Promoter Methylation Regulates SAMHD1 Gene Expression in Human CD4\(^+\) T Cells*

Received for publication, December 19, 2012, and in revised form, February 20, 2013. Published, JBC Papers in Press, February 20, 2013, DOI 10.1074/jbc.M112.447201

Suresh de Silva‡, Heather Hoy‡, Timothy S. Hake§, Henry K. Wong‖‖‖, Pierluigi Porcu‡‡‡, and Li Wu§§‡‡1
From the ‡Center for Retrovirus Research, Department of Veterinary Biosciences, Departments of §§Microbial Infection and Immunity and †Internal Medicine, ‡‡Comprehensive Cancer Center, and Divisions of ‡‡‡Dermatology and †††Hematology, The Ohio State University, Columbus, Ohio 43210

Background: SAMHD1 expression is significantly reduced in CD4\(^+\) T cell lines compared with primary CD4\(^+\) T lymphocytes.

Results: The SAMHD1 promoter contains a CpG island that is prone to DNA methylation.

Conclusion: Methylation of the SAMHD1 promoter contributes to transcriptional repression in CD4\(^+\) T cell lines.

Significance: We identified promoter methylation as a contributing factor to SAMHD1 gene regulation.

The retrovirus restriction factor SAMHD1 is the first identified mammalian dNTP triphosphohydrolase that is highly expressed in human myeloid lineage cells and CD4\(^+\) T lymphocytes. Although SAMHD1 expression is variable in human cell lines and tissue types, mechanisms underlying SAMHD1 gene regulation have not been defined. Recent studies showed that SAMHD1 is highly expressed in human primary CD4\(^+\) T lymphocytes, but not in some CD4\(^+\) T cell lines. Here, we report that SAMHD1 expression varies among four CD4\(^+\) T cell lines and is transcriptionally regulated. Cloning and sequence analysis of the human SAMHD1 promoter revealed a CpG island that is methylated in CD4\(^+\) T cell lines (such as Jurkat and Sup-T1), resulting in transcriptional repression of SAMHD1. We also found that the SAMHD1 promoter is unmethylated in primary CD4\(^+\) T lymphocytes, which express high levels of SAMHD1, indicating a direct correlation between the methylation of the SAMHD1 promoter and transcriptional repression. SAMHD1 expression was induced in CD4\(^+\) T cell lines by blocking DNA methyltransferase activity, suggesting that promoter methylation is one of the key epigenetic mechanisms by which SAMHD1 expression is regulated.

Transcriptional regulation of genes involved in cellular metabolism, such as nucleic acid biosynthesis and breakdown, is critical in maintaining cellular homeostasis. The gene encoding SAMHD1 (SAM and HD domain-containing protein 1) was initially identified in a human dendritic cell cDNA library as a human homolog of the mouse IFN-\(\gamma\)-induced gene MG-11 (1, 2). Structural and biochemical analyses have revealed that SAMHD1 is the first known mammalian dNTP triphosphohydrolase capable of hydrolyzing dNTPs into their constituents (3, 4), thus implicating it in nucleic acid metabolism. A lack of SAMHD1 expression caused by homozygous mutations within SAMHD1 has been found in patients suffering from a rare genetic disorder, Aicardi-Goutières syndrome (5), which is an autoimmune disease likely caused by abnormal metabolism of nucleic acids (6). Furthermore, SAMHD1 was recently identified as an HIV-1 restriction factor in human myeloid lineage cells (7–9) and quiescent CD4\(^+\) T lymphocytes (10, 11), wherein it is responsible for maintaining the cellular dNTP pool at a level that is inadequate for HIV-1 and other retrovirus replication (10, 12, 13).

In their initial report, Li et al. (2) observed a diverse expression profile of SAMHD1, known formerly as DCIP (dendritic cell-derived interferon \(\gamma\)-induced protein), in human cancer cell lines and a range of tissue types. Importantly, SAMHD1 mRNA was detected at relatively high levels in peripheral blood leukocytes, but not in the brain, colon, and thymus (2). Recent reports pertaining to the function of SAMHD as an HIV-1 restriction factor have focused on the expression of SAMHD1 in HIV-1 target cell types, which include CD4\(^+\) T lymphocytes, macrophages, and dendritic cells (14–16). These cell types express relatively high levels of SAMHD1. Interestingly, several reports indicated the lack of SAMHD1 expression in transformed CD4\(^+\) T cell lines (7, 8, 10), which are highly permissive to HIV-1 infection. This observation prompted us to investigate the mechanisms underlying SAMHD1 gene regulation and whether epigenetic modulation might play a role in dictating the diverse expression profile of SAMHD1.

Two major molecular mechanisms that mediate epigenetic regulation of gene expression are DNA methylation and histone modifications (17). Promoter activity affected by these epigenetic modifications plays a critical role in regulating the expression of a specific gene (17). However, it is unknown whether epigenetic modifications of the SAMHD1 promoter are involved in regulating the diverse expression profile of SAMHD1 among different cell types.

