Poster # 207

Vpx-mediated degradation of SAMHD1 increases lentiviral gene transfer into hematopoietic stem and progenitor cells

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Background

Understanding how to achieve efficient transduction of hematopoietic stem/progenitor cells (HSPCs), while preserving their self-renewing capacity, is key for applying lentivirus-based gene engineering methods in Phase I/II clinical trials. The sterile alpha motif (SAM) domain and HD domain-containing protein 1 (SAMHD1) was recently identified as an HIV-1 restriction factor in myeloid cells and resting CD4$^+$ T cells that interferes with reverse transcription by decreasing the nucleotide pools or by its RNase activity. HIV-2 and SIV have evolved to counteract the effects of SAMHD1 by their accessory protein Vpx, which targets SAMHD1 for proteasomal degradation. We hypothesized that SAMHD1 also interferes with HIV-1 vector-based HSPCs transduction.

Methods

Expression of SAMHD1 in HSPCs was quantified by Western blotting and real-time quantitative PCR (qPCR). For HSPC transduction, we used HR-GFP-Vpx+ lentivirus, which carries Vpx and encodes GFP. Integrated provirus and viral DNA intermediates in HSPCs and monocyte-derived macrophages (MDMs) were quantified by qPCR. Transduction efficiency was assessed by flow cytometry.

Results

Our results show that SAMHD1 is highly expressed in HSPCs already at 2 hours culturing in a medium enriched with cytokines conventionally used for transduction of HSPCs. In contrast, fresh HSPCs have poor SAMHD1 expression. Expression levels of SAMHD1 in cultured HSPCs are comparable to those found in MDMs. Following lentiviral based transduction with HR-GFP-Vpx+, we did not observe any increase of proviral DNA in HSPCs, while a significant one in MDMs which served as positive control. Similarly, there was a less than 3-fold increase of DNA intermediates in HSPCs, but a vigorous one in MDMs. HSPCs exposed to HR-GFP-Vpx+ showed a significant increase in the percentage of GFP$^+$ cells which was associated with a decrease in SAMHD1 expression, which apparently did not translate into increases of proviral DNA. GFP$^+$ cells were detected mostly within SAMHD1$^-$ cells. There was an obvious increase of the percentage of GFP$^+$SAMHD1$^-$ cells, while GFP$^+$SAMHD1$^+$ cells decreased 7 days post transduction when compared to cells exposed to HR-GFP-Vpx- viral like particles.

Results

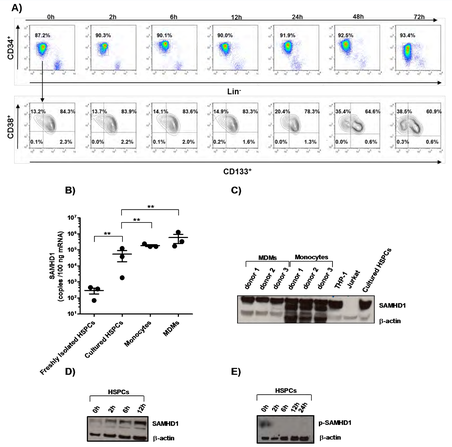


FIG. 1. Characterization of HSPCs and GFP expression in lentiviral-transduced HSPCs by flow cytometry and analysis of SAMHD1 expression in cultured HSPCs. (A) Freshly isolated cord blood-derived HSPCs were cultured in the presence of cytokines and characterized by the cell surface expression of lineage markers, CD34, CD133, CD38 over time. HSPCs were challenged with Len-EF1α-GFP at MOI 100. A representative dataset of three independent experiments is shown. Expression levels of mRNA (B) and protein of SAMHD1 (C and D) and p-SAMHD1 (E) were measured in freshly isolated HSPCs, HSPCs cultured for 3 days (B and C), 0–12 hours (D) or 0-24 hours (E) in a cytokine-enriched milieu, as well as in freshly isolated monocytes and MDMs (C). THP-1 cells and Jurkat cells served as positive and negative controls, respectively, for western blotting (C). RNA was extracted from 1x10^6 cells, cDNA was synthesized, and the levels of SAMHD1 expression were analyzed by RT-qPCR. Data are presented as absolute copy numbers/100 ng of mRNA (n=3). Dots represent individual measurements with mean±SEM indicated. Statistical analysis was done using two-tailed Mann-Whitney test without correcting for multiple comparisons (**P < 0.001). Protein levels of SAMHD1 and p-SAMHD1 were determined by western blotting; β-actin served as loading control. A representative dataset of three independent experiments is shown.

