Investigating the Effects of Chromatin Spatial Folding on HIV-1 Latency

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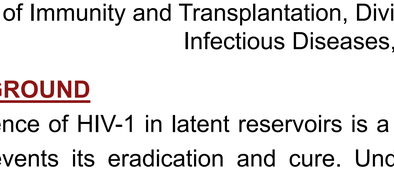
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BACKGROUND

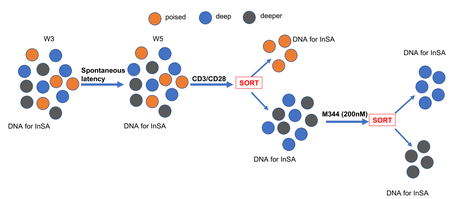
Persistence of HIV-1 in latent reservoirs is a major obstacle that prevents its eradication and cure. Understanding the mechanisms governing HIV-1 latency is crucial to eliminate this reservoir. Spatial folding of chromatin governs cellular gene expression. However, how 3D genome organization affects HIV-1 latency is unknown.

METHODS

- To investigate the impact of chromatin spatial folding on HIV-1 latency, we used a comparative approach that takes advantage of the different integration profiles of wild type (WT) HIV-1 and a capsid mutant virus N74D. We mapped unique integration sites (UISs) for HIV-1 WT and N74D to DamID and HiC data.
- To investigate how different integration site distribution may affect latency, jurkat cells were transduced with either WT or N74D single cycle HIV-1 vectors expressing GFP from the LTR. GFP+ cells were sorted after 48 hrs, and latency was established.



- To identify UISs functionally relevant for latency, latently infected cells (WT and N74D) were stimulated serially by TCR engagement followed by inhibition of HDAC6 using M344 Inhibitor and sorted into reversible (GFP+) and deep (GFP-) latency populations at each step.



Serial sorting into "poised", "deep" and "deeper" latency + Integration site analysis (InSA), using sheared DNA and LM-PCR.

RESULTS

Mapping UISs For WT and N74D HIV-1 Onto Lamina Associated Domains (LADs) And Topologically Associated Domains (TADs), WT HIV-1 Integration Favors Active TADs and A1 compartment

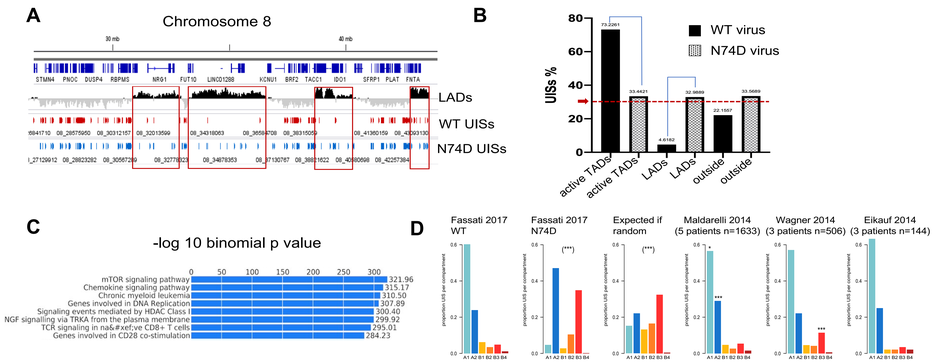


Fig. 1. (A) Representative DamID of chromosome 8 segment, with mapped UISs for WT or N74D viruses. Peaks in boxes represent LADs, red (WT) and blue (N74D) lines below represent UISs. **(B)** Proportion of UISs mapped to active TADs, or LADs or outside either of them (outside). Dotted line shows expected values for random integration UISs were obtained from **Zhyvoloup et al., 2017**, TAD sequences were obtained from GEO: GSE63525. LADs sequences were obtained from GEO: GSE94971. **(C)** Gene Ontology for genes targeted by WT HIV-1 in TADs LADs sequences were obtained from GEO: GSE94971 . Analysis were carried out using BEDtools version 2.28.0. **(D)** Proportion of UIS obtained from Zhyvoloup et al., 2017 mapped to different chromatin compartments. HiC compartment data for GM12878 lymphoid cells were downloaded from Rao et al. Cell 159: 1685 (2014). *In vivo* UIS were obtained from Einkauf, K. B., et al. (2019) *J Clin Invest* 129, 988-998, Wagner, T. A., et al. (2014) *Science* 345, 570-573 and Maldarelli, F., et al. (2014) *Science* 345, 179-183. UISs counts per compartment were obtained using Bedtools intersect tool, and their proportion per compartment displayed as a barplot. Statistical differences between compartment counts in Fassati 2017 WT UISs and N74D or the other *in vivo* studies were assessed using Chi-squared test

N74D Virus Established Latency Faster Than WT

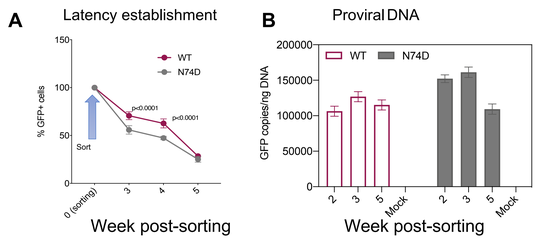


Fig. 2. (A) FACS analysis for GFP expression at different time points after sorting the infected cells. **(B)** Quantification by TaqMan qPCR of proviral DNA copies (GFP primers) in mock-infected cells (not inf.) and sorted GFP- cells at different weeks post-sorting.