Here, we report for the first time the cloning of the human SAMHD1 promoter and the use of CD4\(^+\) T cell lines as a model to study SAMHD1 gene regulation. Sequence analysis of the SAMHD1 promoter revealed the presence of a putative CpG

*This work was supported, in whole or in part, by National Institutes of Health Grants AI098524 and AI102822 (to L. W.).

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\(^\text{TM}\)/EBI Data Bank with accession number(s) KC543354.

1 Supported in part by the Public Health Preparedness for Infectious Diseases Program (PHPID) of The Ohio State University. To whom correspondence should be addressed: Center for Retrovirus Research, Department of Veterinary Biosciences, The Ohio State University, 1900 Coffey Rd., Columbus, OH 43210. Tel.: 614-292-5408; Fax: 614-292-6473; E-mail: wu.840@osu.edu.
island surrounding the transcription start site. In addition, we show that CpG methylation of the SAMHD1 promoter correlates with transcriptional repression of SAMHD1 in CD4+ T cell lines, thus providing insights into epigenetic modulation of the SAMHD1 promoter activity.

EXPERIMENTAL PROCEDURES

**Plasmids**—Based on our bioinformatics analysis of the SAMHD1 gene using the Transcriptional Regulatory Element Database, the promoter region of the SAMHD1 gene includes a 1286-bp fragment upstream of the ATG start codon. The human SAMHD1 promoter sequence from nucleotides −1083 to +202 relative to the transcription start site was amplified using Platinum® PCR High Fidelity Supermix (Invitrogen) from the genomic DNA of HEK 293T cells following a nested PCR-based approach. KpnI and XhoI sites were included at the 5′-ends of the internal forward and reverse primers (sequences are listed in Table 1), respectively, for cloning into the multiple cloning site of the pGL4.10 promoterless luciferase-based reporter plasmid (a kind gift from Jesse Kwiek, The Ohio State University). The primers used for PCR and cloning of the SAMHD1 promoter were as follows: SAMHD1 Pr1_fwd, SAMHD1 Pr1_rev, SAMHD1 Pr2_fwd_KpnI, and SAMHD1 Pr2_rev_XhoI (Table 1).

**Cell Culture and Drug Treatments**—The THP-1 monocytic cell line (ATCC TIB-202) was obtained from American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 medium as described (18). The CD4+ T cell line Sup-T1 (derived from an acute T cell leukemia patient) was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. The cutaneous T cell lymphoma cell line MyLa (20) was a gift from Michael Girardi (Yale University). The HEK 293T cell line and the CD4+ T cell line HuT/CCR5 (derived from the cutaneous T cell lymphoma cell line HuT-78) were kind gifts from Vineet KewalRamani (National Cancer Institute) and were maintained in specific medium as described previously (21). Primary CD4+ T lymphocytes were isolated from peripheral blood mononuclear cells of healthy donors using magnetic beads coated with anti-CD4 antibodies (BD Biosciences) as described (21). For treatment with trichostatin A (TSA2; Cell Signaling), 1 × 10^6 cells were cultured in medium containing 1 μM TSA for 24 h and processed for RNA extraction using the RNeasy minikit (Qiagen). For treatment with 5-aza-2′-deoxycytidine (5-AzadC; Tocris Bioscience), cells were cultured in the presence of 5-AzadC (10 μM) for 72 h and processed for RNA extraction. Combination drug treatments were performed by treating 1 × 10^6 cells with 5-AzadC (10 μM) for 72 h, followed by an 18-h treatment with TSA (1 μM).

**Immunoblotting and mRNA Quantification of SAMHD1**—To assess SAMHD1 protein levels, 1 × 10^6 cells were lysed in cell lysis buffer (Cell Signaling) supplemented with protease inhibitor mixture (Sigma). Following protein quantification using a bicinchoninic acid assay (Thermo Scientific), 20 μg of whole cell lysate was electrophoretically separated on an SDS-polyacrylamide gel and immunoblotted using a mouse anti-human SAMHD1 antibody (Abcam) as described (22). Detection of GAPDH expression was carried out to serve as a loading control as described (22). For SAMHD1 mRNA quantification in cell lines and CD4+ T lymphocytes, total cellular RNA was extracted using the RNeasy minikit according to the manufacturer’s guidelines. 0.25 μg of total RNA from each cell type was used as a template for first-strand cDNA synthesis performed with a Superscript™ III first-strand synthesis kit and oligo(dT) primers (Invitrogen). SYBER Green-based quantitative real-time PCR analysis was performed using the SAMHD1 cDNA-specific primers listed in Table 1. Quantification of GAPDH mRNA was carried out for normalization. GAPDH primer sequences have been published previously (23). Analysis for relative gene expression was performed using the 2^−ΔΔC_T method (24).