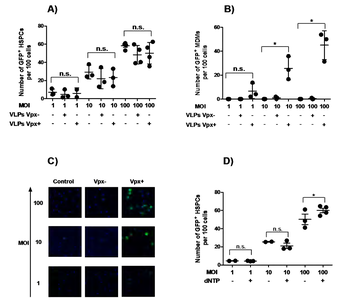


FIG. 2. Vpx+ VLPs pretreatment has no effect on transduction efficiency. HSPCs (A) or MDMs (B and C) were pretreated for 2 hours with Vpx+/- VLPs. In (D), HSPCs were exposed to 0.01 mM dNTPs. Transduction was performed with Len-EF1α-GFP at MOIs of 1, 10 and 100. Transduction efficiency was determined by quantifying the percentage of GFP$^+$ HSPCs by flow cytometry (A and D) and by immunohistochemistry (C and D). Data are from three independent donors of MDMs and HSPCs. Dots represent three individual measurements with mean±SEM indicated. Statistical analysis used the two-tailed T-test. p-value, *P < 0.05.

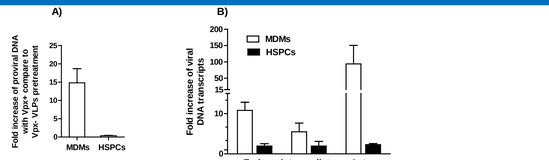


FIG. 3. Vpx+ VLPs treatment will not increase the proviral DNA or the viral DNA intermediates in HSPCs. MDMs and HSPCs were pretreated for 2 hours with Vpx+/- VLPs, followed by Len-EF1α-GFP transduction at an MOI 100, and subsequently cultured for 3 days before harvesting. Data are presented as fold increase of samples pretreated with Vpx+ and Vpx- VLPs (A). MDMs or HSPCs transduced with HR-GFP-Vpx+ (HR-GFP-Vpx+ carries Vpx within the virions and its lentiviral gene encodes GFP) or HR-GFP-Vpx- at an MOI 100, and subsequently cultured for 3 days before harvesting. Fold inductions of early, intermediate and late transcripts from HR-GFP-Vpx+ were determined by the ratio of MNEs of samples exposed to HR-GFP-Vpx+ and HR-GFP-Vpx-. (B) Dots represent three individual measurements with mean±SEM indicated.

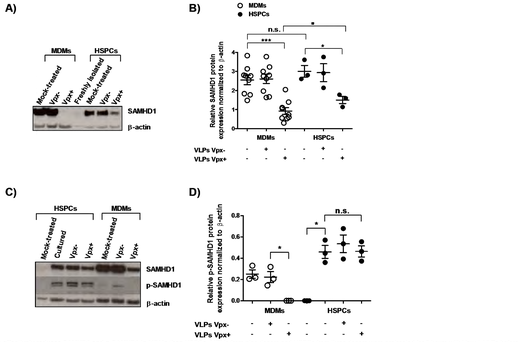


FIG. 4. Vpx+ VLPs treatment more effectively reduces SAMHD1 and p-SAMHD1 in MDMs than HSPCs. MDMs from nine donors and HSPCs from three donors were pretreated for 2 hours with Vpx+/- VLPs before cell lysis. Protein levels of SAMHD1 and p-SAMHD1 were determined by western blotting; β-actin served as loading control. A representative western blot is shown in (A and C) and semi-quantitative analysis of experiments is shown in (B and D). Dots represent individual measurements with mean±SEM indicated in (B and D). Statistical analysis was done using two tailed T-test (* black) and one-tailed Mann-Whitney test (+). * two-tailed P < 0.05, ***P < 0.001, + one-tailed P < 0.05.

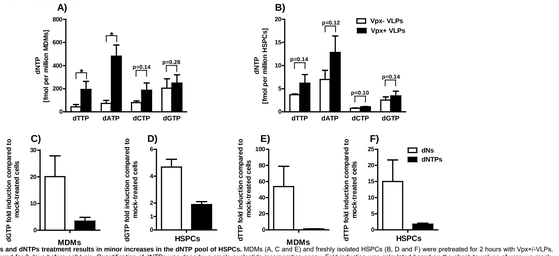


FIG. 5. Vpx+ VLPs, dNs and dNTPs treatment results in minor increases in the dNTP pool of HSPCs. MDMs (A, C and E) and freshly isolated HSPCs (B, D and F) were pretreated for 2 hours with Vpx+/-VLPs, dNs or dNTPs, and cultured for 3 days before cell lysis. Quantification of dNTPs was done by a single nucleotide incorporation assay. Fold induction was calculated based on the absolute values of vero- vs mock-treated samples (C-F). White bars represent Vpx- VLPs pretreated samples, black bars represent Vpx+ VLPs pretreated samples (A-B). White bars represent dNs pretreated samples, black bars represent dNTPs pretreated samples (C-F). Data are from three donors and bars represent mean±SEM. Statistical analysis was done using two-tailed paired T-test, *P < 0.05.

