Powerful gene-editing tool appears to cause off-target mutations in human cells

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In the past year a group of synthetic proteins called CRISPR-Cas RNA-guided nucleases (RGNs) have generated great excitement in the scientific community as gene-editing tools. Exploiting a method that some bacteria use to combat viruses and other pathogens, CRISPR-Cas RGNs can cut through DNA strands at specific sites, allowing the insertion of new genetic material. However, a team of Massachusetts General Hospital (MGH) researchers has found a significant limitation to the use of CRISPR-Cas RGNs, production of unwanted DNA mutations at sites other than the desired target.

"We found that expression of CRISPR-Cas RGNs in human cells can have off-target effects that, surprisingly, can occur at sites with significant sequence differences from the targeted DNA site," says J. Keith Joung, MD, PhD, associate chief for Research in the Massachusetts General Hospital (MGH) Department of Pathology and co-senior author of the report receiving online publication in *Nature Biotechnology*. "RGNs continue to have tremendous advantages over other genome editing technologies, but these findings have now focused our work on improving their precision."

Consisting of a DNA-cutting enzyme called Cas 9, coupled with a short, 20-nucleotide segment of RNA that matches the target DNA segment, CRISPR-Cas RGNs mimic the primitive immune systems of certain bacteria. When these microbes are infected by viruses or other organisms, they copy a segment of the invader's genetic code and incorporate it into their DNA, passing it on to future bacterial

generations. If the same pathogen is encountered in the future, the bacterial enzyme called Cas9, guided by an RNA sequence the matches the copied DNA segment, inactivates the pathogen by cutting its DNA at the target site.

About a year ago, scientists reported the first use of programmed CRISPR-Cas RGNs to target and cut specific DNA sites. Since then several research teams, including Joung's, have succesfully used CRISPR-Cas RGNs to make genomic changes in fruit flies, zebrafish, mice and in human cells – including induced pluripotent stem cells which have many of the characteristics of embryonic stem cells. The technology's reliance on such a short RNA segment makes CRISPR-Cas RGNs much easier to use than other gene-editing tools called zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), and RGNs can be programmed to introduce several genetic changes at the same time.

However, the possibility that CRISPR-Cas RGNs might cause additional, unwanted genetic changes has been largely unexplored, so Joung's team set out to investigate the occurrence of "off-target" mutations in human cells expressing CRISPR-Cas RGNs. Since the interaction between the guiding RNA segment and the target DNA relies on only 20 nucleotides, they hypothesized that the RNA might also recognize DNA segments that differed from the target by a few nucleotides.

Although previous studies had found that a single-nucleotide mismatch could prevent the action of some CRISPR-Cas RGNs, the MGH team's experiments in human cell lines found multiple instances in which mismatches of as many as five nucleotides did not prevent cleavage of an off-target DNA segment. They also found that the rates of mutation at off-target sites could be as high or even higher than at the targeted site, something that has not been observed with off-target mutations associated with ZFNs or TALENs.

"Our results don't mean that RGNs cannot be important research tools, but they do mean that researchers need to account for these potentially confounding effects in their experiments. They also suggest that the existing RGN platform may not be ready for therapeutic applications," says Joung, who is an associate professor of Pathology at Harvard Medical School. "We are now working on ways to reduce these off-target effects, along with methods to identify all potential off-target sites of any given RGN in human cells so that we can assess whether any second-generation RGN platforms that are developed will be actually more precise on a genome-wide scale. I am optimistic that we can further engineer this system to achieve greater specificity so that it might be used for therapy of human diseases."

More information: High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells, DOI: 10.1038/nbt.2623

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