Poster # 169

Vpx-mediated degradation of SAMHD1 does not render hematopoietic progenitor cells more permissive to lentiviral gene transfer

Contact information:
Duo Li
University Hospital Zurich
Rämistrasse 100, 8091 Zurich
Switzerland
duo.li@usz.ch
+41442553367

Duo Li[1], Erika Schlaepfer[1], Baek Kim[2], Oliver T. Keppler[3], Annette Audigé[1], Roberto F. Speck[1]

[1] Division of Infectious Diseases and Hospital Epidemiology, University Hospital of Zurich, University of Zurich, Switzerland
. [2]Center for Drug Discovery, Department of Pediatrics, Emory University, Atlanta, Georgia, US.
[3]Department of Infectious Diseases, Virology, University of Heidelberg, Heidelberg, Germany.

Background

Understanding how to achieve efficient transduction of hematopoietic progenitor cells (HPC), while preserving their long-term ability to self-renew, is key to 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 and resting CD4$^+$ T cells that interferes with reverse transcription by decreasing the nucleotide pool. Vpx originates from HIV-2 or SIV and relieves the block in HIV-1 reverse transcription through the proteosomal degradation of SAMHD1. We hypothesized that SAMHD1 might be operative in HIV-1-vector-based HPC transduction.

Methods

We used Vpx-mediated degradation of SAMHD1, SAMHD1 shRNA, HIV-2 or SIV-based lentiviral vectors or provided an excess of deoxynucleoside triphosphates (dNTPs) or deoxynucleoside (dNs) to study whether the decrease of SAMHD1 or the increase of dNTP pool will lead to a higher transduction rate. In addition, Alu-PCR was performed, which aimed to quantify the integrated provirus in HPC and monocyte-derived macrophages (MDMs).

Results

Our results show that SAMHD1 is highly expressed in HPC cultured in a medium enriched with cytokines. In contrast, uncultured HPC have poor SAMHD1 expression. Expression levels of SAMHD1 in cultured HPC are even comparable with those found in myeloid cells, including monocytes and MDMs. However, Vpx$^+$ virus-like particles (VLPs), dNs, SAMHD1 shRNA, and HIV-2 and SIV-based lentiviral vectors did not result in a higher transduction rate, suggesting that the potential block in reverse transcription in HPC was not relieved. Notably, while Vpx$^+$ VLPs resulted in a considerable decrease of SAMHD1 in HPC, the remaining SAMHD1 level was still comparatively high. Lastly, Vpx$^+$ VLPs led to a striking increase of dNTPs in MDMs. However, we observed only a minor increase in HPC, which corresponded to the observation that Vpx$^+$ VLPs did not result in an accumulation of integrated provirus in HPC.

Results

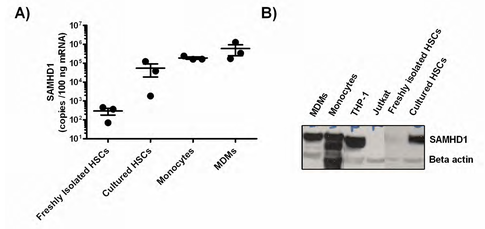


FIG 1 SAMHD1 is highly expressed in cultured HPC mRNA (A) and protein levels (B) were measured in freshly isolated HPC, HPC cultured for three days in a cytokine-enriched milieu, monocytes and MDMs. THP-1 cells and Jurkat cells were included as controls when measuring SAMHD1 protein levels. A) RNA was 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 per 100 ng/mRNA. B) Protein levels of SAMHD1 determined by western blotting; β-actin served as loading control.

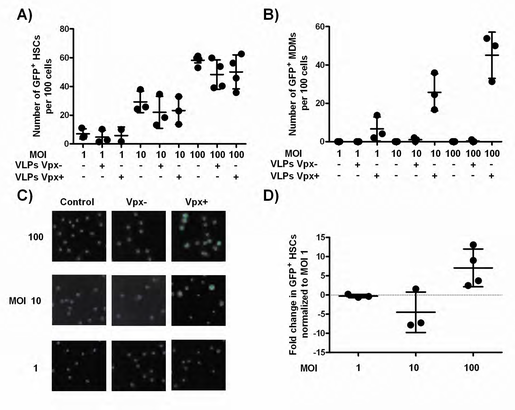


FIG 2 Vpx$^+$ VLPs pretreatment has no effect on transduction efficiency. HPC (A) or MDMs (B and C) were pretreated for two hours with Vpx$^{-/+}$ VLPs or HPC (D) exposed to 0.01 mM dNTPs. Transduction was performed with pLen-EF1a-GFP at MOIs of 1, 10 and 100. Transduction efficiency was determined by quantifying the GFP$^+$ HPC by flow cytometry (A, D) and by immunohistochemistry (B, C). Data are from three independent donors of MDMs and HPC.

