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Downregulation of SAMHD1 expression correlates with promoter DNA methylation in Sézary syndrome patients

Suresh de Silva[1], **Feifei Wang**[1,*], **Timothy S. Hake**[2,*], **Pierluigi Porcu**[2,3,4], **Henry K. Wong**[2,3,5,#], and **Li Wu**[1,2,#]

[1]Center for Retrovirus Research, Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio 43210

[2]Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

[3]Department of Internal Medicine, The Ohio State University, Columbus, Ohio 43210

[4]Division of Hematology, The Ohio State University, Columbus, Ohio 43210

[5]Division of Dermatology, The Ohio State University, Columbus, Ohio 43210

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TO THE EDITOR

While only about 15% of non-Hodgkin lymphomas are T-cell lymphomas, the treatment of this subset of neoplasms remains a significant challenge in the field of hematological cancers owing to unclear mechanisms regulating malignant T-cell growth. Cutaneous T cell lymphoma (CTCL) identifies a group of extranodal T-cell lymphomas characterized by the infiltration of malignant $CD4^+$ T-cells in the skin (Wong *et al.*, 2011). Sézary syndrome (SS) is an aggressive subtype of CTCL defined by diffuse pruritic rash, lymphadenopathy and malignant T-cells in the peripheral blood. The mechanisms underlying the proliferation of neoplastic $CD4^+$ T-cells in SS are not fully understood, but abnormal epigenetic regulation of gene expression including silencing of tumor suppressor genes (TSG) likely plays an important role (van Doorn *et al.*, 2005). Epigenetic mechanisms are critical contributors in cancer initiation and progression through modulation of gene expression, and transcriptional repression of TSG via epigenetic mechanisms occurs in many cancers (Baylin and Jones, 2011).

Sterile alpha motif (SAM) and HD domain containing protein 1 (SAMHD1) is the first identified mammalian triphosphohydrolase that hydrolyzes deoxynucleoside triphosphates (dNTPs), implicating a role in nucleic acid metabolism (Goldstone *et al.*, 2011). SAMHD1 acts as an HIV-1 restriction factor in myeloid-cells and quiescent $CD4^+$ T-cells by diminishing the intracellular dNTP pool to a level that is insufficient for viral replication

[#]To whom correspondence should be addressed: Henry K. Wong, Division of Dermatology, Comprehensive Cancer Center, 2012 Kenny Road, Office 227, Columbus, OH 43221, USA. Tel.: 614-293-4464, Fax: 614-293-8090. henry.wong@osumc.edu. Li Wu, Center for Retrovirus Research, Department of Veterinary Biosciences, The Ohio State University, 1900 Coffey Road, Columbus, Ohio 43210, USA, Tel.: 614-292-5408, Fax: 614-292-6473, wu.840@osu.edu.
[*]These two authors contributed equally to this study.

Conflict of interest
The authors declare that they have no conflict of interests.

(Baldauf *et al.*, 2012; Lahouassa *et al.*, 2012). Non-dividing $CD4^+$ T-cells, monocytes, macrophages and dendritic cells from healthy individuals express high levels of SAMHD1 protein and have significantly lower levels of intracellular dNTPs compared to activated $CD4^+$ T-cells, while several leukemia/lymphoma $CD4^+$ T-cell lines lack SAMHD1 protein expression and have increased dNTP levels necessary for cell division (Baldauf *et al.*, 2012; Hrecka *et al.*, 2011; Laguette *et al.*, 2011). We found that promoter methylation represses *SAMHD1* expression in human leukemia/lymphoma $CD4^+$ T cell lines, while the *SAMHD1* promoter is unmethylated in primary $CD4^+$ T-lymphocytes from healthy donors that express high levels of SAMHD1 protein (de Silva*et al.*, 2013). The role of SAMHD1 in cancer remains unknown.

To explore the potential role of SAMHD1 in CTCL, we measured *SAMHD1* mRNA levels in peripheral blood mononuclear cells (PBMCs) from 14 healthy donors and nine CTCL patients using a real-time quantitative-PCR (qPCR) assay (de Silva *et al.*, 2013). These patients included 8 with SS and one with advanced stage mycosis fungoides (MF) (Table 1), which are two related subtypes of CTCL originating from $CD4^+$ skin-homing T-cells (Wong *et al.*, 2011). Interestingly, qPCR results revealed that PBMCs from the 8 SS patients and an advanced stage MF patient expressed on average 3-fold lower *SAMHD1* mRNA levels (P=0.0013, two-sample t-test) compared to those from 14 healthy donors (Figure 1a and Table 1). These results indicate that *SAMHD1* expression is significantly downregulated in PBMCs from SS patients relative to healthy individuals.