**Exogenous SAMHD1 Promoter Activity**—SAMHD1 promoter activity was assessed by separately nucleofecting pGL4-SAMHD1-luc (0.5–1.5 μg) and pGL4-luc empty vector control plasmid together with 0.1–0.5 μg of the pTK- Renilla plasmid in THP-1 and CD4+ T cell lines using the appropriate Nucleofector. The pTK-Renilla plasmid was included to serve as an internal transfection efficiency control, and transfections were conducted in duplicate. Firefly and Renilla luciferase expression was quantified 24 h post-transfection using a Dual-Luciferase assay kit (Promega).

---

**TABLE 1**

| PCR primer | DNA sequences |
|------------|---------------|
| SAMHD1 Pr1_fwd | 5′-AGATTCACGCGAATCCTGCAG-3′ |
| SAMHD1 Pr1_rev | 5′-AGATCCTCCAGAGACTTGTAACAC-3′ |
| SAMHD1 Pr2_fwd_KpnI | 5′-GGCGGGCGGTACGATCACGCCACTGCACTCCAG-3′ |
| SAMHD1 Pr2_rev_XhoI | 5′-GGCGGCCCTAGGAGCTACAATCCGGGTCGTCG-3′ |
| SAMHD1 cDNA fwd | 5′-GGATATTTGGGTTATTTGAGAATTC-3′ |
| SAMHD1 cDNA rev | 5′-ACCTACACCAGGGAAACACOTA-3′ |
| M_fwd | 5′-GCGGGCGGTACGATCACGCCACTGCACTCCAG-3′ |
| U_fwd | 5′-AGATCAGCGACCTGCCTGCCAGTCGTCG-3′ |
| M_rev | 5′-GCGGGCGGTACGATCACGCCACTGCACTCCAG-3′ |
| U_rev | 5′-GCGGGCGGTACGATCACGCCACTGCACTCCAG-3′ |
| P1 | 5′-GCGGGCGGTACGATCACGCCACTGCACTCCAG-3′ |
| P2 | 5′-GCGGGCGGTACGATCACGCCACTGCACTCCAG-3′ |

2 The abbreviations used are: TSA, trichostatin A; 5-AzadC, 5-aza-2′-deoxycytidine; MSP, methylation-specific PCR.
**SAMHD1 Promoter Regulation by CpG Methylation**

In Vitro Methylation of the SAMHD1 Promoter—In vitro methylation of the SAMHD1 promoter in the pGL4-SAMHD1-luc plasmid was carried out using the CpG methyltransferase M.SssI (New England Biolabs). Plasmid DNA (1 μg) was treated with 1 unit of M.SssI enzyme in a total volume of 20 μl for 1 h at 37 °C according to the manufacturer’s recommendations. Following heat inactivation of the enzyme, the methylated plasmid DNA was purified using DNA Clean and Concentrator™-5 (Zymo Research). Complete methylation of the pGL4-SAMHD1-luc plasmid was confirmed by digestion with the methylase-sensitive endonuclease HpaII (New England Biolabs), followed by agarose gel electrophoresis.

DNA Methylation Analysis of the SAMHD1 Promoter in CD4⁺ T Cells—Two methods were employed to confirm the methylation status of the SAMHD1 promoter in CD4⁺ T cells. 1) For methylation-specific PCR (MSP) (25), genomic DNA was extracted from THP-1, Jurkat, Sup-T1, HuT/CCR5, and MyLa cells using the DNeasy blood and tissue kit (Qiagen) according to the manufacturer’s instructions. Genomic DNA (0.75 μg) was bisulfite-converted using the MethylDetector™ bisulfite modification kit (Active Motif) according to the manufacturer’s guidelines. Next, 1–2 ng of sodium bisulfite-converted genomic DNA was used as a template for PCR analysis using methyl-specific primers, which were designed using the MethPrimer program. The sequences of the methyl-specific primers (M_fwd, M_rev, U_fwd, and U_rev) are listed in Table 1. The PCR conditions for amplifications were 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. A final extension at 72 °C for 10 min was conducted. The PCR products were electrophoresed on a 2% agarose gel and stained with ethidium bromide.

2) For HpaII digestion of genomic DNA, followed by SAMHD1 promoter-specific PCR, genomic DNA (1 μg) was digested with 10 units of HpaII endonuclease (New England Biolabs) for 16 h at 37 °C. The enzyme was heat-inactivated, and DNA was purified using DNA Clean and Concentrator™-5. Purified DNA (50 ng) was used as a template for PCR amplification with the primer pair (P1 and P2) that flanks the predicted CpG island containing the SAMHD1 promoter sequence. To serve as an input control, a 0.25-kb fragment of the GAPDH gene (26) lacking HpaII sites was PCR-amplified using the same template DNA. The PCR conditions and the sequences of the GAPDH gene-specific primers have been described previously (27).

Bisulfite DNA Sequencing—Genomic DNA from THP-1, Jurkat, Sup-T1, HuT/CCR5, and MyLa cells was bisulfite-converted and subjected to PCR amplification using the methyl-specific primers described above. The purified PCR products were sequenced, and sequences were aligned using the ClustalW program.

Statistical Analysis—Data were analyzed using Student’s t test, and statistical significance was defined as p < 0.05.

