C-MYC AND NR4A REGULATE STOCHASTIC REACTIVATION OF LATENT HIV-1

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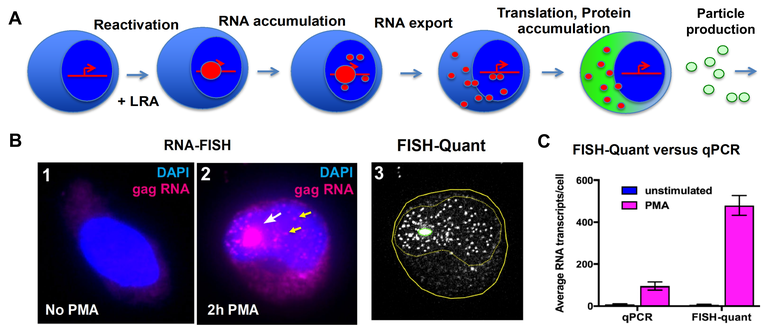
BACKGROUND

The “shock and kill” approach to eliminate HIV-1 latent reservoirs uses Latency Reversing Agents (LRA) to activate the provirus. At present reactivation in 100% of the latent cells is not possible and one reason is the stochasticity or ‘noise’ in pro-viral transcriptional activation. Current strategies do not address stochasticity in reactivation during LRA treatment.

METHODS

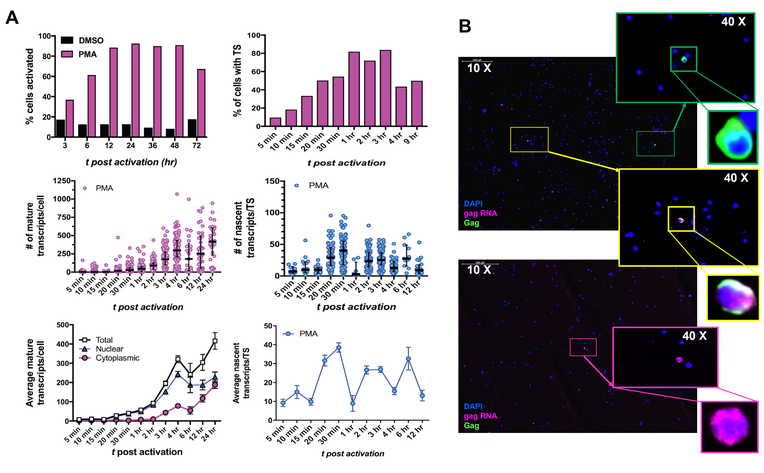
To test stochasticity in latent HIV-1 proviral reactivation, we adopted single molecule RNA fluorescence in situ hybridization (smRNA-FISH) combined with quantitation of single RNA molecules within cells using FISH-Quant. To facilitate the identification of rare, reactivated cells, we utilized a High Speed and High-Resolution-Scanning (HSHRS). To determine the “extrinsic factors” responsible for stochastic activation, we carried out a single-cell RNA-seq (scRNA-seq) analysis, using the 10x genomics. The findings were validated using qRT-PCR.

Figure 1: Development of Stellaris-based smRNA-FISH analysis of latent cells and quantitation of mRNA in reactivated cells



(A) Schematic representation to indicate the stages of proviral reactivation that could be blocked in a latent cell. **(B)** Images of a single ACH2 latent cells before and after PMA treatment. Panel 2 indicates the single molecule detection of Gag RNA. The white arrow points to the transcription site (TS), and the yellow arrow points to single RNA molecules. Panel 3 is the filtered image used for quantitation using FISH-Quant. **(C)** comparison of a number of HIV-1 transcripts obtained using FISH-Quant and in qRT-PCR.

Figure 2: (A) Kinetic analysis of HIV-1 reactivation in latent cells using quantitative smRNA-FISH revealing the stochasticity of reactivation. (B) Stochastic reactivation of latent cells in the absence of LRA.



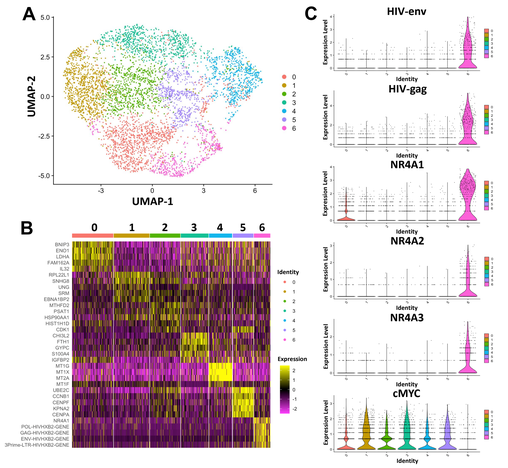
- Quantitative single molecule RNA-FISH revealed that there is a high degree of stochasticity and cell-to-cell variability in the reactivation of latent HIV-1 provirus.
- ~5% of ACH2 cells are stochastically activate in uninduced conditions.
- Single cell RNA-seq analysis of uninduced latent cells identified a pool of cellular transcripts that are differentially regulated selectively in reactivated cells.
- We identified NR4A3 and c-MYC as key extrinsic factors associated with stochastically activated and reactivated HIV-1 latent cell-lines and primary cell models of latency.
- Drug-mediated targeting of cMYC and NR4A indicates that SN-38 might act as a novel LRA.

RESULTS

Kinetic analysis using smRNA-FISH and FISH-Quant indicated that HIV-1 reactivation in latent T-cells is rapid (15-30 min) and that transcription proceeded in waves (Fig. 2A). There was a high degree of stochasticity and cell-to-cell variability in reactivation and the number of transcripts/cells varied from 0-1,000, at any given time. HSHRS analysis of a large pool of uninduced latent cells (>100,000 cells) indicated that ~1-10% of cells are active even in the absence of an inducer (Fig. 2A and B).

