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ORIGINAL INVESTIGATION

Increased SAMHD1 transcript expression correlates with interferon-related genes in HIV-1-infected patients

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Abstract

Purpose To investigate the contribution of SAMHD1 to HIV-1 infection in vivo and its relationship with IFN response, the expression of SAMHD1 and IFN-related pathways was evaluated in HIV-1-infected patients.

Methods Peripheral blood mononuclear cells (PBMC) from 388 HIV-1-infected patients, both therapy naïve ($n = 92$) and long-term HAART treated ($n = 296$), and from 100 gender and age-matched healthy individuals were examined. CD4+ T cells, CD14+ monocytes and gut biopsies were also analyzed in HIV-1-infected subjects on suppressive antiretroviral therapy. Gene expression levels of SAMDH1, ISGs (MxA, MxB, HERC5, IRF7) and IRF3 were evaluated by real-time RT-PCR assays.

Results SAMHD1 levels in HIV-1-positive patients were significantly increased compared to those in healthy donors. SAMHD1 expression was enhanced in treated patients compared to naïve patients ($p < 0.0001$) and healthy donors ($p = 0.0038$). Virologically suppressed treated patients exhibited higher SAMHD1 levels than healthy donors ($p = 0.0008$), viraemic patients ($p = 0.0001$) and naïve patients ($p < 0.0001$). SAMHD1 levels were also increased in CD4+ T cells compared to those in CD14+ monocytes and in PBMC compared to those of GALT. Moreover, SAMHD1 was expressed more strongly than ISGs in HIV-1-infected patients and positive correlations were found between SAMHD1, ISGs and IRF3 levels.

Conclusions SAMHD1 is more strongly expressed than the classical IFN-related genes, increased during antiretroviral therapy and correlated with ISGs and IRF3 in HIV-1-infected patients.

Keywords SAMHD1 · HIV-1 · ISG · IFN · IRF3

Introduction

Sterile alpha motif and HD-domain-containing protein 1 (SAMHD1) is a human triphosphohydrolase able to act as an HIV-1 restriction factor in non-dividing myeloid cells and quiescent CD4+ T cells [1–7]. Its main activity consists in limiting the intracellular dNTP pool required for reverse transcription. Controversial results have been obtained on the ability of SAMHD1 to exhibit an RNAase activity for counteracting HIV-1 infection [8–10]. A significant number of in vitro studies suggest that SAMHD1 may constitute an important mechanism of cellular defence against HIV-1/2, and a broad range of viruses including murine leukemia virus (MLV), human T-cell leukemia virus type 1, vaccinia virus, herpes simplex virus 1 (HSV-1) and hepatitis B virus (HBV) [11–18]. However, few data have addressed the interplay between HIV-1 and SAMHD1 expression

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in vivo and functional regulation of this retroviral restriction factor [19–22]. Indeed, alongside its ability to control cellular dNTP homeostasis, SAMHD1 acts as a critical regulator in innate immune responses due to its association with autoimmune disease leading to aberrant activation of interferon (IFN) [23]. Although the underlying mechanism is still unclear, current knowledge suggests that SAMHD1 is induced in vitro by IFNα/β stimulation in different cell lines such as primary monocytes, U87-MG, HEK293T and HeLa cells [23–25]. By contrast, type I IFNs did not induce SAMHD1 in THP-1, monocyte-derived macrophages (MDMs), primary CD4+ T lymphocytes, monocyte-derived dendritic cells (MDDC) or myeloid and plasmacytoid dendritic cells (DC) [6, 24–26], suggesting that the regulation of SAMHD1 synthesis during HIV-1 infection could vary considerably according to the type of cell studied. Interestingly, IFN regulatory factor 3 (IRF3), a crucial transcription factor in the innate immune response against viral infection, has been proposed to induce direct upregulation of SAMHD1 expression independently from the classical IFN-induced JAK–STAT pathway [27]. Moreover, SAMHD1 Thr592 phosphorylation, which correlates with its loss of ability to restrict HIV-1 replication [28, 29], is reduced after type I IFN treatment, while its expression level remains insensitive, uncovering a new IFN regulation mechanism of restriction factors [29]. Nevertheless, several proinflammatory cytokines can modulate SAMHD1 expression levels or phosphorylation status at threonine 592 in primary lymphoid and myeloid HIV-1 target cells, but it remains unclear whether this translates into any change in the cells susceptibility to HIV-1 [6].

Since an inadequate regulation of type I IFN drives chronic immune activation in HIV-1 infection [30, 31], and considerable controversy exists over whether type I IFN can support SAMHD1 production [27], further research is needed to address possible associations between SAMHD1 levels and those of IFN-related pathways in HIV-1 infection.

Therefore, the aim of this study was to gain new insights into the role played by SAMHD1 in regulating the natural course of HIV-1 infection, evaluating SAMHD1 expression and its relationship with the IFN response in vivo. In particular, we examined SAMHD1 expression in peripheral blood mononuclear cells (PBMC) of naïve HIV-1-infected patients, long-term antiretroviral-treated patients and healthy individuals, and correlated the expression levels with CD4+ T cell counts and viral load. SAMHD1 levels were also analyzed in HIV-1 target cells (CD4+ T cells and CD14+ monocytes) and in the effector sites (lamina propria) of the gastrointestinal tract within a subgroup of HIV-1-infected subjects on suppressive antiretroviral therapy.

Last, to address the in vivo relationship between SAMHD1 and IFN-related pathways, SAMHD1 levels were compared and correlated with those of different IFN-related genes, such as well-established ISGs [myxovirus resistance A (MxA) and B (MxB), HECT and RLD domain-containing E3 ubiquitin protein ligase 5 (HERC5), and IRF7], and IRF3 in HIV-1-positive patients.

Materials and methods

Study population

The blood samples used in this retrospective study derived from routine HIV-1 viraemia testing at Policlinico Umberto I "Sapienza" University Hospital in Rome, Italy. Peripheral blood samples obtained from 388 HIV-1-infected patients, both therapy naïve ($n = 92$) and long-term HAART treated ($n = 296$), and from 100 gender- and age-matched healthy individuals were examined. Treated HIV-1-positive patients were also divided into two groups based on achieving ($n = 243$) or not ($n = 53$) virological suppression (defined as persistent HIV-1 RNA levels below the lower limit of detection of the currently used assay, 37 copies/mL) in response to HAART. CD4+ T cells, CD14+ monocytes and gut biopsies obtained, respectively, from a subgroup of 27 and 7 of HIV-1-infected subjects on suppressive antiretroviral therapy were also analyzed. CD4+ T cells and CD14+ monocytes were also isolated from 22 gender- and age-matched healthy donors. Last, paired peripheral blood samples were also obtained during long-term antiretroviral therapy from seven HIV-1 infected patients whose blood samples had been collected before starting antiretroviral therapy.

The study was approved by the ethics committee of the Policlinico Umberto I Hospital, "Sapienza" University of Rome (ethical approval code Rif#3184), and informed consent was obtained from both HIV-1-positive patients and healthy individuals.

Specimen processing

PBMC were isolated from fresh blood collected from all HIV-1-infected patients and healthy individuals by Ficoll–Hypaque density gradient centrifugation (Sigma-Aldrich; St. Louis, MO, USA) and dry pellets were stored at − 80 °C. CD14+ monocytes and CD4+ T cells were isolated from PBMC collected from 27 long-term-treated HIV-1-infected patients and from 22 healthy donors by positive selection using MACS® Technology (Miltenyi Biotec; Bergisch Gladbach, Germany) according to the manufacturer's protocol. Furthermore, within the group of HIV-1-infected patients on long-term suppressive antiretroviral therapy, seven underwent endoscopic procedures as previously described [32]. Gut biopsies from each intestine site (terminal ileum, cecum, and ascending, transverse, and descending colon) were pooled, washed twice with EDTA wash media, incubated in EDTA solution 5 mM and digested with 1 mg/mL collagenase (Sigma-Aldrich; Milan, Italy) and 1.5 U DNAse I (Sigma-Aldrich; Milan, Italy), allowing the isolation of lamina propria lymphocytes (LPLs) that were filtered through a 70-μm cell strainer as previously described [32]. One aliquot of LPLs was stored at − 80 °C as dried pellets for RNA extraction and gene expression analysis.

Flow cytometry assay

The distribution of cellular populations was evaluated by flow cytometry analysis on freshly isolated PBMC and LPL. Acquisitions were performed on Miltenyi Biotec flow cytometer-MACSQuant Analyzer (eight fluorescence channels, three lasers). Gating analysis and data were analyzed using MACSQuantify software 2.5 (Miltenyi Biotec). The MACSQuant is equipped with three lasers: a 405-nm solid-state diode, a 488-nm solid state and a 633 nm. Cells were washed twice with phosphate buffered saline (PBS) and then resuspended in 250 μL of PBS. Each sample volume was run in flow cytometry using the same gating strategy. Results were obtained as a percentage values on the total cells.

Measurement of HIV-1 RNA and CD4+ T-lymphocyte count

HIV-1 viral load was determined by Versant HIV-1 RNA kPCR assay (Siemens Healthineers, Tarrytown NY, USA) which has a detection limit of 37 copies/mL. Absolute CD4+ T cell count was performed by FACScalibur flow cytometer (Becton Dickinson; San Jose, CA, USA).