Next we examined whether the downregulation of *SAMHD1* mRNA levels would translate to reduced SAMHD1 protein levels in PBMCs of CTCL patients. To this end we measured expression levels of surface CD4 and intracellular SAMHD1 proteins in PBMCs from three CTCL patients with high circulating neoplastic T-cells (patients #3, #4, and #5 in Figure 1b and Table 1) and four healthy donors using immunostaining and flow cytometry (Figure 1b) (Descours *et al.*, 2012). The limited sample size was due to available PBMCs only from three CTCL patients. Total SAMHD1-positive cells in PBMCs from CTCL patients (37±9%) were significantly lower (P=0.0024) than those from healthy donors (71±6%) (Figure 1c). Furthermore, the percentage of SAMHD1-expressing cells in CD4-negative PBMCs from CTCL patients (3±2%) was significantly lower than that from healthy donors (19±8%) (P=0.023). Analysis of SAMHD1 and CD4 double-positive cells in PBMCs from CTCL patients (34±9%) and healthy donors (52±8%) showed a statistical difference (P=0.042), suggesting significant downregulation of SAMHD1 protein expression in the neoplastic $CD4^+$ cell subset from CTCL patients. Given that PBMCs from CTCL patients comprise mainly of $CD4^+$ cells, we also compared the percentage of SAMHD1-expressing cells in the $CD4^+$ gated population between healthy donor samples (81±3%) and CTCL patient samples (46±6%) and found a significant reduction (P=0.0024) in SAMHD1-expressing cells (supplemental Figure S1).

We hypothesized that the *SAMHD1* promoter in PBMCs from the CTCL patients is methylated and thereby inhibits *SAMHD1* expression. To compare the methylation status of the *SAMHD1* promoter in PBMCs from the CTCL patients selected and healthy donors, genomic DNA of PBMCs was treated with the methylation-sensitive HpaII endonuclease, or left untreated, and then subjected to PCR amplification using *SAMHD1* promoter-specific primers as described (de Silva *et al.*, 2013). The *SAMHD1* promoter contains five HpaII sites, and methylation of these sites prevents digestion by HpaII. The intact undigested sequence can serve as a template for PCR amplification to yield a 1.2-kb product. As an input control of genomic DNA, a 0.25-kb region within the *GAPDH* gene lacking HpaII sites was PCR amplified (Figure S2). The *SAMHD1* promoter in PBMCs from eight of nine CTCL patients tested was methylated (Figure S2a, 1.2-kb bands). Strikingly, PBMCs from eight healthy donors showed that the *SAMHD1* promoter was unmethylated (Figure S2b).

The purity of the genomic DNA for complete enzyme digestion as well as the intact nature of the HpaII sites in the SAMHD1 promoter sequence in healthy donor and CTCL patient genomic DNA was confirmed by restriction digestion with MspI (an isoschizomer of HpaII), which cleaves the HpaII site irrespective of its methylation status (Figure S3).

Densitometry analysis of the PCR products in Figure S2 was used to quantify the relative level of methylation of the *SAMHD1* promoter in PBMCs from CTCL patients and healthy donors. Our analysis revealed 51-fold higher average levels of methylation of the *SAMHD1* promoter in PBMCs from 9 CTCL patients (P=0.0052) relative to 8 healthy donors (Figure 1d and Table 1). These results suggest a positive correlation between downregulation of *SAMHD1* expression and methylation of the *SAMHD1* promoter in PBMCs from CTCL patients. However, we observed a lack of correlation between reduced SAMHD1 expression and promoter methylation in patient #3, which suggests that besides promoter methylation, other transcriptional and epigenetic regulatory mechanisms such as microRNA and histone modifications, may also contribute to the regulation of SAMHD1 expression in CTCL patients.

