

# SAMHD1 expression is associated with low immune activation but not correlated with HIV-1 DNA levels in CD4<sup>+</sup> T cells of patients with HIV-1

JIE LI<sup>1\*</sup>, CHUANHUA GAO<sup>2\*</sup>, SHANSHAN HUANG<sup>1</sup>, LONGTENG JIN<sup>3</sup> and CHANGZHONG JIN<sup>4</sup>

<sup>1</sup>Department of Infectious Disease, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325027; <sup>2</sup>Laboratory of Biochemistry and Biomaterials, Department of Materials, College of Chemical and Material Engineering, Quzhou University, Quzhou, Zhejiang 324000; <sup>3</sup>Department of Childhood Infectious Disease, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325027; <sup>4</sup>State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, P.R. China

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**Abstract.** Sterile  $\alpha$  motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1) can inhibit reverse transcription of human immunodeficiency virus-1 (HIV-1) by hydrolyzing intracellular deoxy-ribonucleoside triphosphate. However, its role in HIV-1 disease progression has not been extensively studied. To study the impacts of SAMHD1 on HIV-1 disease progression, especially on DNA levels, we investigated SAMHD1 levels in the peripheral blood of HIV-1 elite controllers (ECs), antiretroviral therapy (ART) naive viremic progressors (VPs) and patients with HIV-1 receiving ART (HIV-ARTs) compared with healthy controls. In addition, the present study analyzed the relationship between SAMHD1 and interferon- $\alpha$ , immune activation and HIV-1 DNA levels. The results of the present study demonstrated elevated SAMHD1 expression in the peripheral blood mononuclear cells of all patients with HIV-1,

but higher SAMHD1 expression in the CD4<sup>+</sup> T cells of only ECs compared with healthy controls. Immune activation was increased in the VPs and decreased in the ECs compared with healthy controls. Substantially lower HIV-1 DNA levels were identified in ECs compared with those in VPs and HIV-ARTs. SAMHD1 expression was associated with low levels of immune activation. No significant correlation was observed between SAMHD1 and HIV-1 DNA levels. Overall, the findings of the present study indicated that SAMHD1 was highly expressed in ECs, which may be associated with low immune activation levels, but was not directly related to HIV-1 DNA levels.

## Introduction

Host restrictive factors can inhibit human immunodeficiency virus-1 (HIV-1) infection at various stages, and these factors include apolipoprotein B mRNA editing enzyme catalytic subunit 3G proteins (1,2), Tripartite motif-containing protein 5  $\alpha$  (3,4), tetherin/bone marrow stromal cell antigen 2 (5) and sterile  $\alpha$  motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1) (6-9). SAMHD1 is a newly discovered HIV-1 host restriction factor, which can be degraded by the viral accessory protein Vpx of HIV-2 and certain simian immunodeficiency viruses (6-10). However, HIV-1 has lost the Vpx protein during evolution (9). Therefore, SAMHD1 is a host restriction factor that cannot be antagonized by HIV-1.

HIV-1 RNA is reversely transcribed into DNA following its fusion to target host cells, and this requires an adequate supply of deoxy-ribonucleoside triphosphate (dNTPs). SAMHD1 is a diguanosine triphosphate-dependent phosphatase that primarily hydrolyzes dNTPs into deoxynucleoside and inorganic triphosphoric acid, thus reducing the nuclear dNTP concentrations and inhibiting the reverse transcription of HIV-1 (11). There are several *in vivo* studies that have investigated SAMHD1 in the peripheral blood of patients

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*Correspondence to:* Dr Changzhong Jin, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, 79 Qingchun Avenue, Hangzhou, Zhejiang 310003, P.R. China  
E-mail: changzhongjin@163.com

Dr Longteng Jin, Department of Childhood Infectious Disease, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, 109 Xueyuanxi Street, Wenzhou, Zhejiang 325027, P.R. China  
E-mail: jlt786@163.com