**RESULTS**

Lower Levels of SAMHD1 Protein and mRNA Expression in CD4⁺ T Cell Lines Compared with the THP-1 Monocytic Cell Line—A previous study indicated that THP-1 monocytic cells express high levels of SAMHD1, whereas the CD4⁺ T cell lines Jurkat and Sup-T1 do not express detectable SAMHD1 protein (7). To distinguish whether the lack of expression observed in CD4⁺ T cell lines is at the level of protein translation or mRNA transcription, we performed immunoblotting to detect SAMHD1 protein levels and real-time PCR analysis to quantify SAMHD1 mRNA in four CD4⁺ T cell lines. Among the cell lines analyzed, Jurkat and Sup-T1 cells lacked SAMHD1 protein expression, whereas HuT/CCR5 and MyLa cells showed relatively low levels of SAMHD1 protein compared with THP-1 cells (Fig. 1A). Real-time PCR results indicated 1100-fold lower levels of SAMHD1 mRNA in Jurkat and Sup-T1 cells, whereas HuT/CCR5 and MyLa cells expressed 29- and 19-fold lower levels of SAMHD1 mRNA compared with THP-1 cells, respectively (Fig. 1B), which correlated with the level of protein detected by immunoblotting (Fig. 1, A and B). These results indicate that SAMHD1 expression is regulated at the transcriptional level in CD4⁺ T cell lines.

Exogenous SAMHD1 Promoter Activity in the THP-1 Cell Line and CD4⁺ T Cell Lines—Because our data suggested that SAMHD1 expression is regulated at the level of transcription, we speculated that reduction in SAMHD1 promoter activity might be responsible for the lack of SAMHD1 expression in Jurkat and Sup-T1 cells. To test this hypothesis, the SAMHD1 promoter sequence (nucleotides −1083 to +202 relative to the transcription start site) was PCR-amplified from the genomic DNA of HEK 293T cells and cloned into the pGL4.10 promoterless luciferase reporter vector (Fig. 2A). HEK 293T cells were chosen for cloning the SAMHD1 promoter because they express endogenous SAMHD1 protein (8, 22) and because they were not derived from a cancer cell line (28), which would avoid potential mutations of the SAMHD1 promoter. The SAMHD1 promoter-containing reporter vector was nucleofected into
Human SAMHD1 promoter reporter (pGL4-SAMHD1-luc)

FIGURE 2. Exogenous SAMHD1 promoter activity in THP-1 cell line and CD4+ T cell lines. A, schematic representation of the SAMHD1 promoter region. Predicted TATA boxes in the SAMHD1 promoter are shown. The SAMHD1 promoter (nucleotides [nt] −1083 to +202 relative to the transcription start site) was amplified from the genomic DNA of HEK 293T cells and cloned into the pGL4.10 vector to generate the pGL4-SAMHD1-luc construct. B, the SAMHD1 promoter–containing firefly luciferase reporter (pGL4-SAMHD1-luc) was nucleofected into the THP-1 monocytic cell line and CD4+ T cell lines along with a Renilla luciferase reporter vector (pTK-Renilla), which served as an internal reference control to normalize for differences in transfection efficiencies. The empty vector (pGL4.10) served as a negative control and was nucleofected in parallel together with the pTK-Renilla vector. SAMHD1 promoter activities were assessed via luciferase activity at 24 h post-nucleofection and are presented relative to the empty vector control. The data represent average results of three or four independent experiments. *p < 0.01 compared with the THP-1 cells nucleofected with the SAMHD1 promoter reporter.

Individual drug treatments of Jurkat cells with the two epigenetic modifiers increased SAMHD1 mRNA expression (48-fold with 5-AzadC and 28-fold with TSA relative to the untreated control), whereas the sequential combined drug treatment significantly increased SAMHD1 mRNA expression by 136-fold in a synergistic manner (Fig. 3A). SAMHD1 gene transcription was also induced in Sup-T1 cells with individual drug treatments (9-fold with 5-AzadC and 20-fold with TSA) (Fig. 3A), albeit to a lesser extent than in Jurkat cells. Similar to Jurkat cells, the sequential combined drug treatment of Sup-T1 cells synergistically enhanced SAMHD1 mRNA levels by 35-fold (Fig. 3A). In HuT/CCR5 and MyLa cells, only TSA treatment had a significant effect on SAMHD1 mRNA levels, whereas 5-AzadC treatment and the sequential combined drug treatment only modestly increased SAMHD1 mRNA levels (Fig. 3A). Although SAMHD1 mRNA expression in TSA-treated HuT/CCR5 cells was markedly up-regulated (Fig. 3B), the combined treatment did not have the same effect (Fig. 3A), suggesting a cell type-specific response to combined epigenetic modifiers. None of the epigenetic modifiers had an effect on expression of SAMHD1 protein. Interestingly, SAMHD1 protein was induced at
a low level in Jurkat cells and to a very low level in Sup-T1 cells compared with untreated controls (Fig. 3B). SAMHD1 protein levels did not change in the THP-1, HuT/CCR5, and MyLa cells upon sequential combined drug treatment (Fig. 3B). Notably, individual drug treatments of each of the cell lines did not induce or increase SAMHD1 protein levels (data not shown), although SAMHD1 mRNA was up-regulated in Jurkat, Sup-T1, and HuT/CCR5 cells (Fig. 3A). This discrepancy suggests that post-transcriptional and/or post-translational regulations might also contribute to SAMHD1 protein expression in different cell types. On the basis of the significant induction of SAMHD1 gene transcription observed in Jurkat and Sup-T1 cells by blocking DNA methylation, we speculate that methylation of the SAMHD1 promoter might be one of the key regulatory mechanisms of SAMHD1 gene expression in CD4+ T cell lines.