Real-time polymerase chain reaction assay

Quantitative RT/real-time PCR for SAMHD1, ISGs (MxA, MxB, HERC5, and IRF7), and IRF3 levels was carried out with the LightCycler480 instrument (Roche, Basel, Switzerland). Total RNA was extracted from PBMC collected from HIV-1-positive patients and healthy individuals using the RNeasy Plus Universal Kit (Qiagen; Hilden, Germany) and reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Foster City, CA, USA) according to the manufacturer's protocols. Primers and probes for each gene were added to the Probes Master Mix (Roche; Basel, Switzerland) at 500 and 250 nM, respectively, in a final volume of 20 μl. The housekeeping gene β-glucuronidase was used as an internal control. Gene expression values were calculated by the comparative Ct method. All the primers/probe sets were purchased from Applied Biosystems (Foster City, CA, USA). The primers and probe sequences used for each gene were the following: SAMHD1: Forward: 5′-TCTCACATGTTTGATGGACGATT-3′; Reverse: 5′-CCTTGTTCATGCGTCCATTTC-3′; Probe: 5′-[6FAM]ATTCCACTTGCTCGCCCGGAGG[TAM]-3′; MxA [33]: Forward: 5′-CTGCCTGGCAGAAAACTTACC-3′; Reverse: 5′-CTCTGTTATTCTCTGGTGAGTCTCCTT-3′; Probe: 5′-[6FAM]CATCACACATATCTGTAAATCTCTGCCCCTGTTAGA[TAM]-3′; IRF3: Forward: 5′-AGCAGAGGACCGGAGCAA-3′; Reverse: 5′-AGAGGTGTCTGGCTGGGAAA[TAM]-3′; Probe 5′-[6FAM]ACCCTCACGACCCACATAAAATCTACGAGTTTG[TAM]-3′; IRF7: Forward: 5′-TGTGCCGAGTGCACCTAGAG-3′; Reverse: 5′-GAAGCACTCGATGTCGTCATAGAG-3′; Probe: 5′-[6FAM]CGCAGCGTGAGGGTGTGTCTTCC[TAM]-3′; β-glucuronidase [34]: Forward 5′-TCTGTCAAGGGCAGTAACCTG-3′; Reverse: 5′-GCCCACGACTTTGTTTTCTG-3′; Probe: 5′-[6FAM]TATGTCTTTCGATATGCAGCCAAGTTTTACCG[TAM]-3′. Primers and probes for MxB and HERC5 were ordered as kits: MxB: assay on demand Hs01550814_m1, HERC5 assay on demand Hs00180943_m1 (Applied Biosystems; Foster City, CA, USA). Validation of amplification efficiency was made for every primer/probe set and was calculated according to the equation ($E = 10 - 1/\text{slope}$). The slope for each gene was about − 3.3, indicating approximately equal amplification efficiency for all target gene and endogenous control.

Statistical analysis

Statistical analyses were performed using Graphpad Prism 5, and data were presented as mean ± SD or median (IQR = interquartile range). The demographic and clinical characteristics of HIV-1-positive patients and healthy donors were compared using Student's t and chi-squared tests. Gene expression differences between HIV-1-positive patients and healthy donors were analyzed using the Mann–Whitney *U* test. The Wilcoxon signed-rank test for paired samples was used to evaluate the gene expression differences between CD4+ T cells and CD14+ monocytes or GALT and PBMC, and the different frequencies of T cells in GALT and PBMC. The same test was used to assess the longitudinal changes of SAMHD1 in HIV-1-positive patients before and after antiretroviral treatment. Spearman's rho coefficient was calculated to assess the correlation between SAMHD1 levels and those of ISGs and IRF3. A *p* value less than 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of HIV-1-infected patients

The demographic and clinical characteristics of HIV-1-infected patients are summarized in Table 1. Overall, 92 naïve and 296 long-term antiretroviral therapy-experienced

Table 1 Demographic and clinical characteristics of treatment-naïve and HAART-treated HIV-1- positive patients and healthy controls

Item[a]	Healthy controls, $n = 100$ (A)	Treatment-naïve HIV-1-positive patients[c], $n = 92$ (B)	Viremic HAART-treated HIV-1 patients, $n = 53$ (C)	Successfully HAART-treated HIV-1 patients, $n = 243$ (D)	A vs B p values	C vs D p values
Age (years)	48 ± 16.6	38 ± 11.81	50 ± 9.41	50 ± 14.02	0.07	0.4
Gender (male/female) (number)	60/40	71/21	38/15	170/73	0.06	0.9
HIV-1 RNA (copies/mL)[b]	NA	34,925 (12,785–111,850)	7862 (3205–56,000)	< 37	NA	< 0.0001
CD4+ T cells/mm^3 at the enrolment	NA	445 (205–590)	362 (130–535)	560 (430–750)	NA	< 0.0001
Years of treatment	NA	NA	10 (5–17)	12 (7–16)	NA	0.6
Therapy class (number)	NA	NA	PI (22) NRTI (15) NNRTI (7) INSTI (9)	PI (100) NRTI (142) NNRTI (51) INSTI (34)	NA	0.1

NA not applicable, *PI* protease inhibitor, *NRTI* nucleoside (or nucleotide) reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *INSTI* integrase strand transfer inhibitor

[a] Data are expressed as mean ± SD or median (IQR). Differences in demographic and clinical characteristics between HIV-1-positive patients and healthy controls were evaluated using Student's t and chi-squared tests

[b] HIV-1 viral load was determined by Versant HIV-1 RNA kPCR assay (Siemens Healthineers, Tarrytown, NY, USA) which has a detection limit of 37 copies/mL

[c] Some of the patients analyzed in this study were included in Immunobiology 221(2):282–90 and clinical data partially reused with permission from Elsevier/Immunobiology and the authors, and expanded by new data

HIV-1-positive patients were included in the study. Untreated HIV-1-positive patients comprised 71 men (77%) with a mean age of 38 years. Plasma viral load ranged from 143 to 1,405,000 copies/mL. Treated HIV-1-infected patients were divided into two groups based on achieving ($n = 243$) or not ($n = 53$) virological suppression in response to HAART. There were no significant differences between the two groups in age, gender or antiretroviral therapy duration. HIV-1-positive patients with a detectable viral load on HAART had a median HIV-1 RNA of 7862 copies/mL (IQR: 3205–56,000). A control group of HIV-uninfected healthy subjects [mean age: 48 years; gender: 60 male (60%)] was also included ($n = 100$).

SAMHD1 mRNA expression in PBMC

We first measured SAMHD1 mRNA levels in PBMC collected from HIV-1-infected patients and age-matched HIV-seronegative healthy individuals (Fig. 1a). SAMHD1 levels were significantly increased in all HIV-1-positive patients compared to the healthy donors ($p = 0.04$). We then evaluated SAMHD1 expression stratifying the HIV-1-positive patients into three different groups: naïve, HAART-experienced patients with detectable viraemia and those with suppressed viral load (< 37 HIV-1 RNA copies/mL). Treated patients showed an increased expression of SAMHD1 compared to naïve patients ($p < 0.0001$) and to healthy donors ($p = 0.0038$). Among treated patients, the virologically suppressed group showed an increased expression of SAMHD1 compared to healthy donors ($p = 0.0008$), viraemic patients ($p = 0.0001$) and naïve patients ($p < 0.0001$). We have also repeated the SAMHD1 gene expression analysis on equivalent groups of comparable size, and the results were confirmed (Supplementary Fig. 1, Panel A–C).

By a longitudinal analysis, we also examined changes in SAMHD1 expression in paired peripheral blood samples obtained during long-term antiretroviral therapy from seven HIV-1-infected patients whose blood samples had been collected before starting antiretroviral therapy. All these patients became aviraemic after antiretroviral therapy (median therapy duration: 7 years) and had a median value of 590 CD4+ T cells/mm^3. No patients had any concurrent acute illness or infection. SAMHD1 levels increased in all patients analyzed with a median (range) SAMHD1 increase of 0.2 Log (0.02–1.38) (Fig. 1b).

Few data are available on the relationship between SAMHD1 levels vs HIV-1 load and CD4+ T cell counts in chronic HIV-1 infection [21, 22, 35]. Thus, we next investigated any correlations in naïve and HAART-treated HIV-1-infected patients but found no association between these factors (CD4+ T cell count: naïve HIV-1-positive patients: $p = 0.06$, $r = 0.22$; treated HIV-1-positive patients with detectable viremia: $p = 0.09$, $r = 0.26$; HIV-1 load: naïve HIV-1-positive patients: $p = 0.13$, $r = -0.2$; treated HIV-1-positive patients with detectable viremia: $p = 0.68$, $r = -0.08$). Furthermore, no differences in SAMHD1