\*Contributed equally

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with HIV-1 and analyzed its role in disease progression. Riveira-Muñoz *et al* (12) studied SAMHD1 expression in the CD4<sup>+</sup> T cells of patients with HIV-1, including elite controllers (ECs) with sustained plasma viral load below the limit of detection in the absence of antiretroviral treatment and viremic progressors (VPs) with high level of viral load (>5,000 cp/ml) and notable loss of CD4<sup>+</sup> T cells (<400/μl); the results demonstrated that higher levels of SAMHD1 were present in ECs compared with the healthy controls (HCs) and VPs, suggesting that SAMHD1 may serve a role in controlling viral replication and slowing the rate of disease progression. However, conflicting results were reported in HIV-exposed seronegative (HESN) individuals in other studies (13,14). Therefore, SAMHD1 expression in the peripheral blood of patients with HIV-1, especially in CD4<sup>+</sup> T cells, should continue to be investigated. Furthermore, since SAMHD1 restricts the reverse transcription of HIV-1, it may be interesting to study the association between SAMHD1 expression and HIV-1 DNA levels in CD4<sup>+</sup> T cells. The present study aimed to investigate the expression of SAMHD1 CD4<sup>+</sup> T cells of patients with HIV-1 receiving antiretroviral therapy (HIV-ARTs), VPs, ECs and HCs, and to study the associations between SAMHD1 expression and immune activation as well as the levels of HIV-1 DNA.

## Materials and methods

**Subjects.** HIV-1 positive patients with the mean age of 47.75 years (range, 27-62 years) were recruited from the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University and the First Affiliated Hospital, School of Medicine, Zhejiang University (Zhejiang, China) between January and September 2018. HIV-1 infection was diagnosed based on the positive results obtained from serological and HIV RNA detection assays. All subjects were volunteers and provided written informed consent to participate in the study. This study was approved by the ethics review boards of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University and the First Affiliated Hospital, School of Medicine, Zhejiang University (Zhejiang, China).

Three groups of patients with HIV-1 were included in the present study: i) 32 VPs who were ART naive and exhibited typical disease progression; ii) 26 HIV-ARTs with suppressed HIV-1 replication, whose viral load was maintained <50 copies/μl or with viral blips <1,000 copies/μl; and iii) 15 ECs with a sustained plasma viral load below the limit of detection in the absence of ART (non-consecutive blips of <2,000 copies/ml were allowed if present in <20% of viral load determinations) (12). In addition, 28 HCs were recruited from community clinics and comprised individuals who had not suffered from infectious or immune diseases during the previous month. The four groups were age- and sex-matched, and there were no significant differences in the mean CD8<sup>+</sup> T cell counts (Table I).

**Detection of SAMHD1 mRNA in the peripheral blood.** Peripheral blood (~5 ml) was collected from the participants into tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated by a density gradient

centrifugation method (1,000 x g for 20-30 min at room temperature) using Ficoll-Paque PLUS (GE Healthcare Life Science). CD4<sup>+</sup> T lymphocytes were purified from the PBMCs using MACS human CD4 microbeads for positive selection (Miltenyi Biotec GmbH). The purity of CD4<sup>+</sup> T lymphocytes was >90%. The total RNA was extracted from the PBMCs and CD4<sup>+</sup> T lymphocytes using TRIzol<sup>®</sup> reagent (Thermo Fisher Scientific, Inc.) and reverse-transcribed into cDNA using a total volume of 20 μl with the temperature protocol of 30°C for 10 min, 42°C for 45 min, and 95°C for 5 min. The quantitative analysis of SAMHD1 mRNA was performed using reverse transcription-quantitative (RT-q) PCR as previously described (15). Briefly, the reaction mixture included 10 μl SYBR<sup>®</sup> Green Master Mix (Takara Biotechnology Co., Ltd.), 0.2 μl each of the forward and reverse primers, and 2 μl cDNA, increased to a final volume of 20 μl with RNase-free water. RT-qPCR was performed using a DNA Engine Chromo 4 Real-time qPCR System (Bio-Rad Laboratories, Inc.) and the thermocycling conditions were as follows: 40 cycles of denaturation at 95°C for 45 sec, annealing at 62°C for 30 sec and extension at 72°C for 30 sec; the dsDNA was measured at 86°C after each cycle. GAPDH mRNA was detected as the internal control. The primers used were as follows: SAMHD1 forward, 5'-AAAACCAGGTTTCAC AACTTCTGC-3' and reverse, 5'-TGCGGCATACAAAC TTTTCTGT-3'; and GAPDH forward, 5'-TGCACCACCAAC TGCTTAGC-3' and reverse, 5'-GGCATGGACTGTGGT CATGAG-3'. The relative expression levels of SAMHD1 and GAPDH were calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method (16). Each sample was amplified three times.

