New CRISPR advance may solve key quandary

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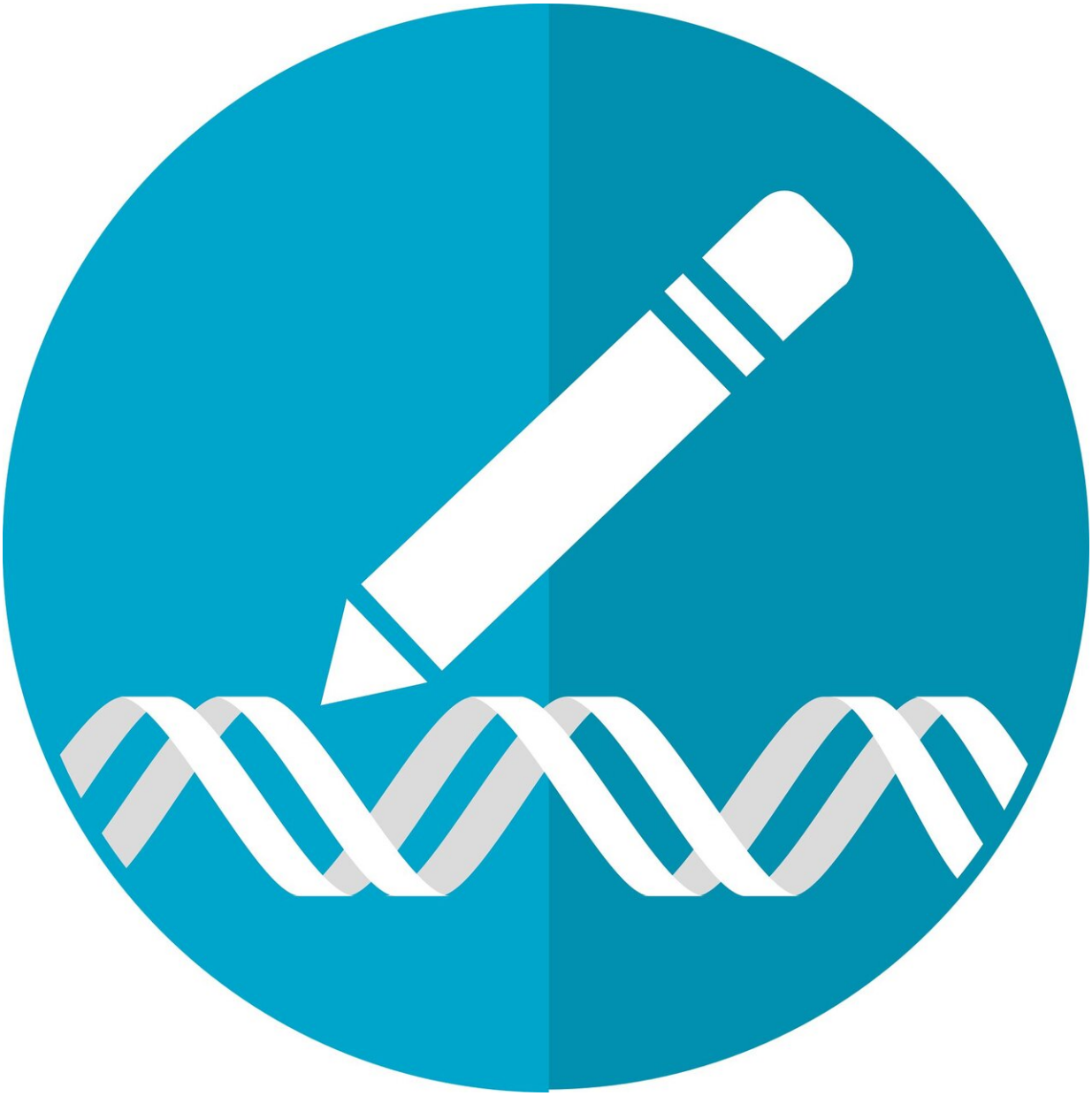

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A mutation unique to certain cancer tumors is a potential homing beacon for safely deploying CRISPR gene editing enzymes to disarm DNA that makes cancer cells resistant to treatment, while ignoring the gene in normal cells where it's critical to healthy function, according to a new study from ChristianaCare's Gene Editing Institute in the journal *Molecular Cancer Research*.

