Inhibition of DNA-PKcs enhances CRISPR/Cas9 inactivation of the HIV-1 provirus

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The use of CRISPR/Cas9 in both HIV-1 latent cell models and HIV-1-infected primary cells has been effective in reducing transcription from the HIV-1 provirus. Importantly, the fraction of CRISPR/Cas9-induced repair profiles which result in genic frameshift mutations or disrupt the HIV-1 promoter contribute largely to the inactivation of the HIV-1 provirus. Therefore, the type of repair profiles and their resulting repair outcomes impact CRISRP/Cas9 editing efficiency. Modulation of double-stranded break (DSB) repair pathways either through chemical perturbation or gene knockdown with CRISPR/Cas9 editing are current approaches that have been used to promote repair of disease alleles, eradicate cancer cells, and enhance gene knockouts. To enhance the occurrence of promoter-disrupting mutations or excision events within the HIV-1 provirus to inactivate it, we inhibited non-homologous end joining (NHEJ)-associated repair proteins LIG4 and DNA-PKcs during CRISPR/Cas9 editing of the HIV-1 provirus in a latently infected CD4 T cell model. We showed that inhibition of DNA-PKcs through small molecule inhibitors like NU7441 and M3814 enhances GFP reduction in TNF-α-stimulated J-Lat 10.6 cells. This enhanced GFP reduction was associated with a change in the repair profiles from insertions to moderate-to-large deletions, an enhancement of excision events, and inhibition of autophosphorylation of DNA-PKcs. However, LIG4 inhibition did not impact GFP reduction nor alter repair profiles in any significant way, likely due to the activity of other ligases substituting. These results serve as proof-of-concept and an early indication of the potential of modulating DSB repair pathways to enhance efficiency of anti-HIV CRISPR therapies.

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