**Detection of plasma interferon (IFN)-α levels.** The plasma IFN-α levels were detected using a human IFN-α ELISA kit (cat. no. 41100-1, R&D Systems, Inc.), according to the manufacturer's instructions. IFN-α concentrations were determined by comparing the samples with a standard curve.

**Quantification of HIV-1 RNA and DNA.** The HIV-1 viral load in the plasma of patients infected with HIV-1 was quantitatively detected using a standardized RT-qPCR (Cobas Amplicor HIV-1 Monitor Test; version 1.5; Ultra-sensitive specimen preparation; Roche Diagnostic Systems Inc) as previously described (17). The detection limit in the plasma was defined as 50 HIV-1 RNA copies/ml.

The total HIV-1 DNA, including the integrated HIV-1 DNA and episomal two long terminal repeat (2LTR) circles, in the peripheral blood was detected using a HIV-1 DNA Detection kit (PCR-Fluorescent Probing; SUPBIO; Guangzhou Hailite Biotechnology Co., Ltd.). Briefly, the total DNA was isolated from the blood using a QIAamp DNA Blood Mini Kit (Qiagen GmbH). The 50 μl reaction system contained 5 μl DNA and 45 μl PCR master mix. The test was performed using a Light Cycler 1.2 (Roche Diagnostics GmbH). The amplification conditions were set as follows: i) five cycles of 37°C for 5 min, 95°C for 10 min; ii) 95°C for 15 sec, 65°C for 15 sec and 72°C for 20 sec; iii) 40 cycles of 95°C for 15 sec, 62°C for 15 sec and 72°C for 20 sec; and iv) 10 cycles of 95°C for 15 sec, 52°C for 15 sec and 72°C for 32 sec. Two standard curves were calculated according to the volume of blood or PBMC counts. The results were expressed as copies/ml and copies/10<sup>6</sup> PBMCs.

Table I. Clinical information of the subjects.

Variable	HCs	VPs	HIV-ARTs	ECs	P-value
Number	28	32	26	15	
Male (%)	17 (60.71%)	19 (59.38%)	17 (65.38%)	4 (80.00%)	0.37
Age, years	31.35±14.63	38.71±7.63	42.66±11.57	36.81±15.19	0.53
Viral load (log <sub>10</sub> )	-	4.35±1.42	1.74±1.01	-	<0.01 <sup>a</sup>
CD4 <sup>+</sup> T cells/μl	702.66±142.82	356.17±188.83	517.05±161.20	637.58±216.33	0.01 <sup>a</sup>
CD8 <sup>+</sup> T cells/μl	674.46±228.94	1,056.81±523.02	835.88±370.36	747.88±298.36	0.17

<sup>a</sup>P<0.05. HIV, human immunodeficiency virus; HCs, healthy controls; VPs, viremic progressors; HIV-ARTs, patients with HIV-1 receiving antiretroviral therapy; ECs, elite controllers.

