Retinoic Acid Reverses HIV Latency through inhibition of PRMT5 and epigenetic modification of H4R3

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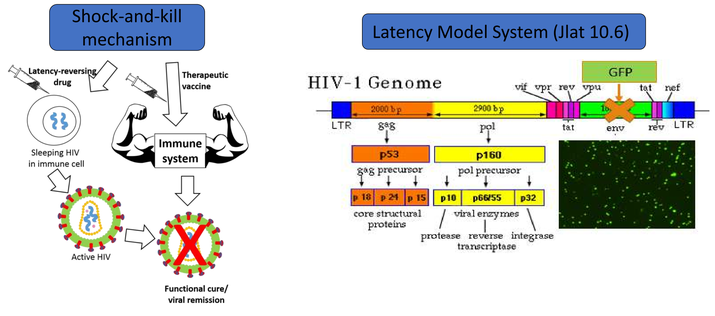
Abstract

Human Immunodeficiency virus (HIV) currently infects 36.9 million people today and there is an active search for better treatment options. HIV is a retrovirus that affects human CD4+ T-cells. When a person becomes infected with HIV, the HIV genome becomes incorporated into the DNA of the host as a provirus. This provirus can either be active or latent. In cells infected with active virus, viral mRNA is being actively expressed and infectious HIV particles continuously bud out of the cell. In latent cells, the provirus is silent and minimal viral mRNA is being produced. The commonly prescribed therapy for AIDS patients antiretroviral therapy (ART) that eliminates cells with active virus. This therapy does not cure patients because it does not eliminate HIV from the body. Hence, it is difficult to treat latent HIV. Therefore, the goal of this study is to investigate the use of retinoic acid in activating HIV from latency to create suitable active cell targets for ART. Our research has shown that retinoic acid can reverse latency and is able to induce transcription of the promoter for the HIV provirus. Retinoic acid can induce HIV transcription through inhibition of protein arginine methyltransferase 5 (PRMT5) leading to the subsequent decrease in its product H4R3me2s on the HIV promoter-associated histone. This histone modification leads to a demethylation of cytosine on the promoter and transcriptional reactivation.

Retinoic Acid and Its Interaction with RAR/RXR

Figure 1: Retinoic Acid Responsive Elements (RARE) with bound Retinoic Acid Receptors (RAR and RXR). Retinoic Acid binds to RAR and RXR

Retinoic Acid Activates Latent HIV



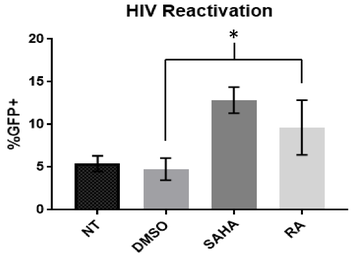


Figure 2: Retinoic Acid (25 uM) can activate latent HIV in the Jlat 10.6 model system SAHA (0.5 uM) is used as a positive control. p-value is 0.05

Retinoic Acid Activates HIV LTR Promoter

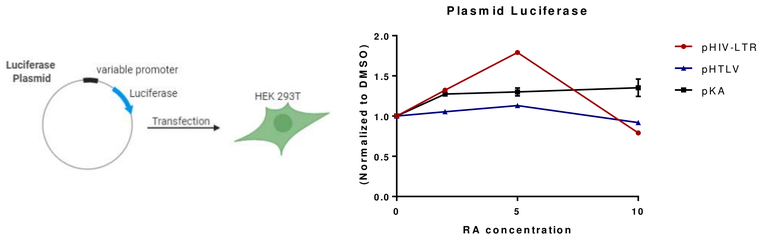
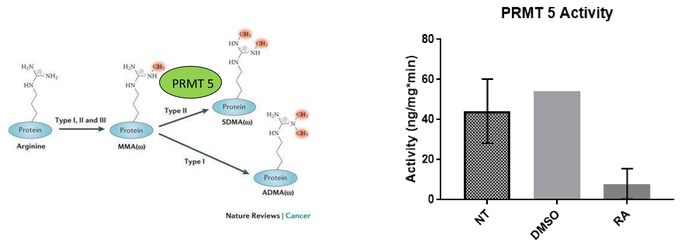


Figure 3: Retinoic Acid activates the pHIV-LTR (n=1) but does not activate pHTLV (n=1) or pKA (n=3). pKA is a cellular promoter and the pHTLV is a viral promoter (Human T-cell Lymphotrophic Virus).