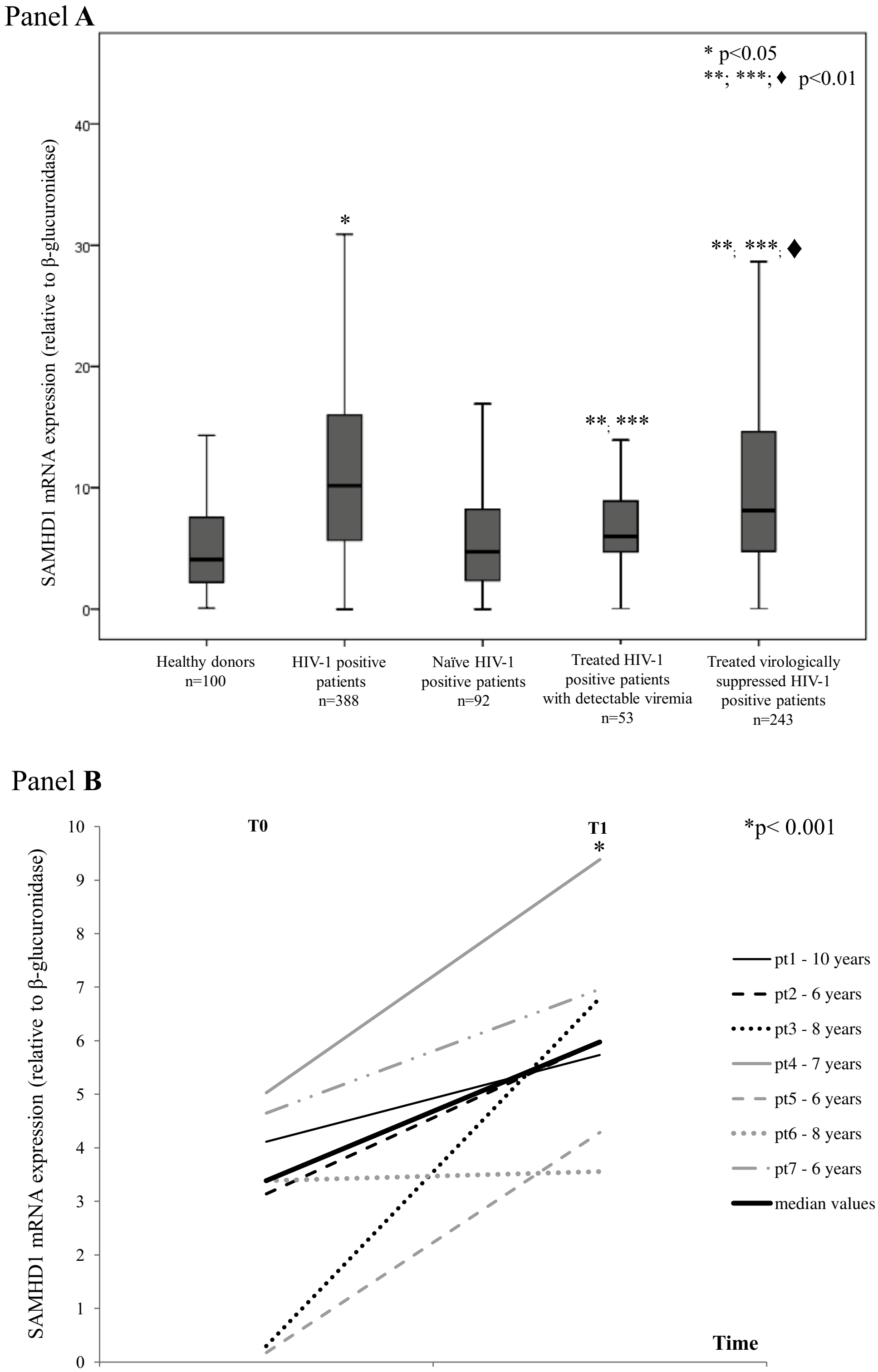

Fig. 1 SAMHD1 mRNA expression in PBMC. **a** SAMHD1 mRNA expression levels were measured in PBMC collected from 92 treatment-naïve HIV-1-positive patients, 243 successfully treated HIV-1-positive patients, 53 viraemic HAART-treated HIV-1-positive patients and 100 age-matched healthy controls. (*) HIV-1-positive patients *vs* healthy donors $p = 0.04$; (**) treated patients *vs* naïve patients $p < 0.0001$; (***) treated patients *vs* healthy donors $p = 0.0038$; (***) virologically suppressed patients *vs* healthy donors $p = 0.0008$; (**) virologically suppressed patients *vs* naïve patients $p < 0.0001$; (♦) virologically suppressed patients *vs* viraemic patients $p = 0.0001$. Data were analyzed using the Mann–Whitney *U* test. **b** Individual and median variation of SAMHD1 expression during long-term HAART in PBMC collected from seven virologically suppressed HIV-1-positive patients. Each patient is represented by a line. The interval time elapsed between T0 (before HAART initiation) and T1 (after HAART initiation) is indicated for every patient near the corresponding line. T0 vs T1 $p < 0.001$. Data were analyzed using the Wilcoxon signed-rank test

expression were recorded between males and females (data not shown).

SAMHD1 mRNA expression in CD4+ T cells, CD14+ monocytes and GALT

As myeloid cells and CD4+ T cells from human peripheral blood are the primary target cells during HIV-1 infection, and SAMHD1 levels were increased in patients maintaining suppression of HIV-1 load to less than 37 copies/mL, we further examined SAMHD1 expression in these cells collected from a subgroup of 27 out of the 296 treated HIV-1-positive patients and from 22 sex- and age-matched healthy donors. SAMHD1 levels were higher in CD4+ T cells than in CD14+ monocytes paired samples (treated patients: $p = 0.038$; healthy donors: $p < 0.0001$; Fig. 2). By comparing SAMHD1 expression in CD4+ T cells and CD14+ monocytes between HIV-1-infected patients and healthy donors, an increased SAMHD1 expression in these cell subsets was recorded in treated HIV-1-positive patients (patients vs healthy donors, CD4+ T cells: $p < 0.0001$; CD14+ monocytes: $p < 0.0001$; Fig. 2).

Since the low expression of SAMHD1 plays an important role in the susceptibility of differentiated T cell subsets to HIV-1 infection in vivo [36], we selected a subgroup of 7 out of 296 treated HIV-1-positive patients (Table 1; Fig. 1) with relatively low PBMC SAMHD1 mRNA expression to explore SAMHD1 levels in GALT. We found twofold higher median values of SAMHD1-mRNAs in PBMC compared to those measured in GALT paired samples ($p = 0.04$; Fig. 3, Panel A). To verify if this discrepancy was due to a different cell composition, GALT and PBMC samples were also analyzed by flow cytometry. Within the GALT and PBMC, T lymphocytes were predominant, and their frequencies were significantly different between these samples (Fig. 3b–d).

Relationship between SAMHD1 and IFN-related pathways

Data on the relationship between type I IFN and SAMHD1 compared to other retroviral restriction factors are conflicting [6, 25, 29]. We, therefore, compared the expression levels of SAMHD1 with those of a well-established type I IFN-induced gene, MxA, both in healthy donors and HIV-1-infected patients. SAMHD1 transcript levels were higher than MxA mRNA in healthy donors and in naïve, viraemic and virologically suppressed HIV-1-infected patients ($p < 0.0001$ for all the analysis, Fig. 4a). Moreover, SAMHD1 transcript levels were compared to those of other ISGs (MxB, HERC5, IRF7) and IRF3, recording an enhanced expression of SAMHD1 transcript levels in both viraemic and virologically suppressed HIV-1-infected patients ($p < 0.0001$ for all the analysis; Fig. 4, Panel B). Comparing the level of these genes between viraemic-treated HIV-1-positive patients and those with suppressed viraemia, a general trend towards increasing ISGs and IRF3 expression was found in viraemic-treated patients; however, the differences were statistically significant for HERC5 ($p = 0.0062$) and IRF3 ($p = 0.0007$) (Fig. 4b). Last, we evaluated whether expression levels of SAMHD1 were correlated with those of the ISGs and IRF3 in HIV-1-positive patients' groups. A coordinated activation between SAMHD1 and MxA was observed as

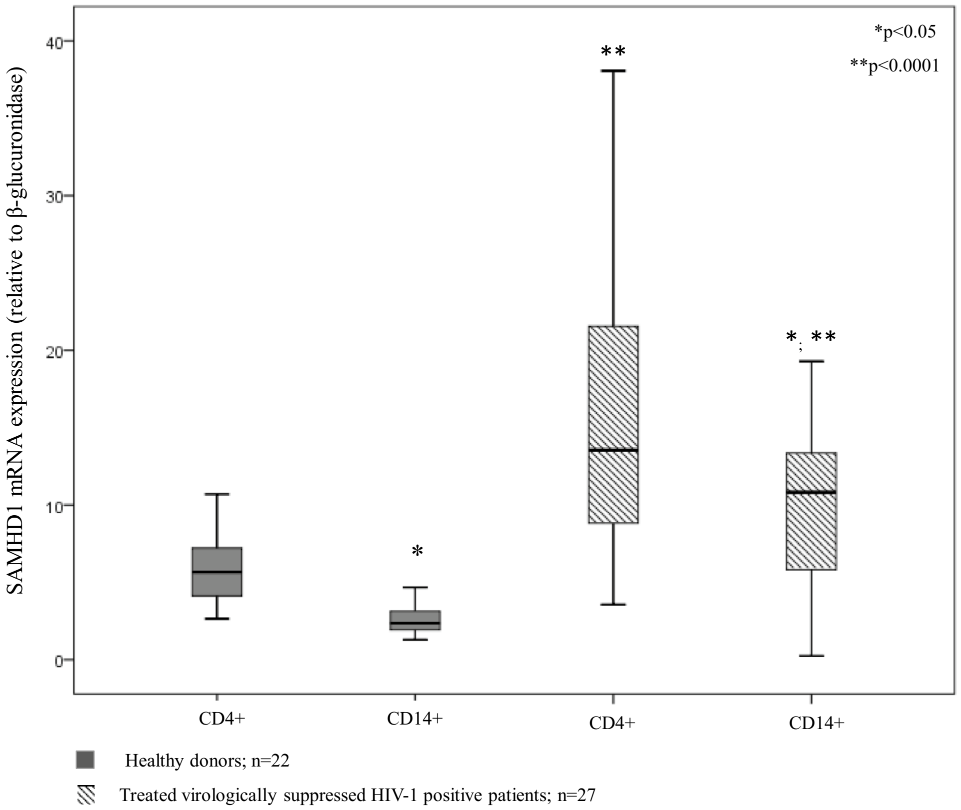

Fig. 2 SAMHD1 mRNA expression in CD4+ T cells and CD14+ monocytes. SAMHD1 mRNA expression levels were measured in CD4+ T cells and CD14+ monocytes collected from 27 of the 296 treated HIV-1-positive patients, and 22 sex- and age-matched healthy donors. (*) CD4+ T cells vs CD14+ monocytes: treated HIV-1-positive patients $p = 0.038$, healthy donors $p < 0.0001$ (Wilcoxon signed-rank test for paired samples). (**) Treated HIV-1-positive patients v*s* healthy donors: CD4+ T cells $p < 0.0001$, CD14+ monocytes $p < 0.0001$ (Mann–Whitney *U* test)