*Flow cytometry.* CD4<sup>+</sup>, CD8<sup>+</sup> and activated CD4<sup>+</sup>CD38<sup>+</sup> human leukocyte antigen (HLA)-DR<sup>+</sup> T cells in the peripheral blood were measured by flow cytometry (FACSCantoII; Becton, Dickinson and Company) using BD FACSCanto II System Software Upgrade (v 3.0); Becton, Dickinson and Company. The cell counts for CD3<sup>+</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD8<sup>+</sup> and activated CD3<sup>+</sup>CD4<sup>+</sup>CD38<sup>+</sup>HLA-DR<sup>+</sup> T cells were determined by a five-color strategy using anti-CD3-allophycocyanin, anti-CD4-fluorescein isothiocyanate, anti-CD8-phycoerythrin-Cy7, anti-CD38-phycoerythrin, and anti-HLA-DR-Peridinin Chlorophyll Protein Complex-Cy5.5 (Becton, Dickinson and Company). Cell staining was performed according to the manufacturer's instructions.

*Statistical analysis.* Data are presented as the mean ± standard deviation. The statistical analyses were performed using SPSS 20.0 (IBM Corp.). One-way analysis of variance with Bonferroni's post hoc test was used when comparing three or more groups. Correlations were tested using the Pearson correlation analysis. All tests were two-tailed. P<0.05 was considered to indicate a statistically significant difference

## Results

*Levels of SAMHD1 increase in the PBMCs of VPs and HIV-ARTs.* SAMHD1 expression was detected in the PBMCs and CD4<sup>+</sup> T cells of patients with HIV-1 and HCs. SAMHD1 mRNA expression was significantly increased in the PBMCs of VPs, HIV-ARTs and ECs compared with that in the HCs, with the highest level exhibited by the EC group; however, no significant differences were observed in the SAMHD1 levels in the PBMCs between the three groups of patients with HIV-1 (Fig. 1A). In addition, the SAMHD1 levels in the CD4<sup>+</sup> T cells were significantly elevated in the ECs compared with those of the VPs, the HIV-ARTs and the HCs, but no significant differences were observed in CD4<sup>+</sup> T-cell expression of SAMHD1 between the VPs, the HIV-ARTs and the HCs (Fig. 1B). The relationship between SAMHD1 levels in CD4<sup>+</sup> T cells, the CD4<sup>+</sup> T cell count and the viral load were also analyzed. No significant correlations were observed between the expression of SAMHD1 in CD4<sup>+</sup> T cells, the CD4<sup>+</sup> T-cell count or the viral load (Table II).

Table II. Correlation between SAMHD1 expression levels in CD4<sup>+</sup> T cells and immunological and virological indexes.

SAMHD1 mRNA in CD4 <sup>+</sup> T cells vs.	R	P-value
CD4 <sup>+</sup> T cells, /μl	0.064	0.683
Viral load, cp/ml	-0.306	0.107
IFN-α, pg/ml	-0.218	0.164
CD4 <sup>+</sup> CD38 <sup>+</sup> HLA-DR <sup>+</sup> T cells, %	-0.401	0.013 <sup>a</sup>
HIV-1 DNA/10 <sup>6</sup> PBMC	-0.168	0.596
HIV-1 DNA/ml blood	0.032	0.357

<sup>a</sup>P<0.05. SAMHD1, sterile α motif and histidine/aspartic acid domain-containing protein 1; HIV, human immunodeficiency virus; IFN, interferon; PBMCs, Peripheral blood mononuclear cells; HLA-DR, human leukocyte antigen-DR.