Retinoic Acid Inactivates PRMT5



PRMTs (Protein Arginine Methyltransferases) can transfer up to two methyl groups on the end of histone arginine residues. While all PRMTs can enzymatically perform the monomethylation, the symmetric and asymmetric demethylation is performed by certain enzymes. PRMT5 specialize in symmetric methylation (Fig.4A). Retinoic Acid can inhibit PRMT5 and decrease the amount of symmetrically methylated products (Fig 4B: n=3)

Retinoic Acid Reduces H4R3me2s on HIV LTR

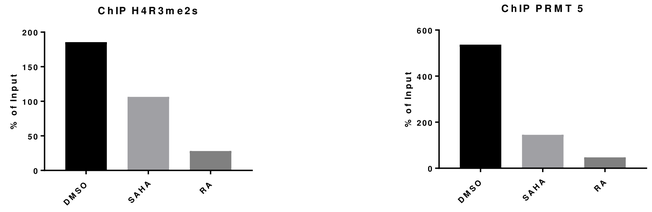
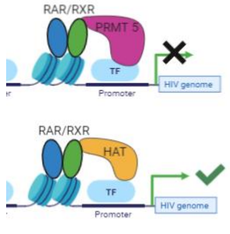


Figure 4: ChIP on PRMT 5 and H4R3me2s. H4R3me2s is in Jlat 10.6 and PRMT 5 is in TZM-bl



On the HIV promoter in Jlat 10.6 T cells, retinoic acid sequesters histone modifiers such as PRMT 5 subsequently preventing modification of histone arginine (H4R3me2s).

Epigenetic Cascade

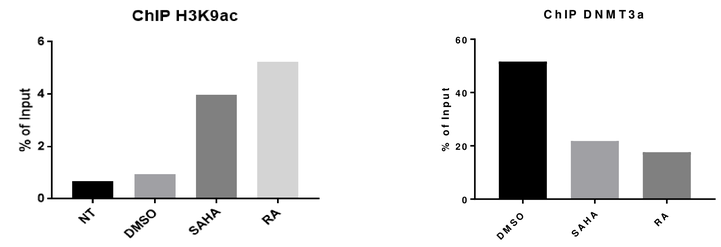
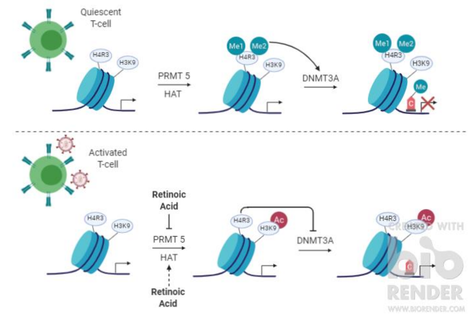


Figure 5: Retinoic acid releases an epigenetic cascade that includes an increase in acetylated lysine (H3K9ac: TZM-bl) and a release of DNA-methyltransferases from the promoter (DNMT3a: TZM-bl)



The decrease in symmetric methylation causes a cascade effect in which DNA methyltransferases are inhibited at the HIV promoter leading to a decrease in 5-methylcytosine at the promoter site[2]. This loss of 5-methylcytosine and gain of acetylated lysine indicates promoter reactivation

Future Directions

Retinoic acid can reactivate HIV at 25 uM. Retinoic acid is specific to the HIV LTR promoter and does not turn on the other cellular and viral promoters tested. However, paradoxical inactivation of retinoic acid was found at higher concentrations of the drug. If the paradoxical inactivation of retinoic acid at higher concentrations is biologically relevant, then the use of 5 uM in the reactivation will be important for future work.

We have also monitored the chemical modification at the HIV promoter due to retinoic acid. Retinoic acid seems to induce loss of PRMT 5 through inhibition and co-repressor replacement. The inhibition of PRMT 5 by retinoic acid will be further investigate to statistically verify the inhibition. This loss of PRMT 5 causes a decrease in H4R3me2s at the HIV promoter in Jlat 10.6 T cells.

The epigenetic cascade will be monitored in Jlat 10.6 cells particularly the DNA modifications. Since DNMT3a is being released from the promoter, the HIV LTR should have less 5-methylcytosine

References

(1) Busby S.A., Burris T.P. (2012) Retinoic Acid Receptors (RARA, RARB, and RARC). In: Choi S. (eds) Encyclopedia of Signaling Molecules. Springer, New York, NY

(2) Zhao, Q.; Rank, G.; Tan, Y. T.; Li, H.; Moritz, R. L.; Richard, J.; Cerruti, L.; Curtis, D. J.; Patel, D. J.; Allis, C. D.; et al. PRMT5-Mediated Methylation of Histone H4R3 Recruits DNMT3A, Coupling Histone and DNA Methylation in Gene Silencing. **2016**, *16* (3), 304–311.