Medical Xpress

Scientists develop a rapid gene-editing screen to find effects of cancer mutations

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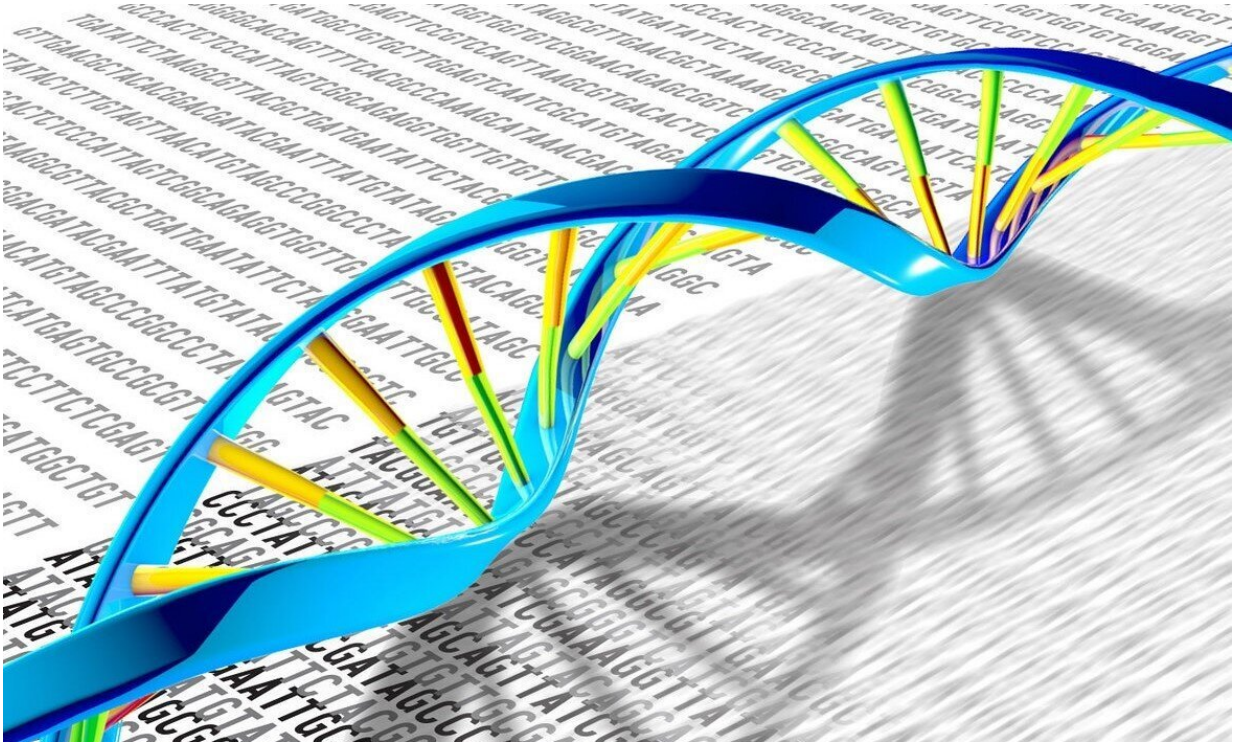

DNA, which has a double-helix structure, can have many genetic mutations and variations. Credit: NIH

Tumors can carry mutations in hundreds of different genes, and each of those genes may be mutated in different ways—some mutations simply replace one DNA nucleotide with another, while others insert or delete larger sections of DNA.

Until now, there has been no way to quickly and easily screen each of those mutations in their natural setting to see what role they may play in the development, progression, and treatment response of a tumor. Using a variant of CRISPR genome-editing known as prime editing, MIT researchers have now come up with a way to screen those mutations much more easily.

The researchers demonstrated their technique by screening cells with more than 1,000 different mutations of the tumor suppressor gene p53, all of which have been seen in cancer patients. This method, which is easier and faster than any existing approach, and edits the genome rather than introducing an artificial version of the mutant gene, revealed that some p53 mutations are more harmful than previously thought.

This technique could also be applied to many other cancer genes, the researchers say, and could eventually be used for precision medicine, to determine how an individual patient's tumor will respond to a particular treatment.

"In one experiment, you can generate thousands of genotypes that are seen in cancer patients, and immediately test whether one or more of those genotypes are sensitive or resistant to any type of therapy that you're interested in using," says Francisco Sanchez-Rivera, an MIT assistant professor of biology, a member of the Koch Institute for Integrative Cancer Research, and the senior author of the study.

