Characterizing CRISPR/Cas9-induced edits to the HIV-1 LTR: Implications for transcriptional output and effects of chromatin structures

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Human immunodeficiency virus type 1 (HIV-1) is a retrovirus that integrates into the host cell's genome as a provirus, establishing a reservoir of latently-infected cells. This presents a major therapeutic challenge, as latently infected cells avoid immune clearance and current antiretroviral drugs cannot target this stage of the replication cycle. Therefore, there is an outstanding need for the development of cure strategies that can clear or inactivate the provirus. One cure strategy is CRISPR/Cas9 gene therapy, whereby the Cas9 endonuclease complexes with a target DNA region through a complementary guide RNA (gRNA) and induces a double-stranded break (DSB) that is repaired by the cell. DSB repair can induce insertions, deletions, and other edits. gRNAs targeting the long terminal repeat (LTR) sequence can disrupt proviral gene expression by introducing edits to transcription factor binding sites, the transcription initiation and termination sequences, and/or the viral transactivation response element (TAR). In the J-Lat 10.6 model system, approximately half of the proviruses were inactivated following treatment with gRNAs targeting the NF-κB binding sites (NF-κB1) or the TAR/polyA loop sequence (SMRT-01). Sequencing of the LTRs post-CRISPR revealed key edits that were associated with full proviral inactivation, while others were found in both active and inactive populations. Further analysis revealed that these edits may be associated with reduced transcriptional output rather than complete inactivation. To test the hypothesis that the lack of complete transcriptional reduction is due to chromatin, J-Lat 10.6 cells were treated with a panel of gRNAs targeting the nucleosome-protected R-U5 region. This panel showed that the chromatin environment at the 5' LTR R-U5 region did not impact edit efficiency, but may instead affect the edit profile and inactivation potency. Taken together, we hypothesize that treating cells with small molecule chromatin modifiers could shift the edit profiles induced by different gRNAs towards complete proviral inactivation.

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