Eradication of integrated HIV-1 genome from latently infected T-cells by targeting LTR sequences using CRISPR/Cas9 gene editing system.

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Abstract

Longevity, homeostatic proliferation and resistance to antiviral therapy of latently infected cells represents the principal challenges toward curing AIDS. The main latent HIV-1 reservoir resides in the subset of CD4+ T-cells called resting, memory T-cells. Using CRISPR/Cas9 gene editing technology to target two unique sequences in US region of HIV-1 LTR (called target A and B), we were able to completely eliminate proviral sequences from the genome of latently infected T-cell lines. We tested and validated two delivery approaches: plasmid DNA transfection/single cell clones selection and inducible lentiviral delivery system. Both strategies resulted in abrogation of HIV-1 reactivation as a result of removal of the proviral sequences from the host cell genome by long range PCR genotyping. This result was further confirmed by sequencing of cleavage lariat from integration locus in chromosome 16. Surveyor assay and sequencing of potential off target sites in the host genome showed no detectable off target effects. Furthermore, removal of the proviral sequence had no significant impact on the expression in neighboring genes. Finally, stable expression of Cas9/gRNA complexes targeting LTR was able to protect cells against new infections. Our results indicate that CRISPR/Cas9 system can be used to specifically remove integrated viral sequences from the genome of latently infected cells. This proof of concept study provides a new avenue to cure AIDS.

Using CRISPR/Cas9 gene editing tool to target integrated HIV-1 genome

Targeting viral LTR sequences with Cas9/gRNA expressing plasmids abrogates reactivation of latent reporter HIV-1 provirus

Analysis of HIV-1 integration site in host genome confirms successful excision of proviral sequences

Stable expression of Cas9/gRNA complexes protects cells against new HIV-1 infection

Conclusions

- Targeting LTR sequences with Cas9/gRNA gene editing machinery successfully eradicates HIV-1 provirus from the genome of latently infected T cells
- Stable expression of Cas9/gRNA provides resistance to new infections
- Lentiviral delivery provides faster, easier and more versatile Cas9/gRNA mediated gene targeting

Future Directions

- Testing Cas9/gRNA mediated HIV-1 eradication in primary cells from infected patients
- Checking efficiency of Cas9/gRNA approach in animal models for HIV-1 disease
- Improving Cas9/gRNA delivery systems

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