**In Vitro Methylation of the SAMHD1 Promoter Leads to Transcriptional Repression**—Promoter regions of human genes often encompass CpG island(s) (31, 32), which, if methylated, could lead to transcriptional repression (33, 34). Thus, we analyzed the SAMHD1 promoter sequence for putative CpG islands using the MethPrimer program. The SAMHD1 promoter contains a single CpG island proximal to the ATG initiation codon located at nucleotides −232 to +17 relative to the transcription start site (Fig. 4A). The presence of a putative CpG island in the SAMHD1 promoter, we speculated that DNA methylation within this CpG island could lead to transcriptional repression of the SAMHD1 gene. To test our hypothesis, the SAMHD1 promoter-containing luciferase reporter plasmid (pGL4-SAMHD1-luc) was methylated in vitro using the DNA methyltransferase M.SssI. The pGL4.10 empty vector was also methylated in parallel to control for methylation effects on the reporter plasmid backbone. Complete methylation of the vectors was confirmed by methylation-sensitive HpaII endonuclease digestion (Fig. 4B). Next, the methylated pGL4-SAMHD1-luc vector and the pGL4.10 empty vector alongside their unmethylated counterparts were nucleofected into THP-1 cells along with their unmethylated counterparts. SAMHD1 promoter activity was assessed via luciferase activity 24 h post-nucleofection. The data represent average results of three independent experiments. *, p < 0.001 compared with the unmethylated control.

**Methylation of the SAMHD1 Promoter Correlates with Transcriptional Repression in CD4+ T Cells**—To examine whether methylation of the SAMHD1 promoter correlates with transcriptional repression in CD4+ T cells, we analyzed the methylation status of the SAMHD1 promoter in each of the CD4+ T cell lines using MSP. PCR amplification of bisulfite-modified genomic DNA using primers designed to discriminate methylated DNA from unmethylated DNA revealed that the SAMHD1 promoter was indeed methylated in Jurkat and Sup-T1 cells (Fig. 5, A and B), which lack SAMHD1 expression (Fig. 1). In contrast, the SAMHD1 promoter in HuT/CCR5 and MyLa cells, which express SAMHD1 at a low level, existed in an unmethylated form.
state. The SAMHD1 promoter in the control THP-1 cell line was also found to be in an unmethylated state similar to the HuT/CCR5 and MyLa cell lines (Fig. 5B).

To further confirm our findings obtained using MSP, genomic DNA from THP-1 cells and each of the CD4+ T cell lines was individually digested with the methylation-sensitive HpaII endonuclease or left untreated and then subjected to PCR amplification using primers complementary to the SAMHD1 promoter sequence. Of note, the SAMHD1 promoter contains five HpaII sites surrounding the transcription start site (Fig. 5C). Methylation of the HpaII sites in the SAMHD1 promoter would prevent digestion by the HpaII endonuclease, and the intact sequence would serve as a template for PCR amplification using SAMHD1 promoter-specific primers (P1 and P2) that flank the HpaII sites (Fig. 5C). To serve as an input control, a 0.25-kb region within the GAPDH gene lacking HpaII sites was PCR-amplified (Fig. 5D, lower panel). A single PCR product corresponding to the size of the SAMHD1 promoter (1.2 kb) was detected in all control genomic DNA samples from the THP-1 and CD4+ T cell lines that were left untreated with HpaII and also in Jurkat and Sup-T1 genomic DNA samples that were digested with HpaII (Fig. 5D, upper panel). These results confirmed that the SAMHD1 promoter is methylated in Jurkat and Sup-T1 cells. The lack of a 1.2-kb PCR product in HpaII-digested HuT/CCR5, MyLa, and control THP-1 cell genomic DNAs confirmed that the SAMHD1 promoter is in an unmethylated state in these cell lines.

**Distribution and Methylation Status of CpG Dinucleotides in the SAMHD1 Promoter in CD4+ T Cell Lines**—To determine the distribution of methylated CpG dinucleotides in the SAMHD1 promoter in the CD4+ T cell lines, we performed bisulfite sequencing of the PCR products that were generated by amplification of bisulfite-converted genomic DNA using methyl-specific primers. Sequence analysis of the SAMHD1 promoter in Jurkat and Sup-T1 cell lines revealed widespread methylation of all CpG dinucleotides within the amplified region of the CpG island (nucleotides −229 to −39 relative to the transcription start site), which directly correlated with transcriptional repression (Fig. 6). Conversely, the same CpG dinucleotides were in an unmethylated state in THP-1, HuT/CCR5, and MyLa cell lines, which express SAMHD1 (Fig. 6).

