children's, show for the first time that the technique can cause large rearrangements of DNA through a process called retrotransposition. Rearrangements occur when breaks in DNA aren't repaired, allowing mismatched ends to join. While retrotransposition events caused by CRISPR were uncommon (occurring up to 5 to 6 percent of the time in the study's experimental model), they can theoretically trigger cancer.

The researchers suggest that tests for retrotransposition be added to safety testing for CRISPR/Cas9 editing systems. Current test technologies either sequence small stretches of DNA to ensure that the desired gene has been added or deleted in the right place, or are designed to detect small gene rearrangements. They don't look for large rearrangements caused by retrotransposition.

"We hope our findings will encourage investigators using CRISR/Cas9 to include a check for insertion of mobile elements," says Chiarle. "CRISPR is really a game-changer in genetic therapy, so it's very important to know exactly what it does to ensure its safety."

Rogue rearrangements

In retrotransposition, DNA sequences known as "mobile elements" move from one location in the genome to another. Using enzymes, they replicate themselves and create a break in both strands of the DNA double helix, where they insert themselves. This happens naturally and is often harmless—in fact, over the course of evolution, mobile elements (also called "jumping genes") have come to make up approximately a third of our genome. But they have also been linked to disease, including cancer.

CRISPR, too, introduces double-strand breaks in DNA. Chiarle, Tao,

and colleagues show that this increases the chances that retrotransposition will happen, causing mobile elements to insert themselves at the very DNA locations intended to be edited by CRISPR, as well as some unintended locations.

"We did CRISPR in multiple cell lines, including those commonly used in many labs, and found an average rate of retrotransposition of up to 5 to 6 percent," says Chiarle, the study's senior investigator. "This is a low number, but many gene therapies are meant to target millions of cells. For example, in blood disorders, CRISPR may be used to edit a few million blood stem cells, which are then reinfused into the patient. To initiate a tumor, you may sometimes need just one cell with a transposition event."

Chiarle stresses that this study was purely experimental, done in cells in the laboratory. "We need to determine how often retrotransposition happens in clinical trials of CRISPR gene therapies," he says.

Tao refined an existing test called PolyA-seq and set up the experimental system to validate it. The test identifies retrotransposition events involving LINE-1, the most common mobile element.

"We think this test could help detect these events more reliably, and it may be more cost-effective than the method that's commercially available," Tao says.

Base editing safer?

Chiarle, Tao, and their colleagues also found that retrotransposition is much rarer during base editing—a newer, more precise technique that chemically changes just one base or "letter" of the genetic code (C or A) without causing a double-strand break in DNA. Retrotransposition events were detected less than 0.01 percent of the time. They were also

less frequent during prime editing, an advanced technique that enables targeted insertions, deletions, and all 12 possible base changes.

"We demonstrate that both base editors and prime editors are much safer and are associated with very low retrotransposition events compared to CRISPR/Cas9," says Chiarle.

More information: Jianli Tao et al, Frequency and mechanisms of LINE-1 retrotransposon insertions at CRISPR/Cas9 sites, *Nature Communications* (2022). DOI: 10.1038/s41467-022-31322-3

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