"This advance addresses a big challenge with using CRISPR in cancer patients, which is ensuring it can distinguish between a tumor cell and a normal cell," said Eric Kmiec, Ph.D., director of ChristianaCare's Gene Editing Institute and principal author of the study.

According to a commentary from journal editors accompanying the study, the process developed by the Gene Editing Institute can "provide an empirical basis for the use of CRISPR-directed gene therapy in solid tumor cells, and continue to advance the use of this technology closer to clinical implementation." Journal editors praised the study for "reporting on the molecular kinetics of CRISPR activity in lung cancer cells for the first time."

Kmiec said the primary focus of the study was to successfully use CRISPR to knock out a gene called NRF2 that protects squamous cell carcinoma lung cancer tumors from being affected by chemotherapy or radiation—but without affecting normal cells. In normal cells, NRF2 can help protect them from various types of damage.

Kmiec said the Gene Editing Institute has done multiple tests in animals to establish that disabling NRF2 with CRISPR increases sensitivity to chemotherapy. They are now conducting tests in animals to further

confirm selective targeting of NRF2 in squamous cell tumors and to assess any safety concerns in order to lay the groundwork for a clinical trial in patients. The trial would test whether using CRISPR to knock out the NRF2 gene in squamous cell carcinoma lung cancer tumors improves the efficacy of conventional chemotherapy and radiation treatments. The study notes that the presence of the NRF2 gene in tumors confers a "dismal prognosis" because it protects tumors from being shrunk or destroyed by these therapies.

But Kmiec said there are several other cancers, including esophageal, head and neck, and certain forms of uterine and bladder cancer, that have similar features. They produce tumors that are frequently protected by the NRF2 gene. And like squamous cell tumors, they also have mutations that create what is technically known as a PAM site (short for protospacer adjacent motif) that can serve as a target for keeping CRISPR edits focused exclusively on tumors.

Kmiec and lead author Kelly Banas said the NRF2 gene typically shows up early in tumor development and can be detected by existing diagnostic tests. They said moving quickly with CRISPR to disable NRF2 could improve the efficacy of conventional treatments and potentially lower the dosages required to shrink tumors.

"In a way, we are trying to use the most advanced tool in medical science to enhance the efficacy of some of the mainstays of conventional cancer treatment," Banas said.

Banas said the inspiration for the study came during a conference of lung cancer specialists that included a discussion of genetic sequences that are unique to squamous cell tumors. She said she then set out to explore whether one of these mutations could serve as a "recognition site" for a CRISPR enzyme.

"I was basically looking for something unique to the NRF2 gene in tumor cells that could essentially tell CRISPR 'here is the site where I am supposed to bind and do my work," she said. "Without any targeted therapy available for this type of lung cancer, the ability to use CRISPR to safely disarm a key mechanism that allows tumors to grow even when being hit with chemotherapy could be an important advance."

CRISPR stands for "clustered regularly interspaced short palindromic repeats." It is a defense mechanism found in bacteria that can recognize and slice up the DNA of invading viruses. Scientists have learned how to modify this mechanism so it can be directed to "edit" specific sequences of DNA code. In patient applications, the goal is to use CRISPR to repair defective genes that can cause disease or eliminate or knock out sequences that are causing problems. But challenges arise when the sequences in question are present in both healthy and diseased cells.

More information: Kelly Banas et al, Kinetics of Nuclear Uptake and Site-Specific DNA Cleavage during CRISPR-Directed Gene Editing in Solid Tumor Cells, *Molecular Cancer Research* (2020). DOI: 10.1158/1541-7786.MCR-19-1208

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