Scientists develop alternative to gene therapy: The technique points to safer, simpler potential HIV treatment

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Scientists at The Scripps Research Institute have discovered a surprisingly simple and safe method to disrupt specific genes within cells. The scientists highlighted the medical potential of the new technique by demonstrating its use as a safer alternative to an experimental gene therapy against HIV infection.

"We showed that we can modify the genomes of cells without the troubles that have long been linked to traditional gene therapy techniques," said the study's senior author Carlos F. Barbas III, who is the Janet and Keith Kellogg II Professor of Molecular Biology and Chemistry at The Scripps Research Institute.

The new technique, reported in Nature Methods on July 1, 2012, employs zinc finger nuclease (ZFN) proteins, which can bind and cut DNA at precisely defined locations in the genome. ZFNs are coming into widespread use in scientific experiments and potential disease treatments, but typically are delivered into cells using potentially risky gene therapy methods.

The Scripps Research scientists simply added ZFN proteins directly to cells in a lab dish and found that the proteins crossed into the cells and performed their gene-cutting functions with high efficiency and minimal collateral damage.

"This work removes a major bottleneck in the efficient use of ZFN proteins as a gene therapy tool in humans," said Michael K. Reddy, who oversees transcription mechanism grants at the National Institutes of Health's (NIH) National Institute of General Medical Sciences, which helped fund the work, along with an NIH Director's Pioneer Award. "The directness of Dr. Barbas's approach of 'simply' testing the notion that ZFNs could possess an intrinsic cell-penetrating ability is a testament to his highly creative nature and further validates his selection as a 2010 recipient of an NIH Director's Pioneer Award."

Questioning Assumptions

ZFNs, invented in the mid-1990s, are artificial constructs made of two types of protein: a "zinc-finger" structure that can be designed to bind to a specific short DNA sequence, and a nuclease enzyme that will cut DNA at that binding site in a way that cells can't repair easily. The original technology to make designer zinc finger proteins that are used to direct nucleases to their target genes was first invented by Barbas in the early 1990s.

Scientists had assumed that ZFN proteins cannot cross cell membranes, so the standard ZFN delivery method has been a gene-therapy technique employing a relatively harmless virus to carry a designer ZFN gene into cells. Once inside, the ZFN gene starts producing ZFN proteins, which seek and destroy their target gene within the cellular DNA.

One risk of the gene-therapy approach is that viral DNA—even if the virus is not a retrovirus—may end up being incorporated randomly into cellular DNA, disrupting a valuable gene such as a tumor-suppressor gene. Another risk with this delivery method is that ZFN genes will end up producing too many ZFN proteins, resulting in a high number of "off-target" DNA cuts. "The viral delivery approach involves a lot of off-target damage," said Barbas.

In the new study, Barbas and his colleagues set out to find a safer ZFN delivery method that didn't involve the introduction of viruses or other genetic material into cells. They experimented initially with ZFN proteins that carry extra protein segments to help them penetrate cell membranes, but found these modified ZFNs hard to produce in useful quantities. Eventually, the scientists recognized that the zinc-finger segments of ordinary ZFNs have properties that might enable the proteins to get through cell membranes on their own.

"We tried working with unmodified ZFNs, and lo and behold, they were easy to produce and entered cells quite efficiently," Barbas said.

New Strategy Against HIV

Next, the team showed how the new technique could be used in a ZFN-based strategy against HIV infection.

The AIDS-causing retrovirus normally infects T cells via a T cell surface receptor called CCR5, and removing this receptor makes T cells highly resistant to HIV infection. In 2006, an HIV patient in Berlin lost all signs of infection soon after receiving a bone marrow transplant to treat his leukemia from a donor with a CCR5 gene variant that results in low expression of the receptor. Disrupting the CCR5 gene in T cells with a ZFN-based therapy might be able to reproduce this dramatic effect.

"The idea is to protect some of the patient's T cells from HIV, so that the immune system remains strong enough ultimately to wipe out the infection," said Barbas.

A gene therapy that uses ZFNs to disrupt CCR5 genes in T cells and reinfuses the modified T cells into patients is currently in clinical trials. Barbas and his team showed that they could achieve the same effect with their simpler ZFN-delivery method. They added ZFN proteins directly

to human T cells in a culture dish and found that within hours, a significant fraction of the ZFN-treated cells showed sharp reductions in CCR5 gene activity.

After several applications of ZFNs, aided by a special cooling method that improves the ability of the proteins to get across cell membranes, the scientists were able to inactivate CCR5 genes with an efficiency approximating that of the gene therapy-based approach, Barbas said.

The new approach also appeared to be safer. A DNA-based method the team used for comparison or the viral-based methods reported in the literature by others ended up producing ZFNs for up to several days, causing a significant amount of off-target DNA damage. But the directly delivered ZFN proteins remained intact within cells for only a few hours, causing minimal off-target damage.

"At some off-target locations where the gene therapy approach frequently causes damage, we saw no damage at all from this new technique," said Barbas.

Hope for 'Tiny Factories' of Health

The team tested its direct ZFN-delivery technique with a variety of other cell types and found that it works with particularly high efficiency in human skin "fibroblast" cells. Researchers now are working on advanced therapies in which they harvest such fibroblasts from patients and reprogram the cells' gene-expression patterns so that they effectively become stem cells. These induced stem cells can then be modified using ZFNs and other genome-editing techniques. When reinfused into a patient, they can produce millions of therapeutic progeny cells over long periods.

Such techniques may one day be used to treat a vast array of diseases.

Barbas, who has been developing anti-CCR5 strategies for more than a decade, wants to start with a ZFN-based therapy that disrupts the CCR5 gene in hematopoietic stem cells. These blood-cell-making stem cells, reinfused into an HIV patient, would become tiny factories for producing HIV-resistant T cells.

"Even a small number of stem cells that carry this HIV-resistance feature could end up completely replacing a patient's original and vulnerable T cell population," he said.

More information: "Targeted gene knockout by direct delivery of ZFN proteins," Thomas Gaj et al., *Nature Methods*, 2012.

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