Inhibition of the IN-LEDGF/p75 interaction by LEDGINs reduces reactivation from latency

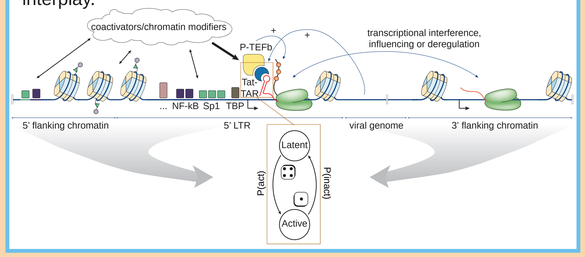
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BACKGROUND

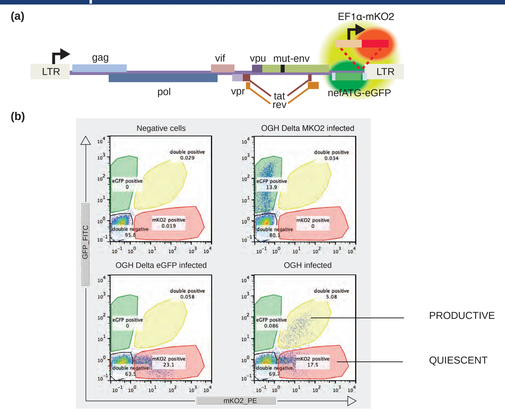
Despite the success of cART, HIV-1 persists in a latent, replication competent state in patients being the main impediment towards a cure for HIV/AIDS. Persistence is a consequence of retroviral integration linking the fate of the provirus to that of its host cell. LEDGF/p75 is the pivotal chromatin tethering factor targeting HIV integration into active transcription units through its interaction with HIV-1 integrase. We investigated the role of integration site selection in the establishment of HIV persistence employing LEDGF/p75 knockout cells and LEDGINs interfering with the LEDGF/p75-IN interplay.



RESULTS

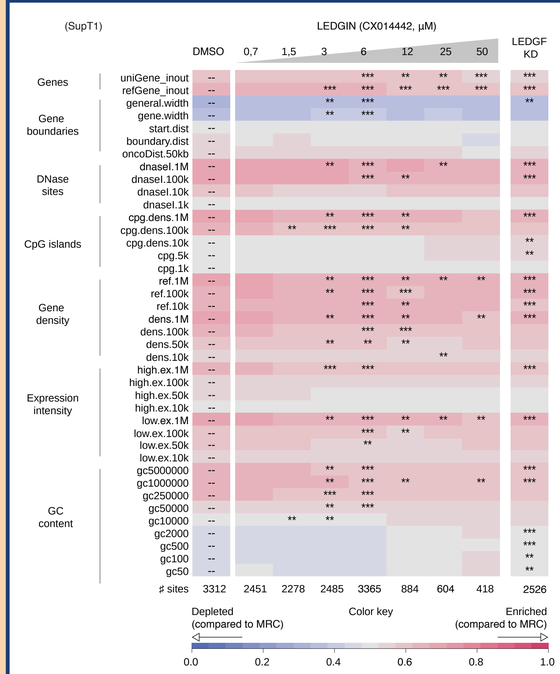
LEDGF/p75 depletion hampers HIV-1 reactivation by latency reversing agents in cell culture. LEDGIN-mediated inhibition of the LEDGF/p75-IN interaction shifts integration out of transcription units and relocalizes the 3D nuclear location of the provirus away from the nuclear rim, comparable with previous observations for LEDGF/p75 depletion. Interestingly, LEDGIN treatment increases the relative fraction of quiescent provirus and blocks HIV reactivation from latency, both in a dose-dependent manner (IC50 CX014442 ≈ 7.24 µM). This approach may represent a viable alternative to the shock-and-kill strategies for functional eradication of HIV in patients as LEDGIN treatment apparently succeeds in rendering almost 100% of the virus into a quiescent state refractory to reactivation.

