Poster 15

Investigating the role of RNA Polymerase-III in regulating HIV-1 replication

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Purpose

Elimination of latent reservoirs is an extremely challenging task due to the involvement of heterogeneous mechanisms in regulating HIV latency. In this study we are investigating the role of RNA polymerase-III (RNA Pol III) enzyme in regulating HIV-1 expression.

Methods

Our preliminary studies demonstrated the enrichment of Pol III transcribed noncoding RNAs in latent cells, namely 7SK, 21A and BC200 that are interspersed among Alu repeats. This is highly relevant to HIV latency because the HIV genome is found to preferentially integrate near Alu repeats. Subsequently, use of an RNA Pol III inhibitor, ML60218, resulted in an unprecedented reactivation (up to 90%) of latent cell lines J89GFP and THP89GFP, in a dose-dependent manner (25 µM-50 µM). Further, we examined the role of RNA Pol III in regulating HIV-1 replication by utilizing two complimentary approaches to diminish RNA Pol III function- i) inhibition of RNA Pol III by its inhibitor ML60218, and ii) genetic knockdown of RNA Pol III using siRNA. These experiments were conducted using HIV1-Bal as well as HIV-1 pseudotyped (DuoFluo) virus in THP-1, HEK293T, SupT, and HeLa cells.

Results

Results suggested that knockdown or inhibition of RNA Pol III significantly induced viral replication as determined by increased HIV-1 specific transcripts. Additionally, we performed pNL4.3 HIV-1 plasmid DNA transfection in HEK293T cells and analyzed the effect of RNA Pol III inhibition/knockdown on HIV transcription. Results showed a consistent increase in HIV-1 transcripts including transcription initiation, elongation and Tat specific transcripts.

Conclusion

Collectively, these results unravel a previously unknown but important role of RNA Pol III in HIV-1 replication. Currently, we are performing experiments using human primary CD4+T cell and macrophages to investigate the role of RNA Pol III inhibitor on HIV expression and latency.