Video Article

Interview: HIV-1 Proviral DNA Excision Using an Evolved Recombinase

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Abstract

HIV-1 integrates into the host chromosome of infected cells and persists as a provirus flanked by long terminal repeats. Current treatment strategies primarily target virus enzymes or virus-cell fusion, suppressing the viral life cycle without eradicating the infection. Since the integrated provirus is not targeted by these approaches, new resistant strains of HIV-1 may emerge. Here, we report that the engineered recombinase Tre (see Molecular evolution of the Tre recombinase , Buchholz, F., Max Planck Institute for Cell Biology and Genetics, Dresden) efficiently excises integrated HIV-1 proviral DNA from the genome of infected cells. We produced loxLTR containing viral pseudotypes and infected HeLa cells to examine whether Tre recombinase can excise the provirus from the genome of HIV-1 infected human cells. A virus particle-releasing cell line was cloned and transfected with a plasmid expressing Tre or with a parental control vector. Recombinase activity and virus production were monitored. All assays demonstrated the efficient deletion of the provirus from infected cells without visible cytotoxic effects. These results serve as proof of principle that it is possible to evolve a recombinase to specifically target an HIV-1 LTR and that this recombinase is capable of excising the HIV-1 provirus from the genome of HIV-1-infected human cells.

Before an engineered recombinase could enter the therapeutic arena, however, significant obstacles need to be overcome. Among the most critical issues, that we face, are an efficient and safe delivery to targeted cells and the absence of side effects.

Video Link

The video component of this article can be found at http://www.jove.com/video/793/

Protocol

Please view related videos entitled Principles of Site-Specific Recombinase (SSR) Technology and Molecular Evolution of the Tre Recombinase for more information about site-specific recombination and engineering the site-specificity of recombinase enzymes.

Disclosures

The authors have nothing to disclose.

