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Unintended Effects of Genetic Manipulation
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Using CRISPR-Cas9 to Genetically Engineer Human Therapies Could Pose Increased Risk of Cancer, Two Studies Suggest

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When the popular new genetic-engineering method called CRISPR-Cas9 is used to genetically modify certain types of human cells in the lab, the cells most likely to survive the process and carry the intended modification are also ones which may be more susceptible to becoming cancerous later, two separate new studies suggest.

The peer-reviewed studies were both published in the same issue of *Nature Medicine* on June 11, 2018. Each explores why using standard CRISPR-Cas9 technology to cut double strands of DNA and then try to remove genetic material and replace it with a new stretch of DNA is less effective with lines of particular types of cultured human cells than with other human and non-human cells. Both studies suggest that genetic engineering with CRISPR-Cas9 can activate in targeted cells a protein called tumor protein 53 (or simply p53).

That protein responds to DNA damage in a cell by setting in motion processes that lead either to the cell's repair or, if it can't be repaired, to the mutated cell's death — providing an important cellular pathway to help prevent the growth of cancers. In fact, p53 is so important for regulating cell division and preventing tumors that it is known as the "guardian of the genome," according to the U.S. National Library of Medicine's online guide to genetics. But its action also poses a new challenge for attempts to engineer cells with CRISPR-Cas9.

In one of the studies, a team of researchers from universities in Sweden, Finland, and the United Kingdom found that applying CRISPR-Cas9 technology to a particular type of human retinal cells cultured in the lab to try to slice out a stretch of DNA and replace it with another one activated the p53 protein. The defensive process thus triggered either foiled the attempted engineering or killed off many of the engineered cells – thereby reducing the "efficiency" of CRISPR-Cas9 for gene "editing," the study concluded. It added: "Thus, cells that do not have p53 or are unable to activate it show better gene editing. Unfortunately, however, lack of p53 is also known to contribute to making cells grow uncontrollably and become cancerous."

"By picking cells that have successfully repaired the damaged gene we intended to fix, we might inadvertently also pick cells without functional p53," explained

Emma Haapaniemi, researcher at the Department of Medicine, Huddinge, Karolinska Institutet and the study's co-first author, in a Karolinska Institutet news release. "If transplanted into a patient, as in gene therapy for inherited diseases, such cells could give rise to cancer, raising concerns for the safety of CRISPR-based gene therapies."

The researchers reported that did not see the same pattern of cells with normally functioning p53 being difficult to engineer when they used CRISPR-Cas9 only to remove a stretch of DNA, without trying to insert new material -- instead relying on the cell to repair the break correctly. However, that simpler process of genetic engineering is "error-prone," they noted, compared to the more precise method of also inserting corrective genetic material.

The second study involved cultured lines of human pluripotent stem cells (hPSCs) — which are self-replicating cells that have the capacity to become many different types of human cells. Such lines used in research originally were derived from human embryos or by being induced in a process called cellular reprogramming. This second study was conducted by researchers at the Novartis Institutes for Biomedical Research in Cambridge, MA, which is part of Novartis International AG, a major global pharmaceutical company.

In their research, they devised a way to achieve a higher rate of success in engineering the particular genetic changes in hPSCs they were intending with the use of CRISPR-Cas9 than has been common in earlier research. But in the process, they discovered that the double-strand breaks in DNA they intentionally caused by using CRISPR-Cas9 activated the p53 protein, leading to the death of most of the cells they had engineered. This was the case whether they were trying to remove a stretch of DNA and replace it with a new, engineered one or whether they were just trying to remove genetic material.

Moreover, hPSCs are known to be subject to a low but still "significant" level of spontaneous mutations that are linked to abnormal p53 response. So the researchers warned that any human therapies using hPSCs engineered with CRISPR-Cas9 would need to carefully evaluate whether surviving engineered cells retained their normal p53 function.

"Our results indicate that Cas9 toxicity creates an obstacle to the high-throughput use of CRISPR/Cas9 for genome engineering and screening in hPSCs," the researchers concluded. "Moreover, as hPSCs can acquire *P53* mutations, cell replacement therapies using CRISPR/Cas9-enginereed hPSCs should proceed with caution, and such engineered hPSCs should be monitored for P53 function."

This second team of researches experimented with temporarily inhibiting the action of p53 in normal cells long enough to engineer them with CRISPR-Cas9. That did increase the number of such normal cells they were able to engineer and that survived. But it also presented its own issues.

The researchers found that even temporarily inhibiting cells' DNA damage signaling in this way "has the potential to increase off-target mutations and poses

a risk for cancer."

They added: "Before engineering patient cells, the risks and benefits must be fully evaluated. It will be imperative to determine the spontaneous mutation rate of *P53* in engineered cells as well as the mutational burden associated with transient *P53* inhibition. As hPSC-based cell therapies using genome-edited cells move into the clinic, it will be critical to ensure that patient cells have a functional *P53* before and after engineering."

Sources

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