SAMHD1 somatic mutations have been identified in patients with lung adenocarcinoma, medulloblastoma, glioblastoma, breast, pancreatic and colorectal cancers, albeit at a very low frequency (Imielinski *et al.*, 2012; Jones *et al.*, 2008; Parsons *et al.*, 2008; Parsons *et al.*, 2011; Sjoblom *et al.*, 2006). Transcriptional repression of TSG through DNA methylation and histone modifications is a common mechanism of gene silencing in numerous types of cancer. Inhibition of epigenetic suppression *in vitro* using specific inhibitors to block DNA methyltransferase and/or histone deacetylase can reactivate expression of TSG silenced in cancer. Downregulation of dNTP catabolic enzymes such as SAMHD1 may lead to imbalances in the intracellular dNTP pool, which can induce mutations and genomic instability as key features of CTCL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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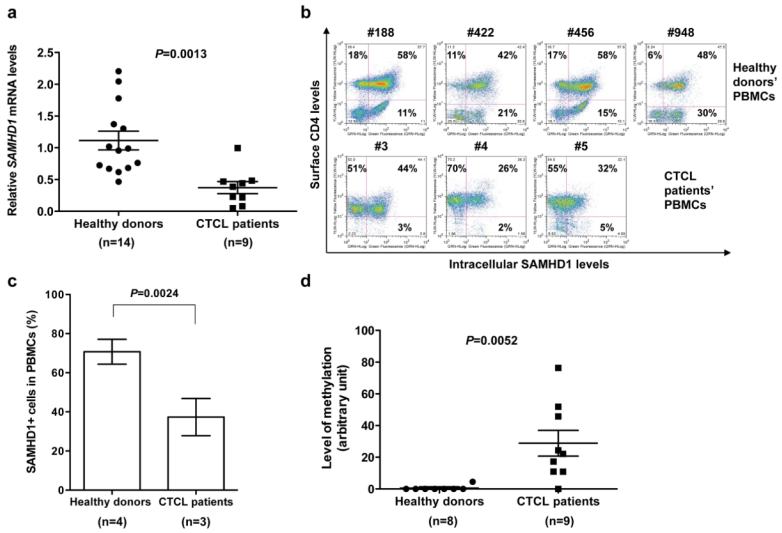

Figure 1. Comparison of SAMHD1 expression levels and the *SAMHD1* promoter methylation in peripheral blood mononuclear cells (PBMCs) from CTCL patients and healthy donors

(a) *SAMHD1* mRNA levels in PBMCs from 14 healthy donors and 9 CTCL patients (refer to Table 1) were measured by qPCR and normalized to *GAPDH* levels. The average levels of *SAMHD1* mRNA levels are shown. The error bars represent standard error of the mean. **(b)** Intracellular SAMHD1 and surface CD4 proteins in PBMCs from CTCL patients and healthy donors were detected by immunostaining and flow cytometry. Donor and patient numbers are indicated above the plots. The percentages of cells positive for CD4 only (upper left quadrant), SAMHD1 only (lower right quadrant), or double positive for CD4 and SAMHD1 (upper right quadrant) are indicated. **(c)** Flow cytometry-based quantification of SAMHD1-expressing (+) cells in PBMCs from healthy donors and CTCL patients. **(d)** Quantification of relative levels of *SAMHD1* promoter methylation in PBMCs from 9 CTCL patients and 8 healthy donors. ImageJ program was used to quantify the intensity of the *SAMHD1* promoter-specific PCR bands in Figure S2 and the relative level of methylation was calculated by setting the intensity of the HpaII-untreated sample as 100 and calculating the percentage intensity of the PCR band in the HpaII-treated samples relative to its matching untreated control. The error bars represent standard error of the mean.

Table 1

Clinical information of CTCL patients and relative levels of *SAMHD1* mRNA and promoter methylation in PBMCs from the patients.

Patient	CTCL subtype [a]	Disease stage	$CD4^+$ cells (%) [b]	Absolute CD4 count	CD4: CD8 ratio	Sézary cell count (%)	Relative *SAMHD1* mRNA levels [c]	Relative *SAMHD1* promoter methylation levels [d]
#1	SS	IVA	98	N/A[e]	N/A	29	0.079	30.74
#2	SS	IVA	93	N/A	N/A	45	0.995	39.31
#3	SS	IVA2	80	N/A	N/A	31	0.217	0.000
#4	SS	IVA	65	N/A	N/A	N/A	0.387	19.43
#5	SS	IVA2	78	N/A	N/A	61	0.469	135.79
#6	MF	IIB	30	163	1.8	N/A	0.048	92.27
#7	SS	IVA	85	1785	28	N/A	0.483	19.34
#8	SS	IVA2	88	3346	82	N/A	0.229	43.29
#9	SS	IVA	82	1466	13	N/A	0.441	81.32

[a] SS: Sézary syndrome, MF: mycosis fungoides. No patient in this group was treated with methotrexate. These patients have been on bexarotene and interferon at some time during their treatments.

[b] $CD4^+$ cells (%) indicate percentage of CD4-positive cells in patient PBMCs by flow cytometry analysis.

[c] Relative levels of *SAMHD1* mRNA in PBMCs from 9 CTCL patients (mean±SD is 0.372±0.285) compared to the average level of 14 healthy donors (1.113±0.553).

[d] Relative levels of *SAMHD1* promoter methylation in PBMCs from 9 CTCL patients (mean±SD is 51.27±43.32) compared to the average level of 8 healthy donors (1.000±1.703).

[e] N/A, not available.