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HIV

Editing HIV entry

A genome-editing approach targeting the CC-chemokine receptor CCR5, which is the main co-receptor for entry of HIV-1 into $CD4^+$ T cells, has the potential to reconstitute immune function in HIV^+ individuals by creating an HIV-resistant $CD4^+$ T-cell population, according to a recent report in *Nature Biotechnology*.

Individuals who are homozygous for the Δ32 deletion in *CCR5* are resistant to HIV-1 infection, which makes CCR5 a potential target for HIV therapy. In this study, Perez *et al.* sought to permanently disrupt the endogenous *CCR5* gene in $CD4^+$ T cells — to mimic the Δ*32CCR5*-null genotype — using zinc-finger nucleases (ZFNs). The engineered ZFNs create double-strand breaks at specific sites in the genome, as determined by the sequence specificity of the DNA-binding domain, that are then imperfectly repaired and permanently disrupt the genomic sequence.

The authors designed ZFNs that target the DNA sequence encoding the first transmembrane domain of CCR5 and showed that in a reporter cell line expressing CCR5, transduction with the *CCR5*-targeted ZFNs (*CCR5*-ZFNs) resulted in high efficiency (50–80%) target-gene mutation and a more than tenfold decrease in cell-surface expression of CCR5. By analysing the consensus sequence of the ZFN-binding site in *CCR5*, 15 putative alternative cleavage sites were identified throughout the genome. In support of the specificity of the engineered ZFNs, there was no detectable ZFN activity at any of these sites with the exception of a low level of modification at *CCR2*, which is not predicted to result in a marked phenotype, and a very low frequency modification of *ABLIM2*, a gene that is not expressed in T cells and that is also not predicted to have adverse effects.

In vitro, cells transduced with the *CCR5*-ZFNs had decreased levels of infection with a CCR5-tropic HIV-1 isolate than did non-transduced cells or cells transduced with a control ZFN. When a $CD4^+$ T-cell line was transduced with suboptimal levels of *CCR5*-ZFNs (to create a low baseline level of endogenous *CCR5* disruption), infection with HIV-1 resulted in a 30-fold increase in the percentage of T cells with ZFN-modified *CCR5* alleles after 52 days of culture. Therefore, ZFN-mediated disruption of *CCR5* confers a long-term survival advantage on $CD4^+$ T cells in the presence of HIV-1 infection. This *in vitro* survival advantage against HIV-1 infection that was provided by the transduction with *CCR5*-ZFNs was confirmed for primary human $CD4^+$ T cells isolated from healthy donors expressing wild-type CCR5.

To explore the clinical feasibility of this approach, primary human $CD4^+$ T cells were transduced with *CCR5*-ZFNs and propagated in culture before adoptive transfer to severely immunodeficient mice. After 1 month of HIV-1 infection, there was a threefold enrichment for ZFN-disrupted *CCR5* alleles in HIV-1-infected mice compared with non-infected mice, which indicates that *CCR5* disruption also confers a survival advantage on $CD4^+$ T cells *in vivo*. Mice engrafted with *CCR5*-disrupted $CD4^+$ T cells compared with control $CD4^+$ T cells had markedly decreased plasma viraemia after HIV-1 infection, which shows that the modified cells confer resistance to HIV-1 infection.

The authors conclude that these results "support the clinical development of adoptive immunotherapy to reconstitute the memory cell pool of HIV-infected patients with ZFN-modified $CD4^+$ T cells", although the approach must first be analysed in terms of chronic infection of non-human primates.

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ORIGINAL RESEARCH PAPER Perez, E. E. *et al.* Establishment of HIV-1 resistance in $CD4^+$ T cells by genome editing using zinc-finger nucleases. *Nature Biotechnol.* **26**, 808–816 (2008)