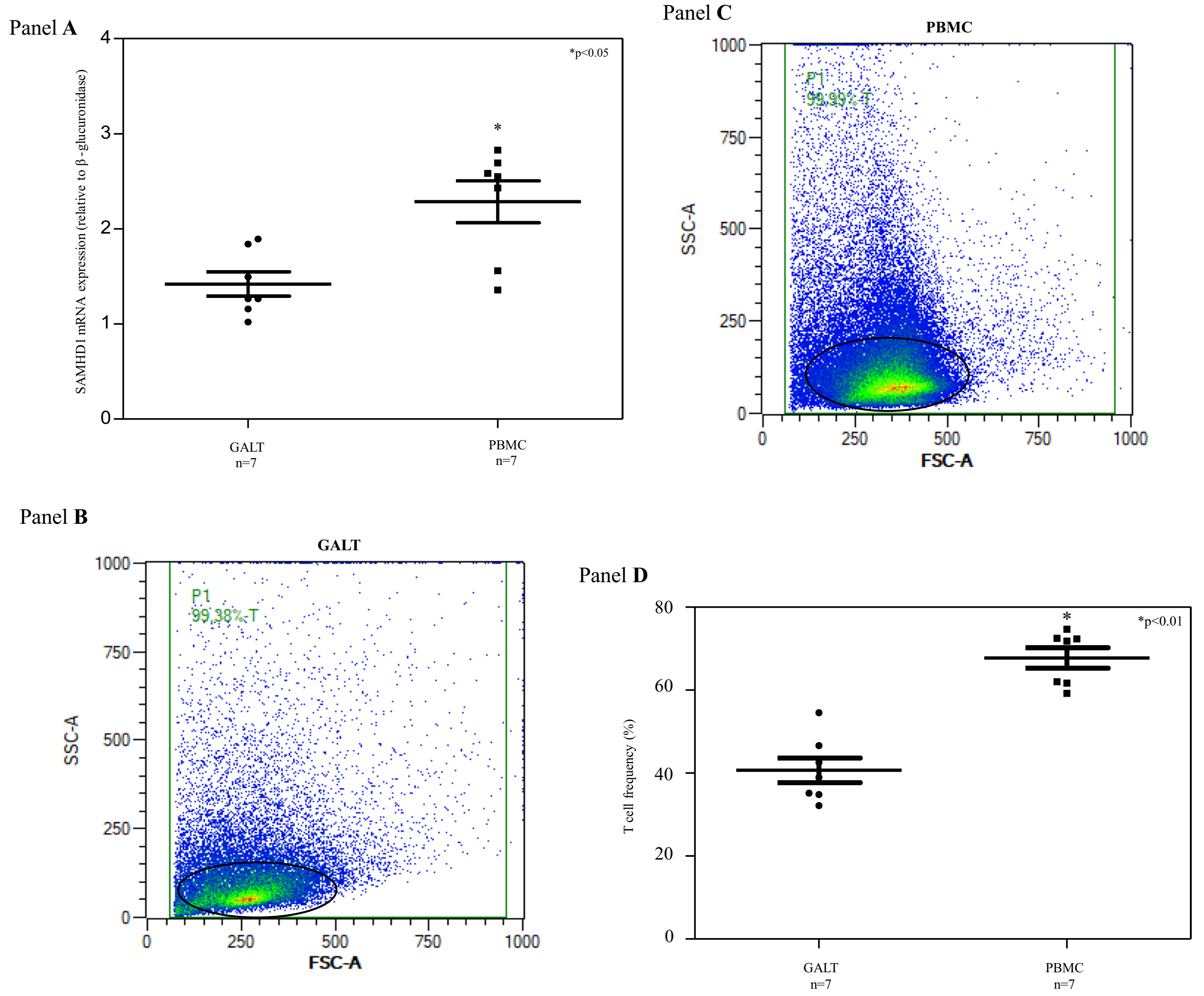

Fig. 3 SAMHD1 expression levels in GALT. **a** SAMHD1 expression levels were measured in paired GALT and PBMC samples collected from 7 of the 296 treated HIV-1-positive patients. *GALT *vs* PBMC $p=0.04$. Data were analyzed using the Wilcoxon signed-rank test for paired samples. **b, c** Representative distribution of cellular populations in forward and side scatter (FSC-SSC) dot plot acquired by FACS. The fraction of T cells found in paired GALT and PBMC samples collected from one out of seven treated HIV-1-positive patients are indicated by a circle. **d** Frequencies of T cells in all paired GALT and PBMC samples ($n=7$). GALT *vs* PBMC *$p=0.01$. Data were analyzed using the Wilcoxon signed-rank test for paired samples

evidenced by the correlation between these two genes in naïve and viraemic-treated HIV-1-infected patients (Table 2). By contrast, SAMHD1 and MxA levels were not correlated with each other in the PBMC of aviraemic HIV-1-infected patients (Table 2). A weak correlation was also found between SAMHD1 levels and those of MxB, HERC5, IRF7 in both viraemic and virologically suppressed HIV-1-infected patients, while SAMHD1 levels were correlated with those of IRF3 only in the virologically suppressed group (Table 2).

Discussion

To determine what happens to SAMHD1 transcript expression during chronic HIV-1 infection, we first analyzed SAMHD1 expression in PBMC from HIV-1-positive patients naïve to HAART and HIV-1-infected patients who received prolonged HAART with or without persistent HIV-1 RNA suppression (plasma viral load < 37 copies of HIV-1/RNA levels). We found that aviraemic-treated patients displayed a significant increase in SAMHD1 levels