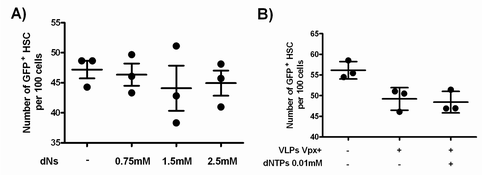


FIG 3 dNs pretreatment and pretreatment with a combination of Vpx$^+$ VLPs and dNTPs have no influence on transduction efficiency. (A) HPC were pretreated for two hours with 0.075, 1.5 or 2.5 mM dNs or (B) with Vpx$^+$ VLPs for two hours before transduction with an HIV-1-based vector. Just before adding the HIV-1-based vector, 0.01 mM dNTPs were added to the culture medium. Transduction was performed at an MOI of 100. Transduction efficiency was determined by quantifying the GFP$^+$ HPC by flow cytometry. The graph shows the data obtained from three independent donors of HPC.

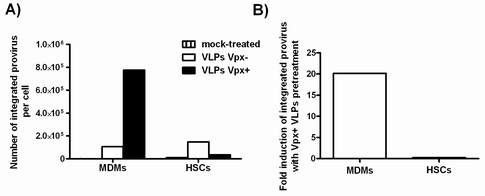


FIG 4 Vpx$^+$ VLPs do not result in accumulation of integrated provirus. MDMs and HPC were pretreated for two hours with Vpx$^{-/+}$ VLPs, followed by Len-EF1a-GFP transduction at an MOI 100, and subsequently cultured for three days before harvesting. Quantification of integrated provirus by Alu-PCR was done as described in Methods. (A) Absolute numbers of integrated provirus copies per cell after 30 cycles of Alu-PCR amplification and (B) fold induction. A representative example out of three is shown.

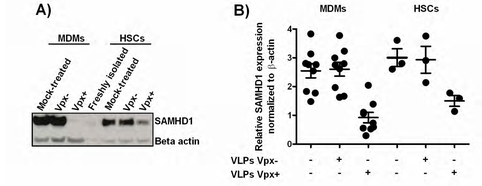


FIG 5 Vpx$^+$ VLPs treatment effectively reduces SAMHD1 in HPC and MDMs. MDMs from nine donors and HPC from three donors were pretreated for two hours with Vpx$^{-/+}$ VLPs before cell lysis. Protein levels of SAMHD1 were determined by western blotting; β-actin served as loading control. (A) A representative western blot, and (B) semi-quantitative analysis of all western blotting.

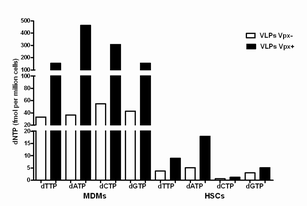


FIG 6 Vpx$^+$ VLPs treatment results in minor increases in the dNTP pool of HPC. MDMs and freshly isolated HPC, were pretreated for two hours with Vpx$^{-/+}$ VLPs and cultured for three days before cell lysis. Quantification of dNTPs was done by a single nucleotide incorporation assay. A representative dataset of three independent experiments is shown.

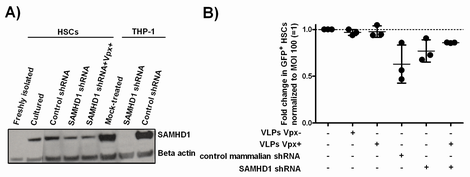


FIG 7 Silencing of SAMHD1 by RNAi does not render HPC more permissive to HIV-1 vector–based transduction. HPC, freshly isolated, cultured for three days, or were pretreated for two hours with Vpx$^{-/+}$ VLPs or with SAMHD1 shRNA and subsequently cultured for three days before cell lysis and western blotting for SAMHD1 levels. (A) SAMHD1 shRNA and control mammalian non-target shRNA transduced THP-1 cells served as controls for demonstrating the efficacy of shRNA to SAMHD1. (B) Transduction efficiency was determined by quantifying GFP$^+$ cells by flow cytometry. A representative dataset of three independent experiments is shown.

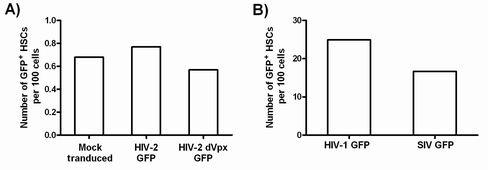


FIG 8 HIV-2 and SIV-based lentiviruses containing Vpx have similar infection rates/transduction efficacy as HIV. (A) HPC from the same donors were challenged with equivalent infectious units of HIV-2 GFP or HIV-2 ΔVpx GFP, (B) or equivalent an MOI (100) of HIV-1 and SIV-based GFP virions and analyzed for the percentage of GFP$^+$ cells. A representative dataset of three independent experiments is shown.

Conclusion

In summary, cytokine exposed HPC, unlike quiescent cells, express high levels of SAMHD1. However, the Vpx-mediated decrease of SAMHD1 levels was not sufficient to increase the lentiviral-based transduction rate. Alternatively, other restriction factors might be operative in HPC.

Acknowledgement: supported by the Swiss National Science Foundation and the Clinical Research Focus Program “Human Hemato-Lymphatic Diseases” of the University of Zurich.