Novel technology vastly improves CRISPR/Cas9 accuracy

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A new CRISPR/Cas9 technology developed by scientists at the University of Massachusetts Medical School is precise enough to surgically edit DNA at nearly any genomic location, while avoiding potentially harmful off-target changes typically seen in standard CRISPR gene editing techniques. By pairing the CRISPR/Cas9 system with a programmable DNA-binding domain (CRISPR/Cas9-pDBD), researchers have created an additional proofreading step that improves the accuracy of the gene editing system and opens the door to potential clinical and gene therapy applications.

"While the standard CRISPR/Cas9 systems are great at making breaks in the genome with a single guide RNA in vitro, this technique is suboptimal for most gene therapy applications involving the editing of a large population of cells where minimizing collateral damage to the genome is critical," said Scot Wolfe, PhD, associate professor of molecular, cell & cancer biology at UMass Medical School. "So we've added an extra proofreading step to the system. By fusing a zinc finger DNA-binding domain to the CRISPR/Cas9 system, it now verifies an additional genetic feature in its intended target site before it will cut the genome. We've shown that this dramatically improves the precision of the CRISPR/Cas9 system by almost 100 fold."

The study was published in *Nature Methods*. UMMS researchers are already developing this nuclease platform to excise latent HIV provirus from the genome of infected cells and potentially correct the genetic mutation that leads to chronic granulomatous disease, an inherited

disease that renders the immune cells incapable of forming the reactive compounds necessary to kill certain bacterial and fungal pathogens.

The CRISPR/Cas9 system is an adaptive immune system used by bacteria to defend itself against bacteriophage and other types of foreign genetic material. It consists of two components: a molecular scalpel—Cas9—that cuts DNA efficiently but is muzzled in its native state and an RNA guide complex that unlocks the scalpel when a matching genetic sequence, defining the exact spot to cut, is found. These RNA guides are produced from clustered regularly interspaced short palindromic repeats or CRISPR arrays, which contain remnants of the genomes of past viral infections. By arming the Cas9 nuclease to target and inactivate these viruses, the CRISPR/Cas9 system provides an adaptive immune defense for the bacterial cells.

Scientists can reprogram the CRISPR/Cas9 system with artificial guide RNAs to cleave sequences within mammalian genomes and enable the surgical insertion of new fragments of genetic information into cells. A simple and efficient way of editing the genome, CRISPR/Cas9 is revolutionizing biomedical research by making it far easier to inactivate or activate genes in a cell line for study. It also simplifies creation of animal disease models that can be used to study human ailments. Work that used to take months or years to perform can now be done in weeks.

Despite the power of the CRISPR/Cas9 system, it isn't perfect. There are times when the RNA guide used to maneuver the cleaving enzyme into the right position within the genome also targets the enzyme to other sequences that are similar but not identical. These mismatched sites, which can occur as many as 100 times across the 6 billion nucleotides that make up the human genome, can sometimes be cleaved, causing unintended damage.

"Though not all of these 100 sites might cleave, if you're altering

millions of cells, like you would in many potential clinical applications, chances are you'll have some with breaks and new insertions at places that weren't intended. This can result in local mutagenesis or genomic re-arrangements that could potentially cause problems, such as cancer," said Dr. Wolfe.

Zinc-finger domains are proteins that can be engineered to bind to specific regions of the genome. By combining the DNA-binding zinc-finger domains with the efficient cleaving mechanism and RNA guide of the CRISPR/Cas9 system, Wolfe and colleagues have created a new system that is both efficient and more accurate. In the current study, his team has shown that off-target cleavage events associated with the targeting of two different genomic locations dropped below detectable levels when CRISPR/Cas9 is combined with a zinc-finger DNA binding domain. At a third target site, the number of off-target cleavage events dropped by 10 fold.

"What we have is like a GPS for CRISPR/Cas9," said Wolfe. "By combing the DNA-binding domain with CRISPR/Cas9, we have created an exciting new technological platform that minimizes the potential risks for patients in need of gene therapy based treatments that could be addressed using artificial nucleases."

Working with Peter E. Newburger, MD, professor of pediatrics and molecular, cell & cancer biology, and Erik J. Sontheimer, PhD, professor of molecular medicine, Wolfe and his team are developing this modified Cas9 system to directly repair ex vivo disease-causing mutations in the hematopoietic stem cells from patients with chronic granulomatous disease. The ultimate goal would be to reintroduce the corrected cells to patients to restore their immune function. Wolfe is also working with Jeremy Luban, MD, the David J. Freelander Professor in AIDS Research and professor of molecular medicine, and a team of colleagues at UMass Medical School to develop these nucleases to

efficiently and precisely excise latent HIV virus out of an infected cell.

More information: Mehmet Fatih Bolukbasi et al. DNA-binding-domain fusions enhance the targeting range and precision of Cas9, *Nature Methods* (2015). DOI: 10.1038/nmeth.3624

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