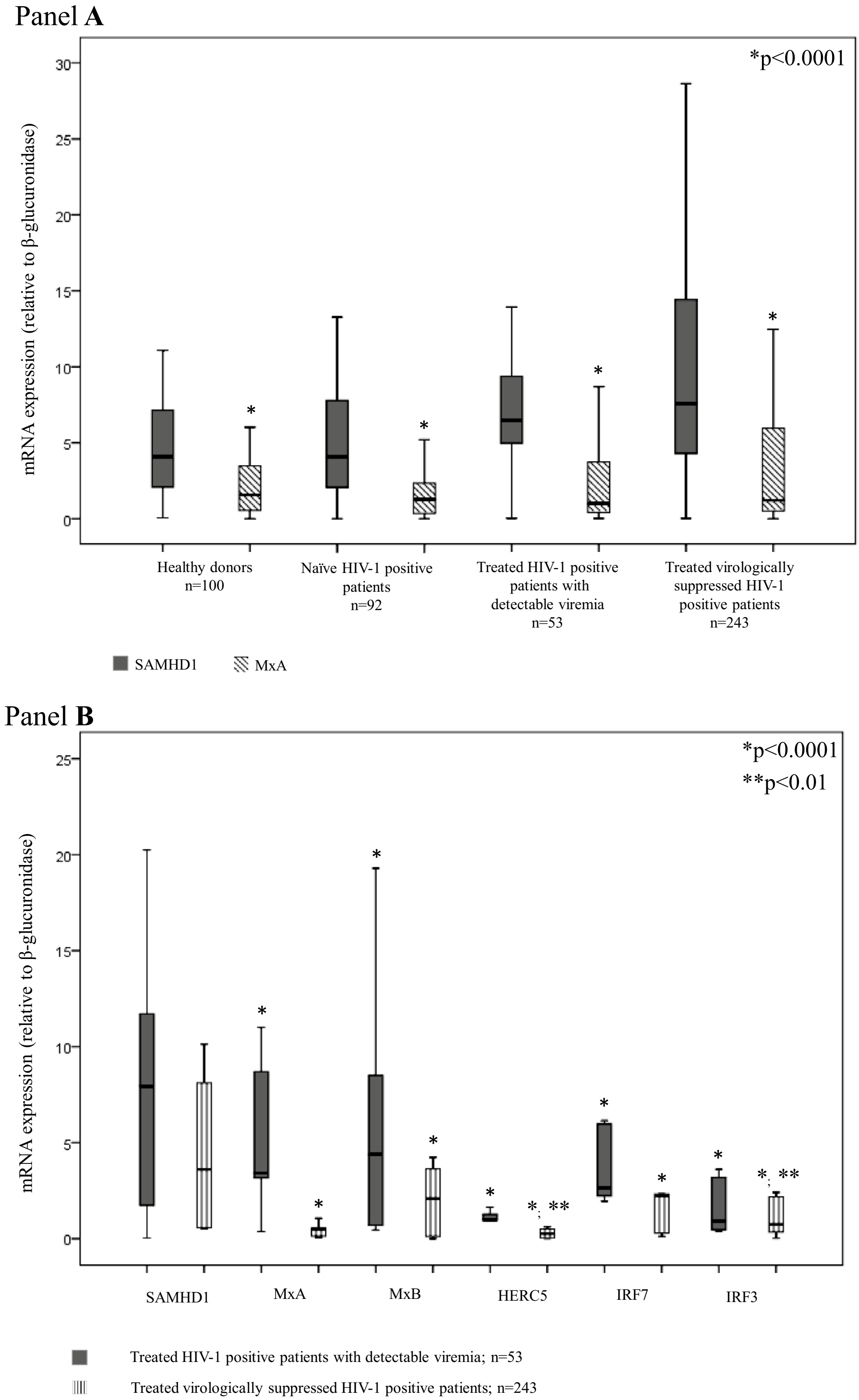

Fig. 4 Comparison between SAMHD1, ISGs and IRF3 mRNA expression levels. **a** SAMHD1 and MxA mRNA expression levels were measured in PBMC collected from 92 untreated HIV-1-infected patients, 243 treated virologically suppressed HIV-1-infected patients, 53 treated HIV-1-infected patients with detectable viraemia and 100 age-matched HIV-seronegative healthy individuals. (*) SAMHD1 vs MxA in naïve, viraemic, virologically suppressed HIV-1-infected patients, and in healthy donors: $p < 0.0001$ for all analyses. Data were analyzed using the Mann–Whitney *U* test. **b** SAMHD1 expression levels were compared to those of ISGs (MxB, HERC5, IRF7) and IRF3 in viraemic ($n = 53$) and virologically suppressed HIV-1-infected patients ($n = 243$). (*) SAMHD1 *vs* ISGs analyzed $p < 0.0001$ for all analyses; (*) SAMHD1 vs IRF3 $p < 0.0001$. Data were analyzed using the Mann–Whitney *U* test. (**) Viraemic patients vs virologically suppressed patients: HERC5 $p = 0.0062$; IRF3 $p = 0.0007$. Data were analyzed using the Mann–Whitney *U* test

compared to naïve patients, viraemic-treated patients, and healthy donors, suggesting that SAMHD1 increased during antiretroviral therapy concomitantly with HIV-1 RNA suppression. This finding obtained from a single time point evaluation was also strongly supported by the significant increase of SAMHD1 levels found in patients on long-term HAART therapy who exhibited permanent viral suppression whose blood samples had been collected before

Table 2 Relationship between SAMHD1, ISGs (MxA, MxB, HERC5, IRF7) and IRF3 mRNA levels in HIV-1-positive patients

	Treatment-naïve HIV-1-positive patients, $n=92$	Viraemic HAART-treated HIV-1 patients, $n=53$	Successfully HAART-treated HIV-1 patients, $n=243$
MxA	$p=\mathbf{0.0001}$, $r=\mathbf{0.99}$	$p=\mathbf{0.012}$, $r=\mathbf{0.34}$	$p=0.925$, $r=-0.006$
MxB	NA	$p=\mathbf{0.00008}$, $r=\mathbf{0.68}$	$p=\mathbf{0.012}$, $r=\mathbf{0.29}$
HERC5	NA	$p=\mathbf{0.012}$, $r=\mathbf{0.47}$	$p=\mathbf{0.001}$, $r=\mathbf{0.32}$
IRF7	NA	$p=\mathbf{0.025}$, $r=\mathbf{0.31}$	$p=\mathbf{0.001}$, $r=\mathbf{0.32}$
IRF3	NA	$p=0.263$, $r=0.15$	$p=\mathbf{0.007}$, $r=\mathbf{0.28}$

SAMHD1, ISGs (MxA, MxB, HERC5, IRF7), and IRF3 mRNA expression were analyzed in PBMC collected from 92 treatment-naive HIV-1-positive patients, 243 successfully treated HIV-1-positive patients, and 53 viraemic HAART-treated HIV-1-positive patients. Spearman's rho coefficient was used to assess the correlation between these two genes. Significant correlations are highlighted in bold

The amount of material obtained from PBMC of naïve HIV-1 positive patients was not sufficient to examine MxB, HERC5, and IRF7 transcript levels

NA not applicable

and after starting antiretroviral therapy. In agreement, HIV-1 infection leads to depletion of SAMHD1 cells in activated CD4+ T cells, but these SAMHD1 aberrations cannot be repaired after long-term HAART-suppressed HIV-1 replication [21]. Interestingly, several studies have shown that SAMHD1 possesses a differential impact on HIV-1 sensitivity to nucleoside analogue reverse transcriptase inhibitors [37–39]. In our study, all the long-term HAART-treated HIV-1-positive patients were exposed to multidrug regimens, thus it was not possible to examine the relationship between SAMHD1 levels and the efficacies of nucleoside reverse transcriptase inhibitors.

Moreover, SAMHD1 regulation was found not to correlate with viral load as previously demonstrated in HIV-1 patients but also in SIV and HIV-2 infection models [21, 22, 35, 40, 41]. Unlike HIV-2, HIV-1 lacks a countermeasure against SAMHD1. Thus, it could be speculated that the chronic immune activation, characterizing even successfully treated HIV-1-infected individuals, might be sufficient to maintain the aberrations in SAMHD1 production. Indeed, several mediators related to the persistent immune activation found in HIV-1-infected patients have been proposed to regulate the production and/or activity of SAMHD1. In particular, IL-2, IL-7, IL-12/IL-18, TNF-α alone and SDF-1α/β, CCL21, XCL1, CCL14, and CCL27 used in combination can modulate the production/function of SAMHD1 [42–44]. T cell activation also contributed to altered SAMHD1 properties in HIV-1-infected patients [21].

In addition, upregulation of the type I IFN pathway is considered one of the markers of persistent immune activation during HIV-1 infection [30, 31]. A relationship between SAMHD1 and the innate immune response was supported by the discovery that toll-like agonists and IFNα/β can induce SAMHD1 expression [1, 23–25, 45, 46]. However, controversial results have emerged on the ability of type I IFNs to induce SAMHD1 directly depending on the type of cell analyzed and the experimental conditions used [6, 23–26, 47]. Our study showed that SAMHD1 was more efficiently expressed during HIV-1 infection than classical ISGs. Although high gene expression does not necessarily imply that a gene is more or less important for a given function, our finding indicates that the strong gene expression of SAMHD1 recorded in HIV-1-positive patients could be due to its constitutive expression throughout the human body [6] and its ability to be induced/regulated by various host factors including microRNA [48]. In support of these findings, our study confirmed that the ISGs levels decreased during HAART [34], while for SAMHD1 an opposite trend was recorded. Moreover, the expression of SAMHD1 and MxA levels was coordinated in naïve and viraemic HIV-1-infected patients, thereby supporting the ability of HIV-1 RNA/DNA to trigger intrinsic antiviral immune response via pattern recognition receptors [49–53]. Similarly, the expression of SAMHD1 was correlated with that of MxB, HERC5, and IRF7 in viraemic HIV-1-infected patients. Unfortunately, the amount of material obtained from PBMC of naïve HIV-1 positive patients was not sufficient to examine MxB, HERC5, and IRF7 transcript levels. However, SAMHD1 has also been implicated in suppressing spontaneous activation of the type I IFN system and reducing T-cell responses [8, 54, 55]. This suggests a potential beneficial effect of SAMHD1 in regulating an abnormal IFN response and might explain in part why SAMHD1 levels were not positively correlated to those of MxA in virologically suppressed patients. In this regard, HIV-1 elite controllers, who are characterized by a similar level of IFN response to healthy donors, maintain higher levels of SAMHD1 transcripts in PBMC than do viraemic progressors [19, 35]. This result is also consistent with the findings of another study, which revealed that SAMHD1-activated CD4+ T cells are more permissive to HIV-1 infection and thus might lead to the loss of this cell subset in HIV-1-infected patients [36].

It is also known that ISGs-mRNAs are not expressed in the same levels during HIV-1 infection [34, 56]. This last aspect might explain in part why SAMHD1 levels were found to be correlated with MxB, HERC5, and IRF7 in virologically suppressed HIV-1 treated patients.

However, our ISG-based experiments had some limitations. First, gene expression may vary between circulating cell populations and so the use of PBMC may show apparent differences in the level of ISG expression that could be due to changes in the size of cell populations. Second, persistent exposure of cells to IFN, as observed during HIV-1 infection, could induce a steady state in which only the un-phosphorylated ISGF3 (U-ISGF3)-dependent genes are expressed [57]. However, the direct quantification of type I IFNs represents an additional problem. There are many different type I IFNs and IFN-α subtypes are also differentially expressed during HIV-1 infection [58]. Moreover, type I IFNs usually exhibit low half-life and their expression is extremely low [59]. Hence, the group of ISGs analyzed in this study does not represent a single immune phenomenon. Indeed, the IFN transcriptome is known to be modular with sets of ISGs that respond not only to type I IFN but also to other type of IFNs (e.g. IFN-λs) [60].