*SAMHD1 expression is associated with the low level of CD4<sup>+</sup> T-cell activation.* The secretion of IFN-α and the level of T cell activation are typically increased during a viral infection (18-20). Considering that SAMHD1 expression is affected by IFNs and immune activation (21), the concentration of IFN-α in the plasma and the percentage of activated CD4<sup>+</sup> T cells in patients with HIV-1 and HCs were assessed. No significant differences were observed in the plasma IFN-α levels between patients with HIV-1 and the HCs (Fig. 2A). Flow cytometric analysis revealed significantly higher immune activation of CD4<sup>+</sup> T cells in the VPs and the HIV-ARTs (Fig. 2B) compared with those in the ECs and the HCs. In addition, the activation of CD4<sup>+</sup> T cells in ECs was lower compared with that in HCs, VPs and HIV-ARTs (Fig. 2B). Correlation analysis revealed that SAMHD1 expression was inversely correlated with the activation of CD4<sup>+</sup> T cells; no correlation was observed between SAMHD1 expression in CD4<sup>+</sup> T cells and plasma IFN-α levels (Table II).

*Total HIV-1 DNA levels in ECs are not correlated with the levels of SAMHD1 expression.* Whether the total HIV-1 DNA levels in the peripheral blood correlated with SAMHD1 expression in CD4<sup>+</sup> T cells was further determined. The total HIV-1 DNA levels in the VPs were 612.86±248.95 copies/10<sup>6</sup> PBMCs or 708.52±427.30 copies/ml blood, which was

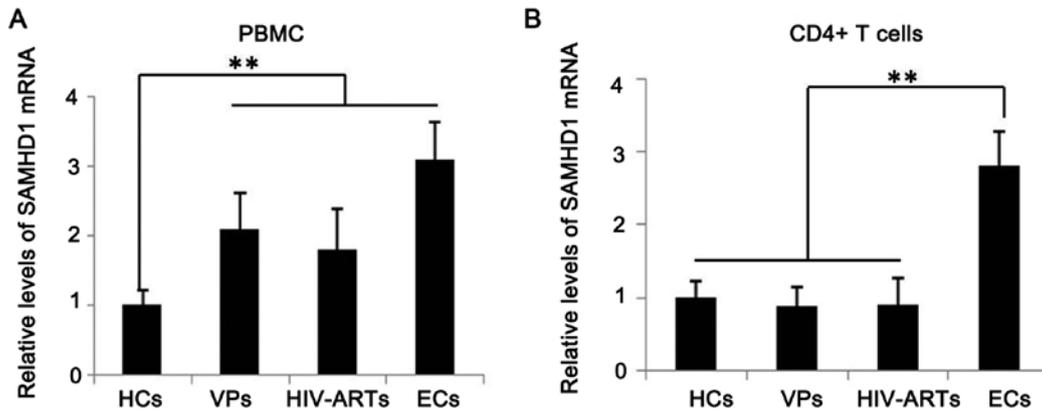


Figure 1. SAMHD1 expression in patients with HIV-1. Relative levels of SAMHD1 mRNA in the (A) PBMCs and (B) CD4<sup>+</sup> T cells of patients with HIV-1 and healthy controls were detected by qRT-PCR. The level of SAMHD1 expression in the healthy controls was set to 1. \*\*P<0.01. SAMHD1, sterile  $\alpha$  motif and histidine/aspartic acid domain-containing protein 1; PBMCs, Peripheral blood mononuclear cells; HIV, human immunodeficiency virus; HCs, healthy controls; VPs, viremic progressors; HIV-ARTs, patients with HIV-1 receiving antiretroviral therapy; ECs, elite controllers.