Figure 1. Reporter constructs



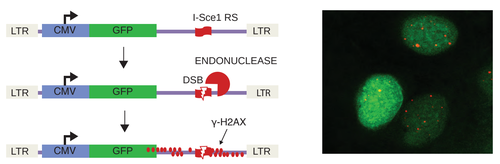
(a) Schematic representation of the LAI-based two-colored reporter virus (HIV OGH) carrying an eGFP driven by the viral LTR promoter in the Nef position and an entire constitutive transcriptional unit (EF1α-mKO2) inserted downstream. (b) Dot plots representing FACS analysis of SupT1 cells infected with the single reporter viral controls (OGH-Delta mKO2, OGH-DeltaeGFP) or the double reporter virus OGH. The different cell populations are highlighted in the representative color. All viruses are VSV-G pseudotyped. GFP, Enhanced Green Fluorescent Protein; mKO2, Mutant Kusubira Orange 2.

Figure 2. LEDGIN inhibition of the LEDGF/p75-IN interaction retargets LV integration



Integration site data sets obtained from SupT1 cells infected with LVP2A and treated with different concentrations of CX014442 were compared to different genomic features. A heat map was generated using the INSIPID software (Bushman Lab, University of Pennsylvania). Tile color depicting the nature of the correlation for an integration data set with the respective genomic/ epigenetic feature (rows, left) relative to matched random controls, as indicated by the colored receiver operating characteristic (ROC) curve area scale at the bottom of the panel. Columns indicate different data sets. Statistical significance (asterisks, ranked Wald tests) is shown relative to the DMSO population (dashes) *p<0.05; **p<0.01; ***p<0.001. Significance is reached when p<0.001, compared to the DMSO control. CX014442 treatment during infection shows a dose dependent shift out of transcriptionally active regions.

Figure 3. LEDGIN treatment shifts HIV-1 proviral localization towards the inner nucleus



U2OS WT or LEDGF/p75 depleted cells were infected with a HIV derived vector pHR-CMV-EGFP with or without an I-SceI site (mock) and γH2AX foci quantified per nucleus 48h post infection after endonuclease digestion. (a) SCIP analysis of proviral DNA corresponding to γH2AX foci (red) in U2OS cells. Bar diagram in de right panel depicts the number of proviruses (H2Aγ foci) detected under each condition. Error bars represent standard deviations from at least three experiments. (b) 3D nuclear localization of HIV-1 provirus relative to the nuclear rim in LEDGF/p75 KD (empty bars) or WT U2OS cells (grey bars) (P<0.001, Kolmogorov-Smirnov test) (n=1000). The right panel depicts the cumulative frequency for the distance relative to the nuclear rim. (c) Cumulative frequency of the 3D nuclear localization of HIV-1 provirus relative to the nuclear rim in U2OS cells treated (grey) with or without (black) LEDGINs. (P<0.001, Kolmogorov-Smirnov test) (n=650). (d) Cumulative frequency of the 3D nuclear localization of HIV-1 provirus relative to the nuclear rim in U2OS cells infected with the HIV-1 INA128T/E170G and treated with (grey) or without LEDGINs (black, CX05045, 3 µM, 50 x IC50). (P>0.05, Kolmogorov-Smirnov test) (n=650). Number of experiments is indicated in each plot (> 100 cells counted/ experiment).

