Researchers identify drugs that block CRISPR-Cas9 genome editing

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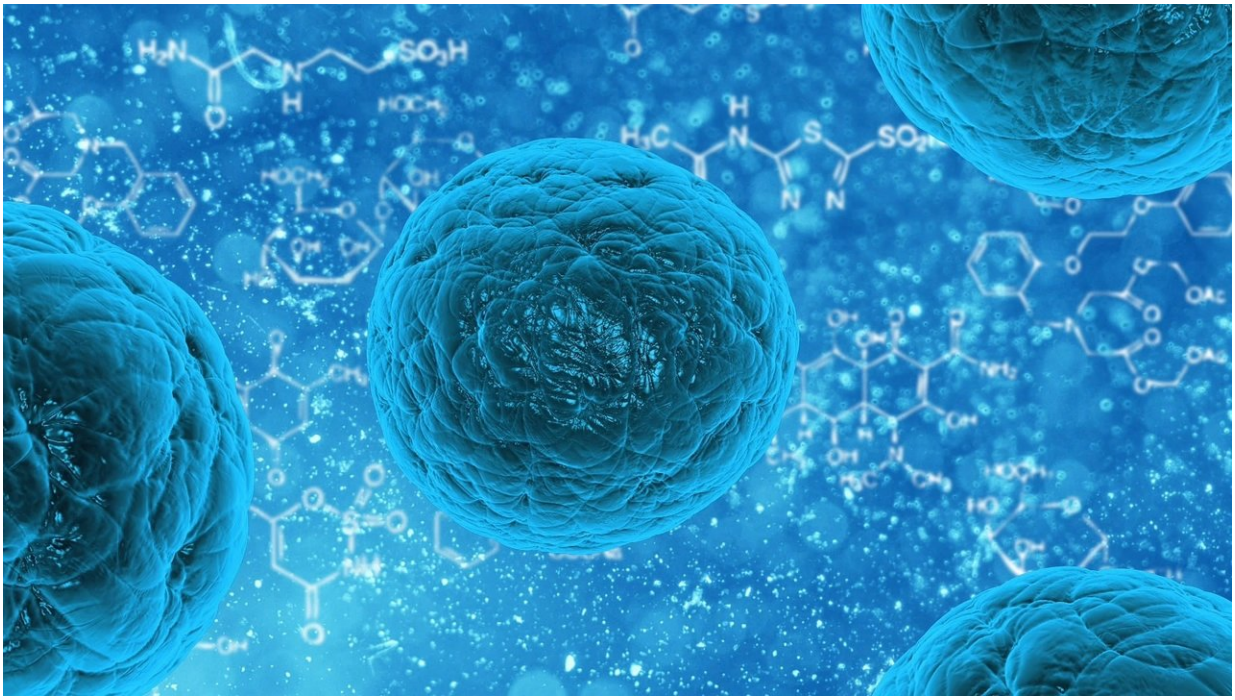

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The discovery of the first small-molecule inhibitors of the Streptococcus pyogenes Cas9 (SpCas9) protein could enable more precise control over CRISPR-Cas9-based genome editing, researchers report May 2nd in the journal *Cell*.

By developing a suite of high-throughput biochemical and cell-based

assays, the researchers screened a diverse collection of small molecules to identify compounds that disrupt the binding of SpCas9 to DNA and thereby interfere with its ability to cut DNA. These first small-molecule CRISPR-Cas9 inhibitors readily enter cells and are much smaller than the previously discovered anti-CRISPR proteins. The new compounds allow for reversible and dose-dependent control of SpCas9-based technologies, including its applications for gene editing, base editing, and epigenetic editing in mammalian cells.

"These studies lay the foundation for the rapid identification and use of small-molecule inhibitors against both SpCas9 and next-generation CRISPR-associated nucleases," says senior author Amit Choudhary of the Broad Institute, Harvard Medical School, and Brigham and Women's Hospital. "Small-molecule inhibitors targeting CRISPR-associated nucleases have the potential for widespread use in basic, biomedical, and defense research, as well as in biotechnological applications."

Currently, SpCas9 is being developed as a gene therapy agent for multiple conditions, including HIV, vision disorders, muscular dystrophy, and other hereditary disorders. But these therapeutic applications would greatly benefit from precise control over the dose and timing of SpCas9 activity to reduce off-target effects. Controlling these aspects of SpCas9 activity could also benefit other applications, such as efficiently editing the DNA of model organisms to model and study disease, and the use of gene drives in genetically engineered mosquitoes engineering mosquitoes to curb the spread of malaria and other mosquito-borne diseases.

The need for dose and temporal control of SpCas9 has created a demand for anti-CRISPR molecules. Although anti-CRISPR proteins that target SpCas9 exist, they are large and impermeable to cells, irreversible in action, can be chewed up by proteases, and may pose the risk of adverse immune reactions in the body. By contrast, small-molecule inhibitors are

proteolytically stable, reversible, and generally non-immunogenic and can easily be delivered to cells through passive diffusion. In addition, they can be synthesized on a large scale at low cost with little batch-to-batch variability.

In the new study, Choudhary and his team introduced a robust, sensitive, and scalable platform for the rapid and cost-efficient identification and validation of small-molecule inhibitors of SpCas9. Measuring CRISPR-Cas9 activity in a high-throughput way that would allow for drug screening has been challenging due to the properties of the SpCas9 enzyme. In the new paper, Choudhary and colleagues developed high-throughput primary and secondary assays for SpCas9-DNA binding and SpCas9 DNA-cutting activity, respectively. For the primary assay, they used a biochemical technique called fluorescence polarization to monitor the interaction between SpCas9 and a fluorophore-labeled DNA segment containing PAM sequences. In the secondary assay, they used automated microscopy to measure fluorescence changes induced by SpCas9-mediated DNA cleavage of a reporter gene in cells.

Using these assays, the researchers first screened representative members of multiple classes of small molecules to identify the class whose members frequently inhibited SpCas9. The team identified two lead compounds that disrupt the ability of SpCas9 to bind DNA and inhibit SpCas9-mediated DNA cleavage in a dose-dependent manner in mammalian cells. Since they block DNA binding by the enzyme, these molecules also inhibit catalytically-impaired technologies of SpCas9, including those for transcriptional activation, and are stable in human plasma.

"These results lay the foundation for precise chemical control over CRISPR-Cas9 activities, enabling the safe use of such technologies," Choudhary says. "However, these molecules are not ready for applications in humans and not tested for efficacy in organisms."

In future studies, the researchers plan to identify the inhibitors' binding sites on the SpCas9:gRNA complex, examine their mechanism of action, and optimize their potency. They will also determine whether the molecules interact with other targets in mammalian cells, and assess their specificity toward other CRISPR-associated nucleases. Early results included in the *Cell* paper indicate that the molecules are quite specific for their target, as they have no effect on a distantly-related CRISPR enzyme, Cas12a.

More information: Maji et al. "A High-Throughput Platform to Identify Small-Molecule Inhibitors of CRISPR-Cas9," *Cell* (2019). DOI: 10.1016/j.cell.2019.04.009

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