Novel animal/virus models for vaccine, cure research, and inhibitor development

PE49

Crispr/Cas9 gene editing eradicates latent and protects cells against new HIV-1 infection

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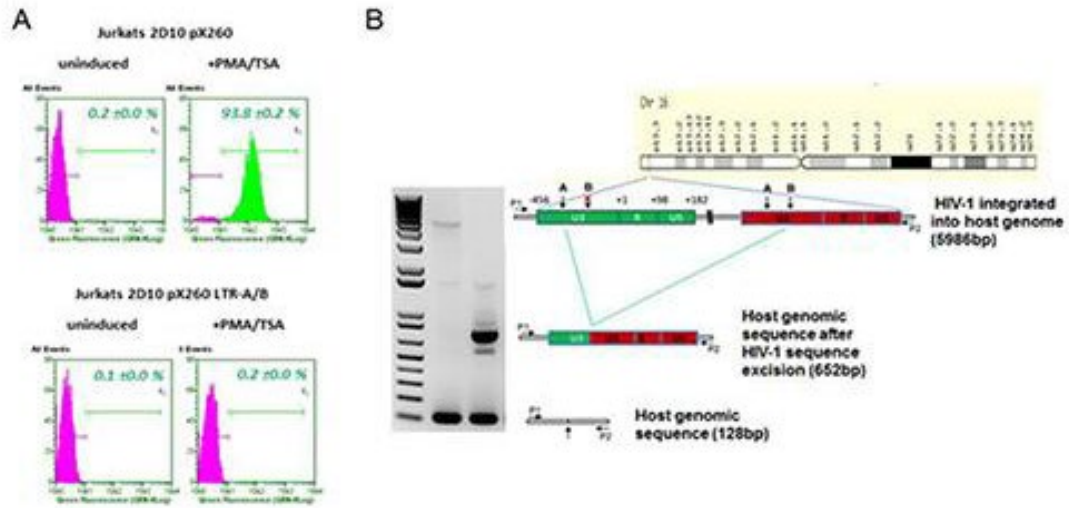
Background: A sterilizing cure for HIV-1/AIDS requires a strategy that eliminates all or at least some critical regions of the HIV-1 genome including the promoter positioned within the 5' LTR of the viral genome from cells serving as a stable reservoir for HIV-1, i.e. resting CD4+ T-lymphocytes, macrophages, and brain microglia, with no adverse impact on the host cells.

Methods: We have tailored CRISPR/Cas9 gene editing by bioinformatic screening, Surveyor assay, whole genome sequencing, and have successfully developed a series of guide RNAs (gRNAs) that, in complex with Cas9 nuclease, effectively and safely eliminate integrated copies of HIV-1 proviral DNA in several human cell culture models. We assessed the impact of our gene editing strategy on viral transcription and replication by measuring the level of a GFP reporter and viral p24, upon reactivation of virus from the latent stage by treatment with PMA and TSA.

Results: We demonstrated inactivation of HIV-1 gene expression and replication in latently infected T-lymphocytes and promonocytic human cell lines as well as microglial cells upon excising the proviral DNA fragment corresponding to the entire coding sequence of HIV-1 spanning the 5' to 3' LTRs from the host chromosome by the CRISPR/Cas9 approach. Further, we demonstrate that the presence of LTR-specific multiplex of guide RNAs in cells expressing Cas9 acts as an efficient inhibitor blocking new HIV-1 infection.



Eradication of HIV-1 DNA in latently infected cells. A. Treatment of latently infected T-lymphocytes with PMA and TSA activates viral gene expression and expression of a GFP reporter in more than 93% of the cells. The presence of gRNAs (LTR A/B) and Cas9 dramatically prevented viral replication. B. Examination of DNA by PCR and direct sequencing verifies removal of integrated proviral DNA from chromosome 16.
[Khalili_IAS_July2015_Fig1]

Conclusions: Our findings suggest that the strategy involving the newly developed CRISPR/Cas9 serves as a promising platform that can be advanced for eradication of HIV-1 and a cure for AIDS.

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