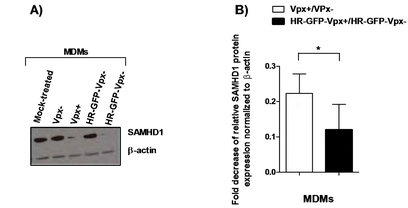


FIG. 6. HR-GFP-Vpx+/- are more efficient to degrade SAMHD1 than Vpx+/- VLPs in MDMs. MDMs from three donors were pretreated for 2 hours with Vpx+/- VLPs or transduced directly with HR-GFP-Vpx+/- VLPs before cell lysis. Protein levels of SAMHD1 were determined by western blotting; β-actin served as loading control. A representative western blot is shown in (A) and semi-quantitative analysis of experiments is shown in (B). Fold induction was calculated based on the absolute values of Vpx+ VLPs vs Vpx- VLPs samples or HR-GFP-Vpx+ samples vs HR-GFP-Vpx- samples. White bars represent Vpx+/- VLPs pretreated samples, black bars represent HR-GFP-Vpx+/- VLPs pretreated samples (B) Data are from three donors and bars represent mean+sSEM (B). Statistical analysis was done using two-tailed paired T-test, *P < 0.05.

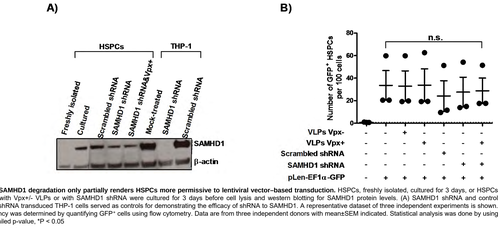


FIG. 7. Vpx-mediated SAMHD1 degradation only partially renders HSPCs more permissive to lentiviral vector–based transduction. HSPCs, freshly isolated, cultured for 3 days, or HSPCs pretreated for 2 hours with Vpx+/- VLPs or with SAMHD1 shRNA were cultured for 3 days before cell lysis and western blotting for SAMHD1 protein levels. (A) SAMHD1 shRNA and control of mammalian non-target shRNA transduced THP-1 cells served as controls for demonstrating the efficacy of shRNA to SAMHD1. A representative dataset of three independent experiments is shown. (B) Transduction efficiency was determined by quantifying GFP$^+$ cells using flow cytometry. Data are from three independent donors with mean±SEM indicated. Statistical analysis was done by using paired T-test with two-tailed p-value, *P < 0.05

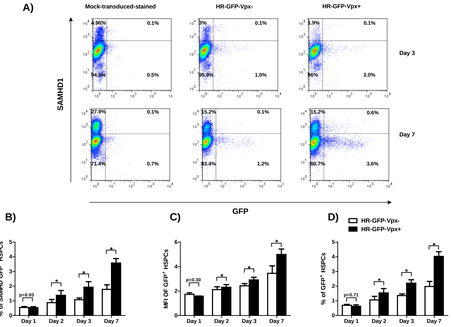


FIG. 7. Vpx partially overcomes restriction to HIV-1-based lentiviral vector transduction in HSPCs. HSPCs were transduced with HR-GFP-Vpx+ or HR-GFP-Vpx- at an MOI 100, and were subsequently cultured for 7 days. SAMHD1 and GFP expression were analyzed by flow cytometry at day 3 and 7 (A). The percentage of SAMHD1$^-$GFP$^+$ cells in total GFP$^+$ cells is shown in (B). MFI and transduction efficiency of HR-GFP-Vpx+ or HR-GFP-Vpx- transduced HSPCs are shown in (C and D) . A representative dataset of three independent experiments is shown. Statistical analysis was done using paired T-test, *P < 0.05, **P < 0.005.

Conclusion

HSPCs cultured in cytokine-enriched medium, unlike uncultured cells, express high levels of SAMHD1. Vpx-mediated decreases of SAMHD1 expression levels enhances transduction rate in HSPCs. Furthermore, the data imply that other blocks mainly at cell entry are the major limiting step for efficient transduction.

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