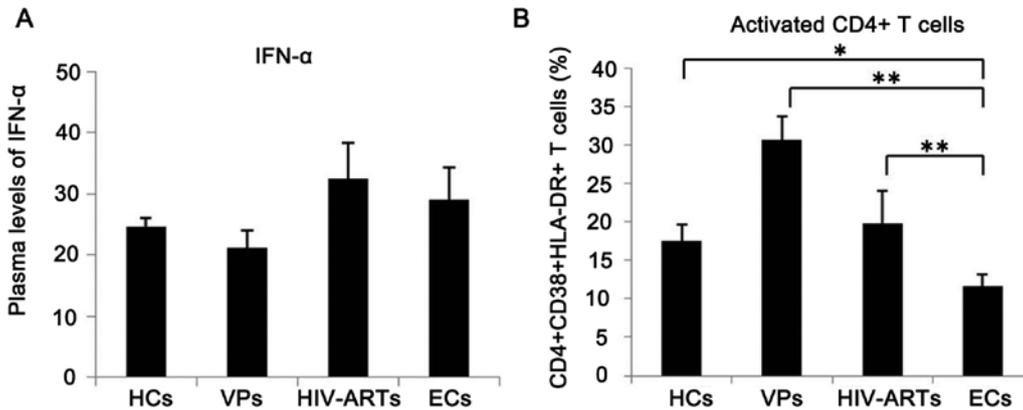


Figure 2. Levels of immune activation markers in patients with HIV-1. (A) Interferon- $\alpha$  in the plasma and (B) percentage of activated CD4<sup>+</sup> T cells in patients with HIV-1 and healthy controls were detected. \*P<0.05, \*\*P<0.01. HIV, human immunodeficiency virus; HCs, healthy controls; VPs, viremic progressors; HIV-ARTs, patients with HIV-1 receiving antiretroviral therapy; ECs, elite controllers.

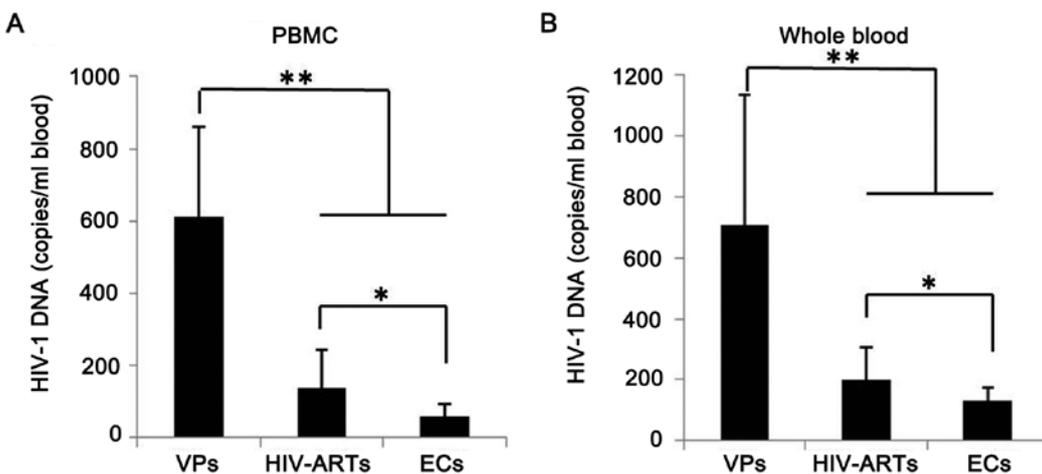


Figure 3. Levels of total HIV-1 DNA in the peripheral blood of patients with HIV-1. The total HIV-1 DNA levels were determined as (A) copies/10<sup>6</sup> PBMCs and (B) copies/ml blood. \*P<0.05, \*\*P<0.01. HIV, human immunodeficiency virus; PBMCs, Peripheral blood mononuclear cells; VPs, viremic progressors; HIV-ARTs, patients with HIV-1 receiving antiretroviral therapy; ECs, elite controllers.

significantly higher compared with those in the HIV-ARTs (137.30±105.04 copies/10<sup>6</sup> PBMC or 201.76±103.04 copies/ml blood) and in the ECs (61.26±30.69 copies/10<sup>6</sup> PBMC and

131.26±42.66 copies/ml blood) (Fig. 3). The total HIV-1 DNA levels exhibited by the ECs were also lower compared those in the HIV-ARTs. The total HIV-1 DNA levels did

not correlate with SAMHD1 expression in CD4<sup>+</sup> T cells (Table II).

