

Suppression of HIV-1C virus production in human PBMCs by dsRNA induced Chromatin remodeling of LTR promoter

A. Singh¹, J.K. Palanichamy², P. Ramalingam¹, M.A. Kassab¹, M. Bhagat¹, R. Andrabi¹, K. Luthra¹, S. Sinha³, P. Chattopadhyay¹

¹All India Institute of Medical Sciences, Biochemistry, New Delhi, India, ²David Geffen School of Medicine at UCLA, Department of Pathology & Laboratory Medicine, Los Angeles, United States,

³National Brain Research Center, Gurgaon, India

Background:

HIV-1 subtype C is highly infectious clade which claims approximately 50% of worldwide HIV-1 infection and predominates in sub-Saharan Africa, China, India and other regions of Asia, has been less studied in comparison to subtype B which accounts for 10% worldwide infection. The 5'LTR of HIV-1 acts as the promoter and regulates the transcription of all downstream viral genes. So, dsRNA mediated transcriptional gene silencing (TGS) of HIV-1C viral genes would be a good approach to suppress viral production. In our study, we have targeted the HIV-1C LTR promoter using dsRNA to induce TGS and decrease viral gene transcription.

Methods:

siHa cells stably expressing luciferase gene under the HIV-1C LTR promoter was used in the presence of Tat protein for the screening of potent siRNA which can induce TGS. Luciferase expression (mRNA) and activity was measured by Real-Time PCR and dual luciferase assay respectively. The chromatin modification at the targeted region after siRNA transfection was studied using CHART-PCR and CHIP assay. HIV-1C infected human PBMCs were transfected with siRNA and Gag-p24 antigen level was determined by ELISA.

Results:

Multiple siRNAs targeting the enhancer and core region of HIV-1C 5'LTR promoter were screened and of these, one siRNA showed significant decrease in luciferase activity and its mRNA expression post transfection. The CHART-PCR and CHIP assay showed that this siRNA mediated TGS was caused by methylation of histone tails like H3k9me2 and H3K27me3, which leads to heterochromatinization of the targeted LTR region. This siRNA mediated TGS also causes the suppression of viral replication and marked reduction in apoptosis of HIV-1C infected human peripheral blood mononuclear cells (PBMCs).

Conclusion:

We have identified a potent siRNA which causes the heterochromatinization of HIV-1C 5'LTR promoter, leading to suppression of viral gene expression and its productive infection for at least 21 days after a single transfection in *ex-vivo* experiments. TGS of HIV-1C causes a long-lasting decrease in viral replication because it operates through heritable epigenetic modification. Thus dsRNA mediated TGS can be used as a therapeutic modality for HIV-1C infection in future.