Our study also extends earlier findings on the major role played by IRF3 in upregulating SAMHD1 [27] by showing a strong positive correlation between SAMHD1 levels and those of IRF3 in virologically suppressed HIV-1-positive patients. This correlation suggests that during HIV-1 infection SAMHD1 expression could also be triggered directly by IRF3 in a mechanism independent of the classical IFN-induced JAK-STAT pathway [27].

To gain new insights into the role played by SAMHD1 in regulating the natural course and pathogenesis of HIV-1, SAMHD1 expression was also evaluated in CD4+ T cells, CD14+ monocytes and GALT in a subgroup of HIV-1-positive patients who had achieved sustained undetectable HIV-1 RNA levels on HAART. Both these HIV-1 target cells carry SAMHD1 as previously reported [6, 61], while an enhanced expression of SAMHD1 was recorded in CD4+ T cells compared to those measured in CD14+ monocytes. The same trend was observed in healthy donors. Interestingly, as observed for PBMC, both CD4+ T cells and CD14+ monocytes isolated from treated HIV-1-positive patients expressed greater SAMHD1-mRNA levels than those from healthy donors. Higher levels of SAMHD1-mRNA were also found in PBMC compared to those measured in GALT. In this context, immunohistochemical staining of anal mucosa and rectum tissue sections of healthy individuals revealed abundant constitutive expression of this restriction factor in infiltrating and resident-nucleated haematopoietic cells in mucosa and submucosa of these transmission site tissues [6]. In contrast, our previous studies recorded an upregulated expression of IFNα, IFNβ and type I IFN receptors in GALT compared to PBMC obtained from HIV-1-infected patients [32, 62], suggesting that the interplay between SAMHD1 and IFN is more complex in the gastrointestinal tract of HIV-1-infected patients. However, it remains unclear which triggers (viral or bacterial) and cell types [e.g. T cells, DCs, epithelial cells] in the gut were driving these gene expression changes in long-term-treated HIV-1-infected patients. In this context, the reduced expression of SAMHD1 in GALT samples could be related to the different amount of T cells recorded in GALT and PBMC, revealing the importance to analyze cell composition in gene expression studies.

Furthermore, it must be highlighted that SAMHD1 expression levels could be not critical for its function. Indeed, it has been demonstrated that SAMHD1 function is regulated by phosphorylation [19, 28, 29, 44, 63–65], i.e., expression levels of SAMHD1 can be similar in distinct cells, but activity might be significantly different depending on its phosphorylation status. Since phosphorylation is tightly linked to cell cycle initiation and progression, a limitation of this study was to not consider this aspect when comparing cell types from different tissues (CD4+ T cells, CD14+ monocytes and GALT) of HIV-1-positive patients. However, measuring mRNA represents a viable strategy for studying a specific phenomenon at the molecular scale, but it cannot be assumed that the mRNA amount is directly correlated with protein expression since post-translational or transcriptional modifications (phosphorylation, ubiquitination, methylation, acetylation or GlcNAcylation) are key to the final synthesis of the protein [66]. Other condition-dependent features of the mRNA such as RNA structure may also change under certain conditions such as translation efficiency [66]. Interestingly, beside phosphorylation, SAMHD1 levels and activity can be regulated by promoter DNA methylation, microRNA-181a and acetylation [67–69]. The latter poses considerable challenges to in vivo analysis of SAMHD1 levels and function, and their relationship with IFN pathways during HIV-1 infection. Moreover, PBMC used in this study were isolated from residual blood samples collected during routine HIV-1 viraemia testing, so the amount of the material was just enough to perform the gene expression experiments. Further studies aimed to evaluate the phosphorylation status of SAMHD1 and its protein level as well as those of IFN-related genes in PBMC, CD4+ T cells, at different time points during HIV-1 infection are needed to better unravel the in vivo relationship between SAMHD1 and IFN response. In this scenario, flow cytometry analysis of cell composition of PBMC should be also performed. Most relevant myeloid lineage targets of HIV-1 in vivo (e.g. macrophages) should be also analyzed.

Conclusions

We have shown that SAMHD1 mRNA levels follow a hierarchical order of virologically suppressed HIV-1 patients > long-term-treated HIV-1 patients with detectable viraemia > therapy-naïve HIV-1-positive patients/HIV-uninfected subjects; SAMHD1 mRNA levels are not correlated with surrogates of HIV-1 disease progression: viral load and CD4+ T count; and an abundant amount of SAMHD1 is present in PBMC and CD4+ T cells compared to that measured in CD14+ monocytes and GALT, respectively. Moreover, this is the first study to investigate the in vivo relationship between IFN's pathways and SAMHD1 indicating an increased expression of SAMHD1 compared to classical ISGs and a coordinated activation among SAMHD1, ISGs (MxA, MxB, HERC5, IRF7), and IRF3 in HIV-1-infected patients. These findings suggest that a relationship between IFN-related pathways and SAMHD1 does exist in vivo. However, there is a multifaceted regulation for the synthesis of ISGs, and the expression of ISGs could provide antiviral defence without the need for inducible IFN production, highlighting the complexity of phenomenon analyzed. Furthermore, SAMHD1 exhibits an opposite trend compared to the IFN-related genes, suggesting that other mechanisms are needed to promote the strong SAMHD1 expression in HIV-1-infected patients with suppressed viraemia. These mechanisms are currently unknown and warrant further investigation.

Author contributions MS wrote the paper, carried out the experiment and performed statistical analysis. LS, AV, CP, and CR collected the samples and participated in carrying out the experiments. IM, GC and GD provided patient's samples and participated in the design and revision of the manuscript. OT and GA participated in the design and revision of the manuscript. CS conceived the study, analyzed the data, wrote the paper and supervised the work. All authors reviewed the work and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflict of interest.

References

1. Antonucci JM, St Gelais C, Wu L (2017) The dynamic interplay between HIV-1, SAMHD1, and the innate antiviral response. Front Immunol 8:1541. https://doi.org/10.3389/fimmu.2017.01541
2. Lahouassa H, Daddacha W, Hofmann H, Ayinde D, Logue EC, Dragin L, Bloch N, Maudet C, Bertrand M, Gramberg T, Pancino G, Priet S, Canard B, Laguette N, Benkirane M, Transy C, Landau NR, Kim B, Margottin-Goguet F (2012) SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. Nat Immunol 13(3):223–228. https://doi.org/10.1038/ni.2236
3. Goldstone DC, Ennis-Adeniran V, Hedden JJ, Groom HC, Rice GI, Christodoulou E, Walker PA, Kelly G, Haire LF, Yap MW, de Carvalho LP, Stoye JP, Crow YJ, Taylor IA, Webb M (2011) HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature 480(7377):379–382. https://doi.org/10.1038/nature10623
4. Jia X, Zhao Q, Xiong Y (2015) HIV suppression by host restriction factors and viral immune evasion. Curr Opin Struct Biol 31:106–114. https://doi.org/10.1016/j.sbi.2015.04.004
5. Baldauf HM, Pan X, Erikson E, Schmidt S, Daddacha W, Burggraf M, Schenkova K, Ambiel I, Wabnitz G, Gramberg T, Panitz S, Flory E, Landau NR, Sertel S, Rutsch F, Lasitschka F, Kim B, König R, Fackler OT, Keppler OT (2012) SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells. Nat Med 18(11):1682–1687. https://doi.org/10.1038/nm.2964
6. Schmidt S, Schenkova K, Adam T, Erikson E, Lehmann-Koch J, Sertel S, Verhasselt B, Fackler OT, Lasitschka F, Keppler OT (2015) SAMHD1's protein expression profile in humans. J Leukoc Biol 98(1):5–14. https://doi.org/10.1189/jlb.4HI0714-338RR
7. Simon V, Bloch N, Landau NR. Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. Nat Immunol 2015, 16(6):546–53. https://doi.org/10.1038/ni.3156
8. Ryoo J, Choi J, Oh C, Kim S, Seo M, Kim SY, Seo D, Kim J, White TE, Brandariz-Nuñez A, Diaz-Griffero F, Yun CH, Hollenbaugh JA, Kim B, Baek D, Ahn K (2014) The ribonuclease activity of SAMHD1 is required for HIV-1 restriction. Nat Med 20(8):936–941. https://doi.org/10.1038/nm.3626
9. Yang Z, Greene WC (2014) A new activity for SAMHD1 in HIV restriction. Nat Med 20(8):808–809. https://doi.org/10.1038/nm.3657
10. Antonucci JM, St Gelais C, de Silva S, Yount JS, Tang C, Ji X, Shepard C, Xiong Y, Kim B, Wu L (2016) SAMHD1-mediated HIV-1 restriction in cells does not involve ribonuclease activity. Nat Med 22(10):1072–1074. https://doi.org/10.1038/nm.4163
11. Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Ségéral E, Yatim A, Emiliani S, Schwartz O, Benkirane M (2011) SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 474(7353):654–657. https://doi.org/10.1038/nature10117
12. Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, Florens L, Washburn MP, Skowronski J (2011) Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature 474(7353):658–661. https://doi.org/10.1038/nature10195
13. Gramberg T, Kahle T, Bloch N, Wittmann S, Müllers E, Daddacha W, Hofmann H, Kim B, Lindemann D, Landau NR. Restriction of diverse retroviruses by SAMHD1. Retrovirology 2013, 10:26. https://doi.org/10.1186/1742-4690-10-26
14. Sze A, Belgnaoui SM, Olagnier D, Lin R, Hiscott J, van Grevenynghe J (2013) Host restriction factor SAMHD1 limits human T cell leukemia virus type 1 infection of monocytes via STING-mediated apoptosis. Cell Host Microbe 14(4):422–434. https://doi.org/10.1016/j.chom.2013.09.009
15. Sze A, Olagnier D, Lin R, van Grevenynghe J, Hiscott J (2013) SAMHD1 host restriction factor: a link with innate immune sensing of retrovirus infection. J Mol Biol 425(24):4981–4994. https://doi.org/10.1016/j.jmb.2013.10.022