## Discussion

In addition to myeloid cells, SAMHD1 is also important for HIV-1 infection of CD4<sup>+</sup> T lymphocytes (22-24). Over expression of SAMHD1 in CD4<sup>+</sup> T cell lines alters the permissibility of HIV-1 infection (22), and higher SAMHD1 expression has been observed in resting CD4<sup>+</sup> T cells that are resistant to HIV-1 infection compared with those in activated CD4<sup>+</sup> T cells (23,24). In addition, Riveira-Muñoz *et al* (12) demonstrated elevated SAMHD1 expression in the CD4<sup>+</sup> T cells of HIV-1-positive ECs compared with the HCs and VPs, indicating an important role for SAMHD1 in HIV-1 resistance. However, inconsistent results have been reported by other researchers; for example, Gonzalez *et al* (13) demonstrated that SAMHD1 expression was higher in the oral and genital mucosa and lower in the PBMCs of HESN individuals who were resistant to HIV-1 compared with HCs. In addition, Santos *et al* (14) reported that no significant differences were observed in the basal SAMHD1 mRNA expression levels in the CD4<sup>+</sup> T cells of HESN individuals compared with those of HCs and HIV-ARTs. The results of the present study revealed higher SAMHD1 expression in the CD4<sup>+</sup> T cells of ECs compared with those of HCs, which was consistent with the findings of the study by Riveira-Muñoz *et al* (12). In addition, there were no differences in the SAMHD1 levels of CD4<sup>+</sup> T cells between the HIV-ARTs, VPs and HCs. Notably, the present study demonstrated higher SAMHD1 expression in the PBMCs of the HIV-ARTs and the VPs compared with the HCs, but no significant difference was observed in the CD4<sup>+</sup> T cells. One reason for these findings may be related to the altered PBMC components present in patients with HIV-1. PBMCs consist of a mixture of different cell types, including myeloid cells with elevated SAMHD1 expression and lymphoid cells with low levels of SAMHD1 (22,25). As a result of the loss of CD4<sup>+</sup> T cells, HIV-1 VPs exhibit a higher percentage of myeloid cells and correspondingly higher SAMHD1 levels in their PBMCs. Other reasons for the higher SAMHD1 levels in the PBMCs of patients with HIV-1 need to be explored in future studies. The use of different subjects (e.g., ECs, HESN individuals, and patients receiving ART or not) and the types of cells investigated (e.g., PBMCs or CD4<sup>+</sup> T cells) may explain the inconsistent results among published studies.

The expression of SAMHD1 can be induced by IFNs (15,26); however, it is unclear whether the *in vivo* expression of SAMHD1 is affected by plasma IFN levels. The present study assessed plasma IFN- $\alpha$  levels and the effects on SAMHD1 expression in CD4<sup>+</sup> T cells. No differences in the plasma IFN- $\alpha$  levels were observed in any of the four subject groups. In addition, there was no correlation between IFN- $\alpha$  and SAMHD1 expression levels. Another potential factor impacting SAMHD1 expression in CD4<sup>+</sup> T cells is immune activation (21). SAMHD1 is highly expressed in resting CD4<sup>+</sup> T cells but is decreased during CD4<sup>+</sup> T cell activation and proliferation (21,23,24). Consistent with previous studies (27,28) indicating that HIV-1 patients have increased CD4<sup>+</sup> T activation, the present study demonstrated that the percentage of activated CD4<sup>+</sup> T cells was increased in the HIV-ARTs and the

VPs, but was decreased in the ECs compared with the HCs. In addition, SAMHD1 expression was inversely correlated with CD4<sup>+</sup> T-cell activation. SAMHD1 is reported to be an important negative regulator of the IFN response and immune activation (29), which may explain the reverse relationship between SAMHD1 and immune activation. Another explanation may be that immune activation induced by HIV-1 infection can increase the proportion of activated CD4<sup>+</sup> T cells with low SAMHD1 expression and decrease the proportion of resting CD4<sup>+</sup> T cells with high SAMHD1 expression (27). The results of the present study suggested that high SAMHD1 expression correlates with a low percentage of activated CD4<sup>+</sup> T cells, and therefore, HIV-1 target cells. Thus, the elevated expression of SAMHD1 in the CD4<sup>+</sup> T cells of ECs may restrict HIV-1 infection by inhibiting viral reverse transcription as well as the extent of immune activation.