T lymphocytes—It has been shown that primary CD4+ T lymphocytes from healthy blood donors express high levels of SAMHD1 mRNA and protein (10, 11, 22, 35). We next studied the methylation status of the SAMHD1 promoter in these cells. Primary CD4+ T lymphocytes were isolated from three healthy donors, and SAMHD1 protein levels and the methylation status of the SAMHD1 promoter were assessed. The methylation status of the SAMHD1 promoter was assessed by digestion of the genomic DNA with HpaII endonuclease, followed by PCR amplification as described above. PCR amplification of HpaII-digested genomic DNA of CD4+ T cells from individual donors revealed that the SAMHD1 promoter was in an unmethylated state (Fig. 7A, upper panel). Immunoblotting results indicated that primary CD4+ T lymphocytes from two donors (cells from donor 2 were not enough due to the sample limitation) expressed higher levels of SAMHD1 protein relative to THP-1 cells (Fig. 7B). These results suggest a direct correlation between the unmethylated status of the SAMHD1 promoter and efficient gene expression.

**DISCUSSION**

Differences in SAMHD1 expression have been observed in human cell lines and tissues, indicating that SAMHD1 expression maybe under transcriptional regulation given its dNTP triphosphohydrolase function. In this study, we used CD4+ T
cell lines as a model to identify mechanisms that regulate SAMHD1 gene expression. Our results indicate that the SAMHD1 promoter contains a CpG island proximal to the initiation codon of the SAMHD1 gene, which, upon DNA methylation, leads to transcriptional repression in certain CD4⁺/H11001 T cell lines.

DNA methylation is an epigenetic process that regulates gene expression during mammalian development. Transcriptional repression of tumor suppressor genes via DNA methylation has been found in certain types of cancer (33, 34). Accordingly, inhibition of epigenetic suppression in vitro using specific drugs can reactivate expression of genes silenced in cancer (30, 36). Our findings indicate that SAMHD1 gene expression can be induced in Jurkat and Sup-T1 CD4⁺ T cell lines upon single treatment with the DNA methyltransferase-blocking agent 5-AzadC or the histone deacetylase inhibitor TSA. Interestingly, the relative level of SAMHD1 induction was higher in Jurkat cells than in Sup-T1 cells, indicating different degrees of transcriptional repression. A synergistic effect on the induction of SAMHD1 expression was observed when Jurkat and Sup-T1 cell lines were treated sequentially with both epigenetic modifiers. Given that DNA methylation and histone deacetylation are dynamically linked processes, it appears that the SAMHD1 promoter is silenced by a combination of both epigenetic processes. We speculate that binding of methyl-CpG-binding proteins to the methylated SAMHD1 promoter could lead to the recruitment of histone deacetylases to further repress SAMHD1 expression by blocking its interaction with transcription factors (37).

We observed that the methylation patterns of the SAMHD1 promoter in THP-1, HuT/CCR5, and MyLa cells are similar, whereas their SAMHD1 expression levels, promoter reporter activity, and responses to 5-AzadC and/or TSA are different. These results suggest that, along with DNA methylation, additional epigenetic mechanisms, such as cell type-specific expression of transcription factors and microRNAs, may also contribute to
the regulation of SAMHD1 gene expression. Furthermore, SAMHD1 gene expression can be regulated at the post-transcriptional level. For example, a recent study identified naturally occurring splice variants of SAMHD1 in several cell lines, including THP-1 cells (38). Thus, given its role in dNTP regulation in cells, it appears that multiple mechanisms are in place to regulate SAMHD1 gene expression.

SAMHD1 is highly expressed in terminally differentiated human myeloid and primary CD4+ T lymphocytes (7, 8, 10, 11, 22), which seems intuitive because these cells have exited the cell cycle and do not require a high level of dNTPs to be maintained in the cell. Thus, SAMHD1 is believed to play a role in dNTP metabolism in non-dividing immune cells. Interestingly, SAMHD1 expression is significantly lower in several cancer cell lines, including CD4+ T cell lines (2). In contrast to myeloid and quiescent CD4+ T lymphocytes, these actively proliferating cancer cell lines maintain a high dNTP pool concentration to facilitate rapid cell division (39). It is conceivable that down-regulating dNTP catabolic enzymes, such as SAMHD1, would enable cancer cells to maintain a high dNTP pool, a prerequisite for rapid cell proliferation. Further studies into the molecular mechanism by which regulates the expression and activity of SAMHD1 will broaden our knowledge of this novel dNTP triphosphohydrolase.

Acknowledgments—We thank Drs. Patrick Green, Jesse Kwiek, and Vineet KewalRamani for the kind gift of reagents. We are grateful to Drs. James DeWille and Corine St. Gelais for critical reading of the manuscript and helpful comments.

