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Abstract

Editing of the Human *TRIM5* Gene Decreases the Permissiveness of Jurkat T Lymphocytic Cells to HIV-1 †

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Abstract: TRIM5α is a cytoplasmic antiviral effector induced by type I interferons (IFN-I) that has the potential to intercept incoming retroviruses by interacting with their capsid core, leading to uncoating induction and the partial degradation of core components. Most HIV-1 strains escape restriction by human TRIM5α due to a lack of interaction between TRIM5α and its viral molecular target. We previously showed, however, that two point mutations, R332G/R335G, in the capsid-binding region confer human TRIM5α with the capacity to target and strongly restrict HIV-1 upon the overexpression of the mutated protein. Here, we explored the possibility to introduce these two mutations in the endogenous human *TRIM5* gene by CRISPR-Cas9-mediated gene editing. For this, we electroporated CRISPR ribonucleoproteins (RNPs) and the donor DNA into Jurkat T lymphocytic cells and isolated clones by limiting dilution. We analyzed 47 clones using specific PCR assays, and found that six clones (13%) contained at least one gene-edited allele. One clone (clone 6) had both alleles edited for R332G, but only one of the two alleles was edited for R335G. Upon challenge with an HIV-1 vector, clone 6 was significantly less permissive compared to unmodified cells, whereas the cell clones with monoallelic modifications were only slightly less permissive. Following IFN-β treatment, the inhibition of HIV-1 infection in clone 6 was significantly enhanced (~50-fold inhibition), whereas IFN-β treatment had no effect on TRIM5α overexpressed by retroviral transduction. Knockdown experiments confirmed that HIV-1 was inhibited by the edited *TRIM5* gene products, whereas quantification of HIV-1 reverse transcription products confirmed that inhibition occurred through the expected mechanism. In conclusion, we demonstrate the feasibility of potently inhibiting a viral infection through the editing of innate effector genes, but our results also emphasize the importance of biallelic modification in order to reach significant levels of inhibition by TRIM5α.

Keywords: TRIM5α; HIV-1; restriction factors; interferon; CRISPR; gene editing