The perils of cutting what you cannot see

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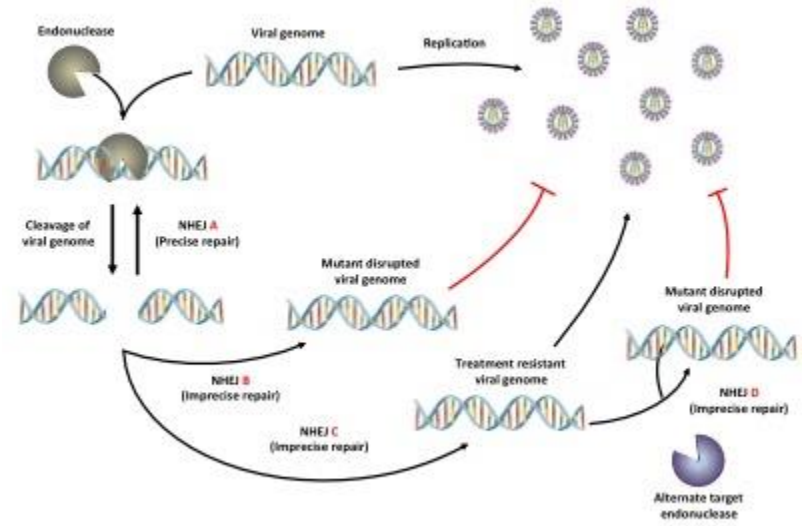

Generation of endonuclease-resistant virus. Upon endonuclease cleavage of an essential viral gene the DNA double strand break can be repaired perfectly (A), mutated to yield a replication incompetent virus (B), mutated to yield a treatment-resistant virus (C), or the genome can be treated with a second endonuclease to prevent treatment resistance (D).

Figure provided by Dr. Daniel Stone

Some sneaky viruses, such as the human immunodeficiency virus (HIV), can integrate their genome into the host's one and hide there for long periods of times (a phenomenon usually described as latency). This strategy protects them from the attacks of the immune system as well as from therapeutic agents. Thus far, complete eradication of such infections cannot be achieved and new therapeutic approaches are sought. A novel possibility consists in inactivating the viral DNA using enzymes that can recognize and cleave specific sequences. There are many variations of these enzymes including zinc finger endonucleases (ZFN), TAL effector nucleases (TALENs) and the CRISPR/Cas9 system. Their use presents several different challenges: the identification of all the sites where the virus establishes latency, the delivery of nucleases inside the cells, and the generation of viral mutants. Mutations can be generated through mechanisms linked to the viral genome replication, especially in the case of HIV, or directly upon imprecise DNA repair following endonuclease cleavage.

In a study led by Ms. Harshana De Silva Feelixge and Dr. Daniel Stone from the Jerome Laboratory at Fred Hutch (Vaccine and Infectious Disease Division), the effects of ZFN-mediated cleavage of the HIV pol gene in generating mutations was evaluated. Four different ZFNs were delivered by adeno-associated virus vectors into cells co-transfected with a plasmid carrying a replication-incompetent NL4-3 derived provirus (pDHIV3). ZFNs were effective in specifically cleaving HIV DNA

and about 10% of sequenced HIV genomes contained mutations in the sequences recognized by ZFN indicating a cleavage event, 30% of which were in frame, which means they could potentially still encode for functional proteins. Researchers consequently evaluated the effects of mutations on viral infectivity. To do so, they selected smaller mutations, which are more likely to encode for a protein retaining its function. Producer cells were transfected with plasmids carrying the mutated genes and subsequently the infectivity of the produced viral particles was evaluated. Most mutants showed no infectivity, although one mutant retained infectivity comparable to the wild-type counterpart. Moreover, cleavage of this mutant sequence by its cognate ZFN was only observed with a much lower efficiency. Introduction of a second mutation in other conserved regions of the gene eliminated the infectivity of the resistant virus, therefore, the use of multiple nucleases could further reduce viral replication.

A further problem that could arise from a process that introduces mutations is reduced sensitivity to antiretroviral drugs. Therefore, the researchers evaluated whether mutated viruses maintained their susceptibility to nucleotide or non-nucleotide reverse transcriptase inhibitors. None of the mutations induced a modification in the susceptibility to the drug.

The study, published in the January issue of Antiviral Research, describes for the first time the possibility that endonuclease treatment can induce mutant viruses to retain their infectivity. "So far targeted endonucleases including CRISPR/Cas9 have been used as antiviral agents against EBV, HBV, HCV, HIV, HPV, HSV, HTLV, and JCV, but this is the first time a treatment resistant virus has been detected. Fortunately, we should be able to negate treatment resistance by using multiple endonucleases that target different regions of the viral genome" said Dr. Stone, opening the possibility for a safe utilization of this approach. Ms. De Silva concluded with another positive note: "With new endonuclease platforms like CRISPR/Cas9 we would be able to deliver a single endonuclease to target multiple regions and therefore, overcome resistance emerging due to selective pressure but also due to endonuclease therapy itself."

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De Silva Feelixge HS, Stone D, Pietz HL, Roychoudhury P, Greninger AL, Schiffer JT, Aubert M, Jerome KR. 2016. Detection of treatment-resistant infectious HIV after genome-directed antiviral endonuclease therapy. *Antiviral Res*. 126:90-8.