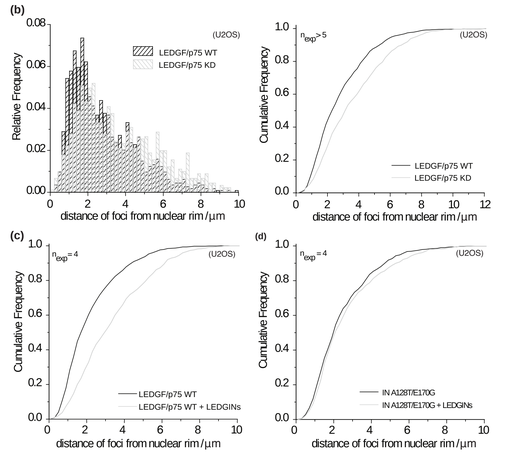
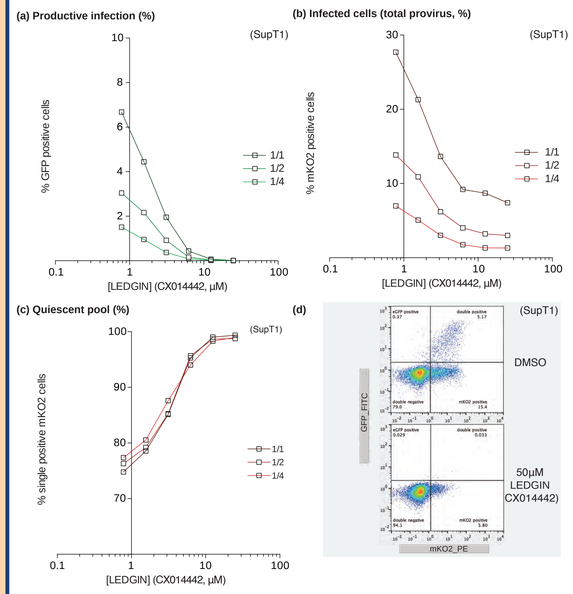
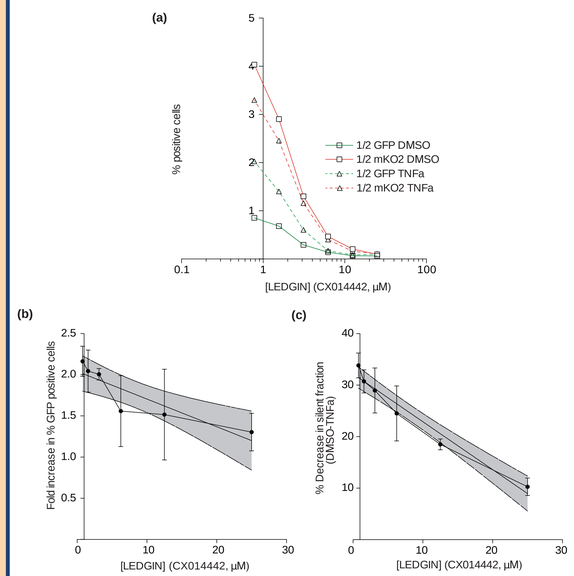


Figure 4. LEDGIN mediated retargeting of integration increases the quiescent reservoir



SupT1 cells were infected with three different dilutions of HIV OGH (a) Dose-response curve showing a decrease in % eGFP positive cells with increasing LEDGIN concentration. Three different virus concentrations are depicted in green. (b) Dose-response curve showing a decrease in % mKO2 positive cells with increasing LEDGIN concentration. Three different virus concentrations are depicted in red. (c) The fraction of single positive mKO2 cells or silently infected cell population increases upon addition of LEDGINs. Three different vector dilutions are depicted in red. All viruses are VSV-G pseudotyped. (d) Respresentative dot plots depicting the different cell populations under two different conditions. All vectors are VSV-G pseudotyped. GFP, Enhanced Green Fluorescent Protein; mKO2, Mutant Kusubira Orange 2

Figure 5. LEDGIN treatment reduces reactivation from latency



SupT1 cells were infected with single round double reporter virus (OGH) and treated with different concentrations of CX014442 (as indicated). % eGFP - % mKO2 positive cells were monitored, respectively. 11 days post infection cells were reactivated using TNFalpha (10 ng/mL). (a) Dose-response curve showing the % eGFP and % mKO2 positive cells after reactivation with DMSO or TNFalpha. (b) Average fold increase in percentage eGFP positive cells after stimulation with TNF alpha relative to the DMSO treated condition. (c) Average decrease in % mKO2 single positive cells or silent reservoir fraction. Data in (b) and (c) represent averages of 3 different vector dilutions and error bars indicate the standard deviation. The straight lines represent the linear regression calculations together with the 95% confidence band plotted in grey. TNFalpha; Tumor Necrosis Factor alpha, DMSO; Dimethyl sulfoxide, GFP; Enhanced Green Fluorescent Protein, mKO2; Mutant Kusubira Orange 2, All viruses are VSV-G pseudotyped.

CONCLUSIONS

LEDGF/p75 determines the integration site pattern of HIV. The presented data in cell lines and in primary cells support our hypothesis that LEDGINs, small molecules that inhibit the interaction between integrase and LEDGF/p75, retarget integration away from the nuclear rim and in genomic regions that are less sensitive to reactivation. If further corroborated in clinical trials, this strategy may pave the way towards a functional cure. Pushing the provirus into quiescence could drive the basic reproduction number of HIV below a threshold required for sustained infection even after treatment interruption.

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