REFERENCES
1. Lafuse, W. P., Brown, D., Castle, L., and Zwilling, B. S. (1995) Cloning and characterization of a novel cDNA that is IFN-γ-induced in mouse perito-
neal macrophages and encodes a putative GTP-binding protein. J. Leukoc. Biol. 57, 477–483
2. Li, N., Zhang, W., and Cao, X. (2000) Identification of human homologue of mouse IFN-γ-induced protein from human dendritic cells. ImmunoLett. 74, 221–224
3. Goldstone, D. C., Ennis-Adeniran, V., Hedden, I. J., Groom, H. C., Rice, G. I., Christodoulou, E., Walker, P. A., Kelly, G., Haire, I. F., Yap, M. W., de Carvalho, L. P., Stoye, J. P., Crow, Y. J., Taylor, I. A., and Webb, M. (2011) HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature 480, 379–382
4. Powell, R. D., Holland, P. J., Hollis, T., and Perrino, F. W. (2011) Aicardi-Goutières syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. J. Biol. Chem. 286, 43596–43600
5. Rice, G. I., Bond, J., Asipu, A., Brunette, R. L., Manfield, I. W., Carr, I. M., Fuller, J. C., Jackson, R. M., Lamb, T., Briggs, T. A., Ali, M., Cormall, H., Couthard, L. R., Aeby, A., Attard-Montalto, S. P., Bertini, E., Bodemer, C., Brockmann, K., Brueton, L. A., Corry, P. C., Desguerre, I., Fazzi, E., Caizoria, A. G., Genier, B., Hamel, B. C., Heiberg, A., Hunter, M., van der Knaap, M. S., Kumar, R., Lagae, L., Landrieu, P., Lourenco, C. M., Marom, D., McDermott, M. F., van der Merwe, W., Orcesi, S., Prendiville, I. S., Rasmussen, M., Shalev, S. A., Soler, D. M., Shiwazi, M., Spiegel, R., Tan, T. Y., Vander Averbeke, W. L. S., Wassmer, M., Whittaker, E., Lebon, P., Setton, D. B., Bonhoeff, D. T., and Crow, Y. J. (2009) Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. Nat. Genet. 41, 829–832
6. Crow, Y. J., and Rehwinkel, J. (2009) Aicardi-Goutières syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity. Hum. Mol. Genet. 18, R130–R136
7. Laguette, N., Sobhian, B., Casartelli, N., Ringead, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emsiliani, S., Schwartz, O., and Benkirane, M. (2011) SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 474, 654–657
8. Hreka, K., Hao, C., Gierszewinska, M., Swanson, S. K., Keski-Broda, M., Srivastava, S., Flores, L. W., Washburn, M. P., and Skowronski, J. (2011) Vpx releases inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature 474, 658–661
9. Berger, A., Sommer, A. F., Zwarg, I., Handorf, M., Welzel, K., Esky, N., Panitz, S., Reuter, A., Ramos, I., Jatiani, A., Muller, L. C., Fernandez-Sesma, A., Rutsch, F., Simon, V., König, R., and Flory, E. (2011) SAMHD1-deficient CD14+ cells from individuals with Aicardi-Goutières syndrome are highly susceptible to HIV-1 infection. PLoS Pathog. 7, e1002425
10. Baldauf, H. M., Pan, X., Erikson, E., Schmidt, M., Suddahca, W., Burggraf, M., Schwengova, K., Ambi, I., Wabnitz, G., Gramberg, T., Panitz, S., Flory, E., Landau, N. R., Sertel, S., Rutsch, F., Lasitschka, F., Kim, B., König, R., Fackler, O. T., and Kepler, O. T. (2012) SAMHD1 restricts HIV-1 infection in resting CD4+ T cells. Nat. Med. 18, 1682–1687
11. Descours, B., Cribier, A., Chable-Bessia, C., Aïnède, D., Rice, G., Crow, Y., Yatim, A., Schwartz, O., Laguette, N., and Benkirane, M. (2012) SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4+ T-cells. Retrovirology 9, 87
12. Lahouassa, H., Daddacha, W., Hofmann, H., Aïnède, D., Logue, E. C., Dragnin, L., Bloch, N., Maudet, C., Bertrand, M., Gramberg, T., Pancino, G., Priet, S., Canard, B., Laguette, N., Benkirane, M., Transy, C., Landau, N. R., Kim, B., and 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, 223–228
13. White, T. E., Brandariz-Nuñez, A., Carlos Valle-Casuso, J., Amie, S., Nguyen, L., Kim, B., Brojatsch, J., and Diaz-Griffero, F. (2013) Contribution of SAM and HD domains to retroviral restriction mediated by human SAMHD1. Virology 436, 81–90
14. St Gelais, C., and Wu, L. (2011) SAMHD1: a new insight into HIV-1 restriction in myeloid cells. Retrovirology 8, 55
15. Aïnède, D., Casartelli, N., and Schwartz, O. (2012) Restricting HIV the SAMHD1 way: through nucleotide starvation. Nat. Rev. Microbiol. 10, 675–680
16. Wu, L. (2012) SAMHD1: a new contributor to HIV-1 restriction in resting CD4+ T-cells. Retrovirology 9, 88
SAMHD1 Promoter Regulation by CpG Methylation

17. Jaenisch, R., and Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* **33**, 245–254.

18. Wu, L., Martin, T. D., Carrington, M., and KewalRamani, V. N. (2004) Raji B cells, misidentified as THP-1 cells, stimulate DC-SIGN-mediated HIV transmission. *Virology* **318**, 17–23.