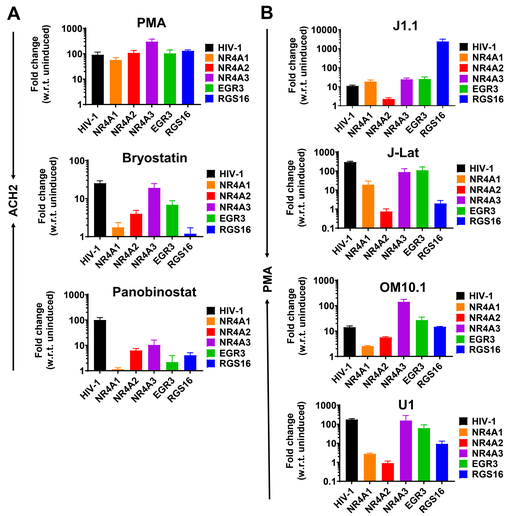
To determine factors responsible for stochastic reactivation, we used scRNA-seq analysis and identified differentially regulated cellular genes (DEGs) present selectively in reactivated cells within the pool of un-induced cells (Fig. 3). The DEGs were validated using qRT-PCR (Fig. 4). We found that c-MYC and NR4A (Orphan nuclear receptor 4A) are correlated to reactivation in latent cell lines and in patient-derived latent cells (Fig. 4, 5 and 6). SN-38, an active metabolite of the topoisomerase-I inhibitor irinotecan (an FDA-approved drug) inhibits cMYC via activation of the NR4A pathway. We found that SN-38 reactivated the HIV-1 latent provirus in cell lines and patient samples (Fig. 7), suggesting the significance of cMYC in regulating proviral reactivation.

Figure 3: scRNA-seq analysis of ACH2 cells: Clustering of cells positive for HIV-1



(A) UMAP plot analysis to indicate seven distinct clusters in latent ACH2 cells. **(B)** Heat map analysis of top 5 upregulated genes in each cluster. **(C)** Violin plot to indicate the distribution of HIV-1, NR4A, and cMYC expression in the seven groups.

Figure 4: qRT-PCR analysis to correlate NR4A and cMYC expression in various latent and reactivated cells.



(A) The DEGs identified in stochastically activated cells (Fig. 3) are also activated upon LRA treatment, along with HIV-1, in ACH2 cells. **(B)** NR4A3 and cMYC are consistently associated with HIV-1 reactivation in other latent cell lines.

Figure 5: Down-regulation of cMYC is correlated to reactivation of latent HIV-1.

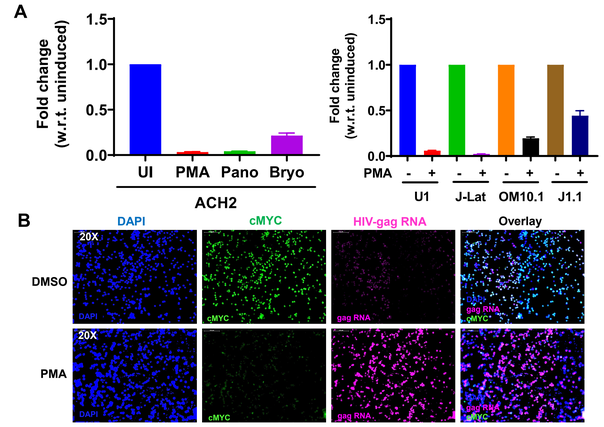


Figure 6: Upregulation of NR4A3 and downregulation of cMYC in reactivated HIV-1 latent cells derived from patients.

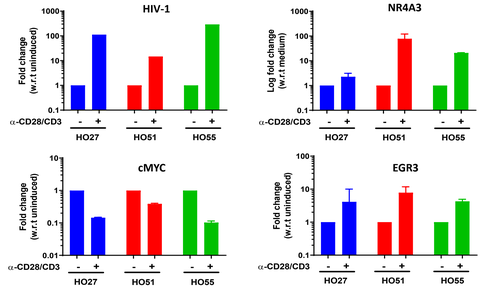
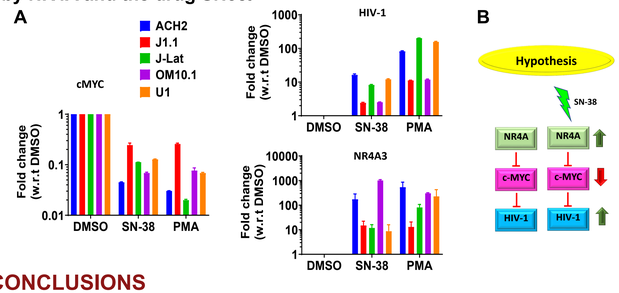


Figure 7: (A) SN-38, an inhibitor of cMYC reactivates HIV-1 and is correlated to the upregulation of NR4A3 and downregulation of cMYC. (B) Schematic representation of a hypothesis for regulation of HIV-1 by NR4A and the drug SN38.



CONCLUSIONS

- HIV-1 reactivation from latent cells is stochastic as revealed by quantitative smRNA-FISH.
- NR4A3 is upregulated while c-MYC is downregulated in cells where latent HIV-1 provirus is reactivated. These could represent extrinsic factors associated with stochastic HIV-1 reactivation.
- The drug SN-38, that inhibits cMYC via NR4A can reactivate latent HIV-1 provirus in cell lines and in patient samples.
- SN-38 could be useful as a drug that might reduce stochastic activation.

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