16. Chen Z, Zhang L, Ying S (2012) SAMHD1: a novel antiviral factor in intrinsic immunity. Future Microbiol 7(9):1117–1126. https://doi.org/10.2217/fmb.12.81
17. Kim ET, White TE, Brandariz-Núñez A, Diaz-Griffero F, Weitzman MD (2013) SAMHD1 restricts herpes simplex virus 1 in macrophages by limiting DNA replication. J Virol 87(23):12949–12956. https://doi.org/10.1128/JVI.02291-13
18. Chen Z, Zhu M, Pan X, Zhu Y, Yan H, Jiang T, Shen Y, Dong X, Zheng N, Lu J, Ying S, Shen Y (2014) Inhibition of Hepatitis B virus replication by SAMHD1. Biochem Biophys Res Commun 450(4):1462–1468. https://doi.org/10.1016/j.bbrc.2014.07.023
19. Riveira-Muñoz E, Ruiz A, Pauls E, Permanyer M, Badia R, Mothe B, Crespo M, Clotet B, Brander C, Ballana E, Esté JA (2014) Increased expression of SAMHD1 in a subset of HIV-1 elite controllers. J Antimicrob Chemother 69(11):3057–3060. https://doi.org/10.1093/jac/dku276
20. Bolduan S, Koppensteiner H, Businger R, Rebensburg S, Kunze C, Brack-Werner R, Draenert R, Schindler M (2017) T cells with low CD2 levels express reduced restriction factors and are preferentially infected in therapy naïve chronic HIV-1 patients. J Int AIDS Soc 20(1):21865. https://doi.org/10.7448/IAS.20.1.21865
21. Fu W, Qiu C, Zhou M, Zhu L, Yang Y, Qiu C, Zhang L, Xu X, Wang Y, Xu J, Zhang X (2016) Immune Activation Influences SAMHD1 Expression and Vpx-mediated SAMHD1 Degradation during Chronic HIV-1 Infection. Sci Rep 6:38162. https://doi.org/10.1038/srep38162
22. Kiselinova M, De Spiegelaere W, Buzon MJ, Malatinkova E, Lichterfeld M, Vandekerckhove L (2016) Integrated and Total HIV-1 DNA Predict Ex Vivo Viral Outgrowth. PLoS Pathog 12(3):e1005472. https://doi.org/10.1371/journal.ppat.1005472
23. Berger A, Sommer AF, Zwarg J, Hamdorf M, Welzel K, Esly N, Panitz S, Reuter A, Ramos I, Jatiani A, Mulder LC, Fernandez-Sesma A, Rutsch F, Simon V, König R, Flory E (2011) SAMHD1-deficient CD14 + cells from individuals with Aicardi-Goutières syndrome are highly susceptible to HIV-1 infection. PLoS Pathog 7(12):e1002425. https://doi.org/10.1371/journal.ppat.1002425
24. Goujon C, Schaller T, Galão RP, Amie SM, Kim B, Olivieri K, Neil SJ, Malim MH (2013) Evidence for IFNα-induced, SAMHD1-independent inhibitors of early HIV-1 infection. Retrovirology 10:23. https://doi.org/10.1186/1742-4690-10-23
25. St Gelais C, de Silva S, Amie SM, Coleman CM, Hoy H, Hollenbaugh JA, Kim B, Wu L (2012) SAMHD1 restricts HIV-1 infection in dendritic cells (DCs) by dNTP depletion, but its expression in DCs and primary CD4 + T-lymphocytes cannot be upregulated by interferons. Retrovirology 9:105. https://doi.org/10.1186/1742-4690-9-105
26. Bloch N, O'Brien M, Norton TD, Polsky SB, Bhardwaj N, Landau NR (2014) HIV type 1 infection of plasmacytoid and myeloid dendritic cells is restricted by high levels of SAMHD1 and cannot be counteracted by Vpx. AIDS Res Hum Retroviruses 30(2):195–203. https://doi.org/10.1089/AID.2013.0119
27. Yang S, Zhan Y, Zhou Y, Jiang Y, Zheng X, Yu L, Tong W, Gao F, Li L, Huang Q, Ma Z, Tong G (2012) Interferon regulatory factor 3 is a key regulation factor for inducing the expression of SAMHD1 in antiviral innate immunity. Sci Rep 6:29665. https://doi.org/10.1038/srep29665
28. Harris RS, Hultquist JF, Evans DT (2012) The restriction factors of human immunodeficiency virus. J Biol Chem 287(49):40875–40883. https://doi.org/10.1074/jbc.R112.416925
29. Cribier A, Descours B, Valadão AL, Laguette N, Benkirane M (2013) Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. Cell Rep 3(4):1036–1043. https://doi.org/10.1016/j.celrep.2013.03.017
30. Sandstrom TS, Ranganath N, Angel JB (2017) Impairment of the type I interferon response by HIV-1: Potential targets for HIV eradication. Cytokine Growth Factor Rev 37:1–16. https://doi.org/10.1016/j.cytogfr.2017.04.004
31. Soper A, Kimura I, Nagaoka S, Konno Y, Yamamoto K, Koyanagi Y, Sato K (2018) Type I Interferon Responses by HIV-1 Infection: Association with Disease Progression and Control. Front Immunol 8:1823. https://doi.org/10.3389/fimmu.2017.01823
32. d'Ettorre G, Ceccarelli G, Andreotti M, Selvaggi C, Giustini N, Serafino S, Schietroma I, Nunnari G, Antonelli G, Vullo V, Scagnolari C (2015) Analysis of Th17 and Tc17 Frequencies and Antiviral Defenses in Gut-Associated Lymphoid Tissue of Chronic HIV-1 Positive Patients. Mediators Inflamm 2015:395484. https://doi.org/10.1155/2015/395484
33. Pierangeli A, Degener AM, Ferreri ML, Riva E, Rizzo B, Turriziani O, Luciani S, Scagnolari C, Antonelli G (2011) Interferon-induced gene expression in cervical mucosa during human papillomavirus infection. Int J Immunopathol Pharmacol 24(1):217–223. https://doi.org/10.1177/039463201102400126
34. Scagnolari C, Monteleone K, Selvaggi C, Pierangeli A, D'Ettorre G, Mezzaroma I, Turriziani O, Gentile M, Vullo V, Antonelli G (2016) ISG15 expression correlates with HIV-1 viral load and with factors regulating T cell response. Immunobiology 221(2):282–290. https://doi.org/10.1016/j.imbio.2015.10.007
35. Buchanan EL, McAlexander MA, Witwer KW (2015) SAMHD1 expression in blood cells of HIV-1 elite suppressors and viraemic progressors. J Antimicrob Chemother 70(3):954–956. https://doi.org/10.1093/jac/dku428
36. Ruffin N, Brezar V, Ayinde D, Lefebvre C, Schulze Zur Wiesch J, van Lunzen J, Bockhorn M, Schwartz O, Hocini H, Lelievre JD, Banchereau J, Levy Y, Seddiki N (2015) Low SAMHD1 expression following T-cell activation and proliferation renders CD4 + T cells susceptible to HIV-1. AIDS 29(5):519–530. https://doi.org/10.1097/QAD.0000000000000594
37. Huber AD, Michailidis E, Schultz ML, Ong YT, Bloch N, Puray-Chavez MN et al (2014) SAMHD1 has differential impact on the efficacies of HIV nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother 58:4915–4919. https://doi.org/10.1128/AAC.02745-1
38. Ballana E, Badia R, Terradas G, Torres-Torronteras J, Ruiz A, Pauls E et al (2014) SAMHD1 specifically affects the antiviral potency of thymidine analog HIV reverse transcriptase inhibitors. Antimicrob Agents Chemother 58:4804–4813. https://doi.org/10.1128/AAC.03145-14
39. Ordonez P, Kunzelmann S, Groom HC, Yap MW, Weising S, Meier C et al (2017) SAMHD1 enhances nucleoside-analogue efficacy against HIV-1 in myeloid cells. Sci Rep 7:42824. https://doi.org/10.1038/srep42824
40. Buchanan EL, Espinoza DA, McAlexander MA, Myers SL, Moyer A, Witwer KW (2016) SAMHD1 transcript upregulation during SIV infection of the central nervous system does not associate with reduced viral load. Sci Rep 6:22629. https://doi.org/10.1038/srep22629
41. Yu H, Usmani SM, Borch A, Krämer J, Stürzel CM, Khalid M, Li X, Krnavek D, van der Ende ME, Osterhaus AD, Gruters RA, Kirchhoff F (2013) The efficiency of Vpx-mediated SAMHD1 antagonism does not correlate with the potency of viral control in HIV-2-infected individuals. Retrovirology 10:27. https://doi.org/10.1186/1742-4690-10-27
42. Coiras M, Bermejo M, Descours B, Mateos E, García-Pérez J, LóHYP-Huertas MR, Lederman MM, Benkirane M, Alcamí J (2016) IL-7 Induces SAMHD1 Phosphorylation in CD4 + T Lymphocytes, Improving Early Steps of HIV-1 Life Cycle. Cell Rep 14(9):2100–2107. https://doi.org/10.1016/j.celrep.2016.02.022
43. Liao W, Bao Z, Cheng C, Mok YK, Wong WS. Dendritic cell-derived interferon-gamma-induced protein mediates tumor necrosis factor-alpha stimulation of human lung fibroblasts. Proteomics 2008, 8(13):2640–50. https://doi.org/10.1002/pmic.200700954