19. Raghavendra, N. K., Shkriabai, N., Graham, R. L. J., Hess, S., Kvaratskhelia, M., and Wu, L. (2010) Identification of host proteins associated with HIV-1 preintegration complexes isolated from infected CD4⁺ cells. *Retrovirology* **7**, 66.

20. Kaltoft, K., Bisballe, S., Dyrberg, T., Boel, E., Rasmussen, P. B., and Thes-strup-Pedersen, K. (1992) Establishment of two continuous T-cell strains from a single plaque of a patient with mycosis fungoides. *In Vitro Cell. Dev. Biol.* **28A**, 161–167.

21. de Silva, S., Planelles, V., and Wu, L. (2012) Differential effects of Vpr on single-cycle and spreading HIV-1 infections in CD4⁺ T-cells and dendritic cells. *PLoS ONE* **7**, e35385.

22. St Gelais, C., de Silva, S., Amie, S. M., Coleman, C. M., Hoy, H., Hollenbaugh, J. A., Kim, B., and 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.

23. Dong, C., Janas, A. M., Wang, J. H., Olson, W. J., and Wu, L. (2007) Characterization of human immunodeficiency virus type 1 replication in immature and mature dendritic cells reveals dissociable cis- and trans-infection. *J. Virol.* **81**, 11352–11362.

24. Livak, K. J., and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻ΔΔCt method. *Methods* **25**, 402–408.

25. Herman, J. G., Graff, J. R., Myöhänen, S., Nelkin, B. D., and Baylin, S. B. (1996) Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9821–9826.

26. Ercolani, L., Florence, B., Denaro, M., and Alexander, M. (1988) Isolation and complete sequence of a functional human glyceraldehyde-3-phosphate dehydrogenase gene. *J. Biol. Chem.* **263**, 15335–15341.

27. Hermankova, M., Siliciano, J. D., Zhou, Y., Monie, D., Chadwick, K., Margolick, J. B., Quinn, T. C., and Siliciano, R. F. (2003) Analysis of human immunodeficiency virus type 1 gene expression in latently infected resting CD4⁺ T lymphocytes in vivo. *J. Virol.* **77**, 7383–7392.

28. DuBridge, R. B., Tang, P., Hsia, H. C., Leong, P. M., Miller, J. H., and Calos, M. P. (1987) Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. *Mol. Cell. Biol.* **7**, 379–387.

29. Christman, J. K. (2002) 5-Azacytidine and 5-aza-2’-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* **21**, 5483–5495.

30. Cameron, E. E., Bachman, K. E., Myöhänen, S., Herman, J. G., and Baylin, S. B. (1999) Synergy of demethylation and histone deacetylation inhibition in the re-expression of genes silenced in cancer. *Nat. Genet.* **21**, 103–107.

31. Gardiner-Garden, M., and Frommer, M. (1987) CpG islands in vertebrate genomes. *J. Mol. Biol.* **196**, 261–282.

32. Larsen, F., Gundersen, G., Lopez, R., and Prydz, H. (1992) CpG islands in vertebrate genomes. *Adv. Cancer Res.* **72**, 141–196.

33. Chan, M. F., Liang, G., and Jones, P. A. (2000) Relationship between transcription and DNA methylation. *Curr. Top. Microbiol. Immunol.* **249**, 75–86.

34. Poole, S., Wang, D., and Jones, P. A. (2000) Methyl-CpG-binding proteins. Targets for cancer therapy. *Cancer Res.* **60**, 1963–1966.

35. Poole, S., Wang, D., and Jones, P. A. (2000) Synergistic effect of histone hyperacetylation and inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* **21**, 5483–5495.

36. Chiurazzi, P., Pomponi, M. G., Pietrobono, R., Bakker, C. E., Neri, G., and Oostra, B. A. (1999) Synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of the FMR1 gene. *Hum. Mol. Genet.* **8**, 1565–1573.

37. Ballestar, E., and Wolffe, A. P. (2001) Methyl-CpG-binding proteins. Targets for cancer therapy. *Cancer Res.* **60**, 1963–1966.

38. Poole, S., Wang, D., and Jones, P. A. (2000) Synergistic effect of histone hyperacetylation and inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* **21**, 5483–5495.

39. Poole, S., Wang, D., and Jones, P. A. (2000) Synergistic effect of histone hyperacetylation and inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* **21**, 5483–5495.

40. Poole, S., Wang, D., and Jones, P. A. (2000) Synergistic effect of histone hyperacetylation and inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* **21**, 5483–5495.