The inhibition of reverse transcription by SAMHD1 results in the reduced production of total HIV-1 DNA (30), which contains two components: Integrated HIV-1 DNA and episomal 2LTR circles, which are transient by-products of failed HIV-1 DNA integration (31). The total level of HIV-1 DNA has been demonstrated to be inversely correlated with the time to viral rebound in patients treated early with interrupted ART (32). The results of the present study demonstrated lower total HIV-1 DNA levels in the peripheral blood of the ECs compared with the VPs and the HIV-ARTs; however, no correlation was observed between SAMHD1 expression and the total HIV-1 DNA levels. The function of SAMHD1 is affected by post-transcriptional and post-translational modifications (33). Phosphorylation of SAMHD1 T592 can abolish the dNTPase activity of SAMHD1 (33). In addition to SAMHD1, the total HIV-1 DNA levels can be impacted by a variety of other factors. For example, high HIV-1 RNA production leads to high total HIV-1 DNA levels, and the number and percentage of activated CD4<sup>+</sup> T cells can also impact the total level of HIV-1 DNA (34,35). Additionally, the total HIV-1 DNA level can be affected by the formation of episomal 2LTR circles (36). Cytosolic DNA (e.g. double-stranded HIV-1 DNA, including episomal 2LTR circles) is sensed by the cyclic GMP-AMP synthase/stimulator of interferon genes cytosolic DNA-sensing pathway in HIV-1 infected cells, resulting in a spontaneous IFN response and subsequent immune activation (37). Therefore, reduced production of HIV-1 DNA by SAMHD1 may limit the magnitude of IFN and effector T cell responses.

The limitations associated with the present study were as follows: i) The levels of integrated HIV-1 DNA and episomal 2LTR circles were not investigated separately due to technological limitations; ii) the number of ECs was small; and iii) the mRNA rather than protein levels of SAMHD1 in purified CD4<sup>+</sup> T cells from patients with HIV-1 were only investigated due to limited volume of blood samples. Future studies with an expanded sample size should be carried out to further explore the expression of SAMHD1 in patients with HIV-1 exhibiting differential disease progression as well as the relationship between SAMHD1 and HIV-1 DNA.

In summary, the results of the present study indicated that SAMHD1 was highly expressed in ECs and may contribute to low total HIV-1 DNA levels and low immune activation observed in individuals infected with HIV-1 compared with

those in HCs. However, no correlation was observed between SAMHD1 expression and the HIV-1 DNA levels. The results of the present study also suggested that SAMHD1 may restrict HIV-1 infection *in vivo* by inhibiting immune activation and thus reducing the number of HIV-1 target cells instead of directly inhibiting HIV-1 reverse transcription. This study also emphasized the importance of reducing the immune activation levels of CD4<sup>+</sup> T cells for patients with HIV-1. To the best of our knowledge, this is the first *in vivo* study on the relationship between SAMHD1 and HIV-1 DNA levels in CD4<sup>+</sup> T cells.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Authors' contributions

JL, CG and CJ contributed to study concept and design. SH and LJ contributed to the clinical sampling. JL, CG, SH and CJ performed the experiments, and acquired, analyzed or interpreted data. JL, CG and CJ drafted and revised the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

This study was approved by the ethics review boards of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University (No. 201700837) and the First Affiliated Hospital, School of Medicine, Zhejiang University (approval no. IIT20171207A). Written informed consent to participate was obtained from all subjects.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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