HIV-1 Integration Site Selection Affects Reactivation

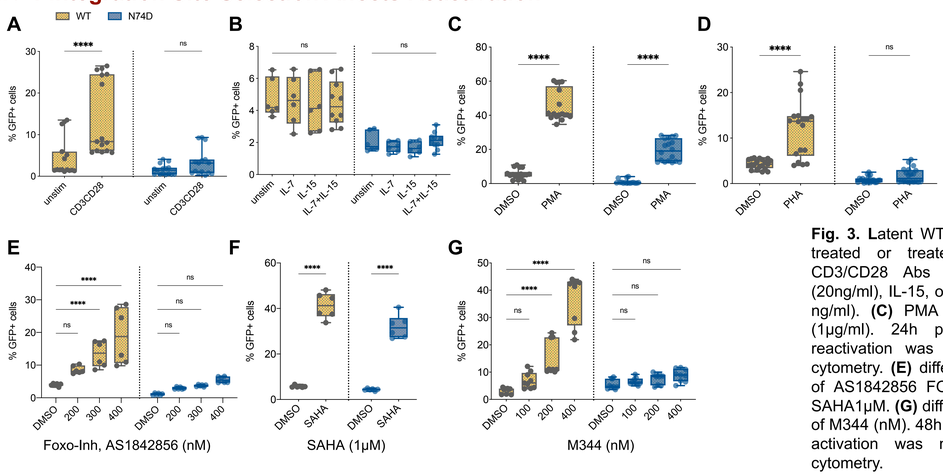


Fig. 3. Latent WT/N74D were DMSO treated or treated with **(A)** anti-CD3/CD28 Abs (1µg/ml). **(B)** IL-7 (20ng/ml), IL-15, or IL-7 plus IL-15 (20 ng/ml). **(C)** PMA (5ng/ml). **(D)** PHA (1µg/ml). 24h post-treatment, viral reactivation was measured by flow cytometry. **(E)** different concentrations of AS1842856 FOXO 1 inhibitor. **(F)** SAHA1µM. **(G)** different concentrations of M344 (nM). 48h post-treatment, viral activation was measured by flow cytometry.

Chromatin histone marks at the site of HIV integration and their role in latency

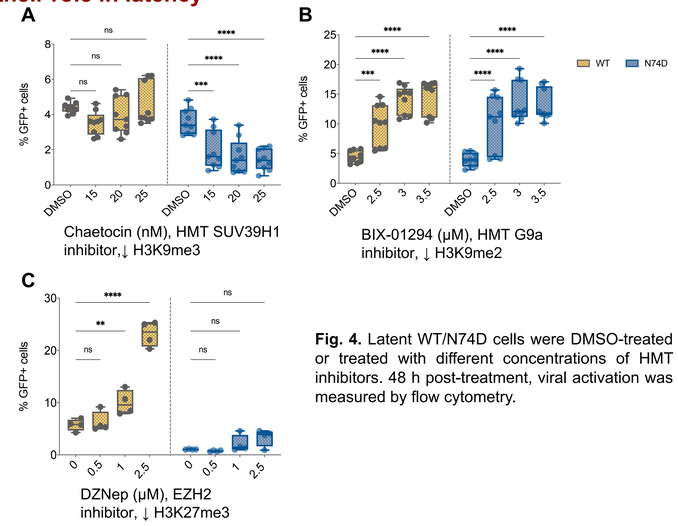


Fig. 4. Latent WT/N74D cells were DMSO-treated or treated with different concentrations of HMT inhibitors. 48 h post-treatment, viral activation was measured by flow cytometry.

WT UIS distribution into compartments in latent and re-activated cells (preliminary analysis 10,000 UIS)

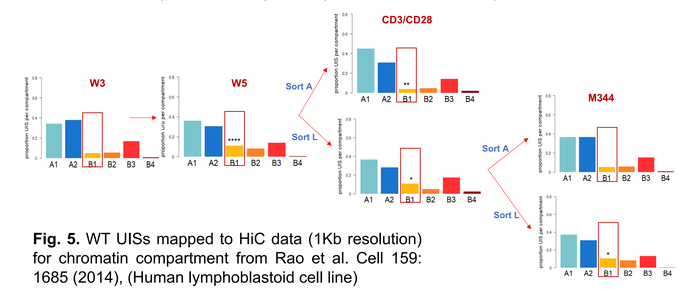


Fig. 5. WT UISs mapped to HiC data (1Kb resolution) for chromatin compartment from Rao et al. Cell 159: 1685 (2014), (Human lymphoblastoid cell line)

CONCLUSIONS

- Mapping (UISs) to chromatin domains showed that WT HIV-1 preferentially integrates into active (TADs) but avoids (LADs). The reverse was true for N74D virus.
- Different integration pattern into 3D chromatin influences latency, whereby WT HIV-1 is silenced more slowly than N74D virus and can be reactivated more efficiently.
- We propose that deep latency may be linked to integration into particular 3D chromatin regions enriched for certain epigenetic marks and gene clusters, which might be specifically targeted to either induce, or prevent, virus reactivation.

ADDITIONAL KEY INFORMATION

- This research is funded by the Government of Sultanate of Oman.

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