MIT graduate student Samuel Gould is the lead author of the paper, which appears today in *Nature Biotechnology*.

Editing cells

The new technique builds on research that Sanchez-Rivera began 10 years ago as an MIT graduate student. At that time, working with Tyler

Jacks, the David H. Koch Professor of Biology, and then-postdoc Thales Papagiannakopoulos, Sanchez-Rivera developed a way to use CRISPR genome-editing to introduce into mice genetic mutations linked to lung cancer.

In that study, the researchers showed that they could delete genes that are often lost in lung tumor cells, and the resulting tumors were similar to naturally arising tumors with those mutations. However, this technique did not allow for the creation of point mutations (substitutions of one nucleotide for another) or insertions.

"While some cancer patients have deletions in certain genes, the vast majority of mutations that cancer patients have in their tumors also include point mutations or small insertions," Sanchez-Rivera says.

Since then, David Liu, a professor in the Harvard University Department of Chemistry and Chemical Biology and a core institute member of the Broad Institute, has developed new CRISPR-based genome editing technologies that can generate additional types of mutations more easily. With base editing, developed in 2016, researchers can engineer point mutations, but not all possible point mutations. In 2019, Liu, who is also an author of the *Nature Biotechnology* study, developed a technique called prime editing, which enables any kind of point mutation to be introduced, as well as insertions and deletions.

"Prime editing in theory solves one of the major challenges with earlier forms of CRISPR-based editing, which is that it allows you to engineer virtually any type of mutation," Sanchez-Rivera says.

When they began working on this project, Sanchez-Rivera and Gould calculated that if performed successfully, prime editing could be used to generate more than 99% of all small mutations seen in cancer patients.

However, to achieve that, they needed to find a way to optimize the editing efficiency of the CRISPR-based system. The prime editing guide RNAs (pegRNAs) used to direct CRISPR enzymes to cut the genome in certain spots have varying levels of efficiency, which leads to "noise" in the data from pegRNAs that simply aren't generating the correct target mutation. The MIT team devised a way to reduce that noise by using synthetic target sites to help them calculate how efficiently each guide RNA that they tested was working.

"We can design multiple prime-editing guide RNAs with different design properties, and then we get an empirical measurement of how efficient each of those pegRNAs is. It tells us what percentage of the time each pegRNA is actually introducing the correct edit," Gould says.

Analyzing mutations

The researchers demonstrated their technique using p53, a gene that is mutated in more than half of all cancer patients. From a dataset that includes sequencing information from more than 40,000 patients, the researchers identified more than 1,000 different mutations that can occur in p53.

"We wanted to focus on p53 because it's the most commonly mutated gene in human cancers, but only the most frequent variants in p53 have really been deeply studied. There are many variants in p53 that remain understudied," Gould says.

Using their new method, the researchers introduced p53 mutations in human lung adenocarcinoma cells, then measured the survival rates of these cells, allowing them to determine each mutation's effect on cell fitness.

Among their findings, they showed that some p53 mutations promoted

cell growth more than had been previously thought. These mutations, which prevent the p53 protein from forming a tetramer—an assembly of four p53 proteins—had been studied before, using a technique that involves inserting artificial copies of a mutated p53 gene into a cell.

Those studies found that these mutations did not confer any survival advantage to cancer cells. However, when the MIT team introduced those same mutations using the new prime editing technique, they found that the mutation prevented the tetramer from forming, allowing the cells to survive. Based on the studies done using overexpression of artificial p53 DNA, those mutations would have been classified as benign, while the new work shows that under more natural circumstances, they are not.

"This is a case where you could only observe these variant-induced phenotypes if you're engineering the variants in their natural context and not with these more artificial systems," Gould says. "This is just one example, but it speaks to a broader principle that we're going to be able to access novel biology using these new genome-editing technologies."

Because it is difficult to reactivate tumor suppressor genes, there are few drugs that target p53, but the researchers now plan to investigate mutations found in other cancer-linked genes, in hopes of discovering potential cancer therapies that could target those mutations. They also hope that the technique could one day enable personalized approaches to treating tumors.

"With the advent of sequencing technologies in the clinic, we'll be able to use this genetic information to tailor therapies for patients suffering from tumors that have a defined genetic makeup," Sanchez-Rivera says. "This approach based on prime editing has the potential to change everything."

More information: High-throughput evaluation of genetic variants with prime editing sensor libraries, *Nature Biotechnology* (2024). DOI: 10.1038/s41587-024-02172-9

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