Virus-Free Gene Therapy for HIV Shows Promise

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An experimental gene therapy for HIV may actually be easier—and even safer—than experts originally hoped. Experimenting with zinc finger nucleases (ZFNs), researchers at the Scripps Research Institute in La Jolla, California, have discovered it's possible to cut out an undesirable "middle man"—a virus (not HIV) that carries the gene-altering payload to cells in the body. Until now, such viral vectors were considered a necessary element of gene therapy.

The new ZFN technique, tested by Carlos Barbas, PhD, of Scripps and his colleagues, is reviewed in the July issue of the journal Nature Methods.

"We showed that we can modify the genomes of cells without the troubles that have long been linked to traditional gene therapy techniques," said Barbas in an accompanying news announcement.

ZFNs—including SB-278, being developed by Sangamo BioSciences—are of particular interest to HIV researchers and have garnered a lot of attention by AIDS treatment activists and the community press.

How do ZFNs work? First, some background: After HIV binds to the CD4 protein on CD4 cells, the virus must then latch onto another receptor on the cell's surface—either CCR5 or CXCR4. Usually, when people contract HIV, their virus starts off using the CCR5 receptor. Later on, as HIV disease progresses, the virus can switch to the CXCR4 receptor—this occurs in about 50 percent of treatment-experienced patients.

Selzentry (maraviroc), an antiretroviral approved by the U.S. Food and Drug Administration, works by blocking the interaction between CCR5 and HIV, ultimately retarding the virus's ability to infect CD4 cells. ZFNs like SB-728 can go one step further—they can block the gene responsible for making CCR5, mimicking a naturally occurring human mutation that renders individuals largely resistant to the virus.

This mutation, dubbed CCR5 delta-32, appears to have no harmful effect in the human body. There's also the case of Timothy Brown, a.k.a. the Berlin Patient, an HIV-positive leukemia patient who was cured of both diseases when he received a bone marrow transplant from a "matched" donor who had inherited this delta-32 CCR5 mutation from both parents. (When the mutation is

inherited from one parent, CCR5 is produced, but it's at low quantities and is associated with slower HIV disease progression. When the mutation is inherited from both parents, which is very rare, little or no CCR5 is expressed on CD4 cells, rendering the cells impervious to forms of HIV that use the CCR5 receptor to enter cells.)

ZFNs have both therapeutic and curative potential. At present, the most widely known research involves Sangamo's therapeutic-focused CCR5-knockout ZFN, which is dubbed SB-728-T. Therapy involves removing CD4 cells from patients' blood, treating the cells with SB-728-T to knock out the CCR5 gene, multiplying the cells in the lab, then transplanting the HIV-resistant genetically modified cells back into the body.

The latest breakthrough involves the actual cell treatment process. Scientists had assumed that ZFN proteins cannot cross cell membranes, so the standard ZFN delivery method has been a gene-therapy technique in which a relatively harmless virus is used 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 potential risk of this 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.

Finally, gene therapies that employ viral DNA may not be effective—or may need to be delayed—in people with antibodies to the virus being used. For example, if a common cold virus is used to deliver the gene therapy to cells, possible candidates may be excluded from clinical trials because they have high levels of virus-specific antibodies that may attack the ZFNs.

Barbas and his colleagues set out to find a safer ZFN delivery method that didn't introduce 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 these modified ZFNs turned out to be 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.

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

Using Sangamo's SB-728-T results as a comparison, 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 CD4 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 similar to 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 and 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,” Barbas said.

Barbas and his colleagues are currently experimenting with a variety of other cell types, including hematopoietic stem cells. Similar to what Sangamo is attempting with its lead candidate SB-728, Barbas hopes to explore his team’s viral DNA-free ZFN approach to turn stem cells into “tiny factories” for producing HIV-resistant CD4 cells, potentially facilitating the cure for people living with the virus.

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