44. Pauls E, Jimenez E, Ruiz A, Permanyer M, Ballana E, Costa H, Nascimiento R, Parkhouse RM, Peña R, Riveiro-Muñoz E, Martinez MA, Clotet B, Esté JA, Bofill M (2013) Restriction of HIV-1 replication in primary macrophages by IL-12 and IL-18 through the upregulation of SAMHD1. J Immunol 190(9):4736–4741. https://doi.org/10.4049/jimmunol.1203226
45. Buitendijk M, Eszterhas SK, Howell AL (2014) Toll-like receptor agonists are potent inhibitors of human immunodeficiency virus-type 1 replication in peripheral blood mononuclear cells. AIDS Res Hum Retroviruses 30(5):457–467. https://doi.org/10.1089/AID.2013.0199
46. Sang M, Liu JB, Dai M, Wu JG, Ho WZ (2014) Toll-like receptor 3 signaling inhibits simian immunodeficiency virus replication in macrophages from rhesus macaques. Antiviral Res 112:103–112. https://doi.org/10.1016/j.antiviral.2014.10.008
47. Dragin L, Nguyen LA, Lahouassa H, Sourisce A, Kim B, Ramirez BC, Margottin-Goguet F (2013) Interferon block to HIV-1 transduction in macrophages despite SAMHD1 degradation and high deoxynucleoside triphosphates supply. Retrovirology 10:30. https://doi.org/10.1186/1742-4690-10-30
48. Riess M, Fuchs NV, Idica A, Hamdorf M, Flory E, Pedersen IM, König R (2017) Interferons Induce Expression of SAMHD1 in Monocytes through Down-regulation of miR-181a and miR-30a. J Biol Chem 292(1):264–277. https://doi.org/10.1074/jbc.M116.752584
49. Altfeld M, Gale M Jr (2015) Innate immunity against HIV-1 infection. Nat Immunol 16(6):554–562. https://doi.org/10.1038/ni.3157
50. Blasius AL, Beutler B (2010) Intracellular toll-like receptors. Immunity 32(3):305–315. https://doi.org/10.1016/j.immuni.2010.03.012
51. Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34(5):637–650. https://doi.org/10.1016/j.immuni.2011.05.006
52. Ramos HJ, Gale M Jr (2011) RIG-I like receptors and their signaling crosstalk in the regulation of antiviral immunity. Curr Opin Virol 1(3):167–176. https://doi.org/10.1016/j.coviro.2011.04.004
53. Loo YM, Gale M Jr (2011) Immune signaling by RIG-I-like receptors. Immunity 34(5):680–692. https://doi.org/10.1016/j.immuni.2011.05.003
54. White TE, Brandariz-Nuñez A, Martinez-Lopez A, Knowlton C, Lenzi G, Kim B, Ivanov D, Diaz-Griffero F (2017) A SAMHD1 mutation associated with Aicardi-Goutières syndrome uncouples the ability of SAMHD1 to restrict HIV-1 from its ability to downmodulate type I interferon in humans. Hum Mutat 38(6):658–668. https://doi.org/10.1002/humu.23201
55. Oh C, Ryoo J, Park K, Kim B, Daly MB, Cho D, Ahn K (2018) A central role for PI3K-AKT signaling pathway in linking SAMHD1-deficiency to the type I interferon signature. Sci Rep 8(1):84. https://doi.org/10.1038/s41598-017-18308-8
56. Liu SY, Sanchez DJ, Aliyari R, Lu S, Cheng G (2012) Systematic identification of type I and type II interferon-induced antiviral factors. Proc Natl Acad Sci USA 109(11):4239–4244. https://doi.org/10.1073/pnas.1114981109
57. Cheon H, Holvey-Bates EG, Schoggins JW, Forster S, Hertzog P, Imanaka N, Rice CM, Jackson MW, Junk DJ, Stark GR (2013) IFNβ-dependent increases in STAT1, STAT2, and IRF9 mediate resistance to viruses and DNA damage. EMBO J 32(20):2751–2763. https://doi.org/10.1038/emboj.2013.203
58. Scagnolari C, Antonelli G (2018) Type I interferon and HIV: Subtle balance between antiviral activity, immunopathogenesis and the microbiome. Cytokine Growth Factor Rev 40:19–31. https://doi.org/10.1016/j.cytogfr.2018.03.003
59. Rodero MP, Decalf J, Bondet V, Hunt D, Rice GI, Werneke S, McGlasson SL, Alyanakian MA, Bader-Meunier B, Barnerias C, Bellon N, Belot A, Bodemer C, Briggs TA, Desguerre I, Frémond ML, Hully M, van den Maagdenberg AMJM, Melki I, Musset L, Pelzer N, Quartier P, Terwindt GM, Wardlaw J, Wiseman S, Rieux-Laucat F, Rose Y, Neven B, Hertel C, Hayday A, Albert ML, Rozenberg F, Crow YJ, Duffy D (2017) Detection of interferon alpha protein reveals differential levels and cellular sources in disease. J Exp Med 214(5):1547–1555. https://doi.org/10.1084/jem.20161451
60. El-Sherbiny YM, Psarras A, Yusof MYM, Hensor EMA, Tooze R, Doody G, Mohamed AAA, McGonagle D, Wittmann M, Emery P, Vital EM (2018) A novel two-score system for interferon status segregates autoimmune diseases and correlates with clinical features. Sci Rep 8(1):5793. https://doi.org/10.1038/s41598-018-24198-1
61. Descours B, Cribier A, Chable-Bessia C, Ayinde D, Rice G, Crow Y, Yatim A, Schwartz O, Laguette N, Benkirane M (2012) SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4(+) T-cells. Retrovirology 9:87. https://doi.org/10.1186/1742-4690-9-87
62. Pinacchio C, Scheri GC, Statzu M, Santinelli L, Ceccarelli G, Innocenti GP, Vullo V, Antonelli G, Brenchley JM, d'Ettorre G, Scagnolari C (2018) Type I/II interferon in HIV-1-infected patients: expression in gut mucosa and in peripheral blood mononuclear cells and its modification upon probiotic supplementation. J Immunol Res 2018:1738676. https://doi.org/10.1155/2018/1738676
63. Badia R, Pujantell M, Torres-Torronteras J, Menéndez-Arias L, Martí R, Ruzo A, Pauls E, Clotet B, Ballana E, Esté JA (2017) Riveira-Muñoz E. SAMHD1 is active in cycling cells permissive to HIV-1 infection. Antiviral Res 142:123–135. https://doi.org/10.1016/j.antiviral.2017.03.019
64. Ruiz A, Pauls E, Badia R, Torres-Torronteras J, Riveira-Muñoz E, Clotet B, Martí R, Ballana E, Esté JA (2015) Cyclin D3-dependent control of the dNTP pool and HIV-1 replication in human macrophages. Cell Cycle 14(11):1657–1665. https://doi.org/10.1080/15384101.2015.1030558
65. Pauls E, Ruiz A, Badia R, Permanyer M, Gubern A, Riveira-Muñoz E, Torres-Torronteras J, Alvarez M, Mothe B, Brander C, Crespo M, Menéndez-Arias L, Clotet B, Keppler OT, Martí R, Posas F, Ballana E, Esté JA (2014) Cell cycle control and HIV-1 susceptibility are linked by CDK6-dependent CDK2 phosphorylation of SAMHD1 in myeloid and lymphoid cells. J Immunol 193(4):1988–1997. https://doi.org/10.4049/jimmunol.1400873
66. Maier T, Güell M, Serrano L (2009) Correlation of mRNA and protein in complex biological samples. FEBS Lett 583(24):3966–3973. https://doi.org/10.1016/j.febslet.2009.10.036
67. Kohnken R, Kodigepalli KM, Mishra A, Porcu P, Wu L (2017) MicroRNA-181 contributes to downregulation of SAMHD1 expression in CD4+ T-cells derived from Sèzary syndrome patients. Leuk Res 52:58–66. https://doi.org/10.1016/j.leukres.2016.11.010
68. Wang JL, Lu FZ, Shen XY, Wu Y, Zhao LT (2014) SAMHD1 is down regulated in lung cancer by methylation and inhibits tumor cell proliferation. Biochem Biophys Res Commun 455(3–4):229–233. https://doi.org/10.1016/j.bbrc.2014.10.153
69. Lee EJ, Seo JH, Park JH, Vo TTL, An S, Bae SJ, Le H, Lee HS, Wee HJ, Lee D, Chung YH, Kim JA, Jang MK, Ryu SH, Yu E, Jang SH, Park ZY, Kim KW (2017) SAMHD1 acetylation enhances its deoxynucleotide triphosphohydrolase activity and promotes cancer cell proliferation. Oncotarget 8(40):68517–68529. https://doi.org/10.18632/oncotarget.19704