Published in final edited form as:

Leuk Res. 2017 January ; 52: 58–66. doi:10.1016/j.leukres.2016.11.010.

MicroRNA-181 contributes to downregulation of SAMHD1 expression in CD4+ T-cells derived from Sèzary syndrome patients

Rebecca Kohnken^a, Karthik M. Kodigepalli^a, Anjali Mishra^{b,c}, Pierluigi Porcu^{b,c,d}, and Li Wu^{a,b,e,*}

^aCenter of Retrovirus Research, Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, 43210, USA

^bComprehensive Cancer Center, The Ohio State University, Columbus, OH, 43210, USA

^cDivision of Hematology, The Ohio State University, Columbus, OH, 43210, USA

^dDepartment of Internal Medicine, The Ohio State University, Columbus, OH, 43210, USA

^eDepartment of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, 43210, USA

Abstract

Sézary syndrome (SS) is a rare subtype of cutaneous T-cell lymphoma (CTCL) that is characterized by aggressive spread of neoplastic CD4+ T-cells from the skin into the bloodstream with metastasis to visceral organs. The deoxynucleoside triphosphohydrolase SAMHD1 is highly expressed in normal CD4+ T-cells, while its expression is down-regulated in CD4+ T-cells from SS patients. MicroRNA (miR) dysregulation is an important epigenetic mechanism in the pathogenesis and progression of SS. MiR-181 has been shown to inhibit SAMHD1 expression in cell lines and was identified as an important prognostic biomarker in CTCL. However, whether SAMHD1 is down-regulated by miR-181 in primary CD4+ T-cells of SS patients is unknown. Compared to normal CD4+ T-cells, SAMHD1 protein expression is significantly reduced in transformed CD4+ T-cell lines and CD4+ T-cells from SS patients, which inversely correlates with increased miR-181 levels in these cells. Over-expression of miR-181b in primary CD4+ T-cells from healthy donors significantly decreased SAMHD1 protein level, but not mRNA level. In contrast, inhibition of miR-181 in a CD4+ T-cell line significantly increased the level of SAMHD1 protein expression. Our results demonstrate that miR-181 is an important regulator of SAMHD1 protein expression in neoplastic CD4+ T-cells, likely through a mechanism of translational inhibition.

*Correspondence author: Professor Li Wu, PhD. wu.840@osu.edu, 466 Veterinary Medical Academic Building, 1900 Coffey Rd, Columbus, OH 43210, Phone: (614) 292-5408.

Conflicts of interest

The authors state no conflicts of interest.

Keywords

Cutaneous T-cell lymphoma; MicroRNA; SAMHD1; Sèzary syndrome

1. Introduction

Cutaneous T-cell lymphoma (CTCL) is characterized by infiltration of neoplastic T-cells into the skin, and presents clinically as rash and plaque lesions [1]. The disease affects approximately 3,000 new people every year in the United States [2]. It usually progresses in an indolent clinical course, but can transform to a highly aggressive disease, Sèzary syndrome (SS), with involvement of blood and visceral organs [1, 2]. SS is a leukemic disease with high proportions of neoplastic CD4⁺ T-cells in the peripheral circulation. The cause of CTCL is unknown, and the pathogenesis of SS remains a focus of intensive research. Epigenetic regulation, genomic instability, and microRNA (miR) dysregulation are thought to be common features in CTCL progression [1, 3].

Sterile alpha motif (SAM) and histidine/aspartic acid (HD) domain-containing protein 1 (SAMHD1) is the first identified mammalian deoxynucleoside triphosphohydrolase (dNTPase) [4–7]. In recent years, many critical cellular functions have been attributed to SAMHD1, including restriction of infection by a diverse array of viruses such as HIV-1 [5, 6, 8–10], regulation of innate immune signaling [11, 12], maintenance of nucleotide homeostasis (reviewed in [13, 14]), repression of retroelement activity [15, 16], and modulation of DNA damage signaling [11, 17]. Furthermore, SAMHD1 mutations could be a founder event in chronic lymphocytic (B-cell) leukemia (CLL) [18], and its potential role in cancer pathogenesis has been investigated following revelations that the *SAMHD1* gene is recurrently mutated in CLL [17, 18]. In a recent sequencing analysis of 80 SS patients, mutations and alterations in copy number at the SAMHD1 locus were identified in >10% of patients [19].

Mechanisms that regulate SAMHD1 function are primarily via post-translational modifications, such as phosphorylation in dividing cells [20–23]. Down-regulation of SAMHD1 expression in cancer cells occurs partially through promoter DNA methylation in CTCL [24, 25] and lung cancer [26]. A previous report showed direct regulation of levels of SAMHD1 mRNA and protein by miR-181a in myeloid leukemia-derived THP-1 and CD4⁺ lymphoma-derived Jurkat cell lines [27]. Recently, miR-181a was shown to down-regulate SAMHD1 mRNA and protein expression [28], and mediate interferon-induction of SAMHD1 expression in astrocytes [29]. MiRs are small non-coding RNAs that play a significant role in regulation of gene expression, and are commonly located within introns and at fragile genomic sites, resulting in dysregulation in almost all cancers [30, 31]. MiRs function to regulate gene expression by binding to the 3' untranslated region (UTR) of the target mRNA in complex with an RNA-interference silencing complex [30, 32, 33]. Mature miR binding predominantly results in translational repression, which may be followed by degradation of the target mRNA [34]. The miR-181 family includes four members (miR-181a-d) produced from three polycistronic clusters [23, 35]. MiR-181 is dysregulated in many cancers [36–38], including function as a tumor suppressor in CLL [39], and as an

oncogene in breast [40] and colorectal cancers [36]. MiR-181 also plays a significant role in T-cell development and differentiation [23, 27, 35, 36, 41]. MiR profiles are increasingly used in diagnosis and prognostication of human cancers including CTCL [42–44].

A binding site for the miR-181 cluster at the 3' UTR of the *SAMHD1* mRNA has been published [27]. Though other miRs showed partial complementarity to the 3' UTR of *SAMHD1* using two online programs (GeneCopoeia and TargetScan), miR-181 was the only common one in both analyses, has significant function in T cell development, and has been strongly implicated in the pathogenesis of CTCL and SS [45]. However, the regulatory function of miR-181 on SAMHD1 in primary CD4+ T-cells and in CTCL-derived cells is unknown. Clarifying the regulatory mechanisms by which miR-181 down-modulates SAMHD1 expression in CTCL cells will help to elucidate the potential role of SAMHD1 in cancer pathogenesis.

Here we investigated the role of the miR-181 family in regulating SAMHD1 expression in CD4+ T-cell lines derived from CTCL patients, as well as primary CD4+ T-cells from SS patients. We demonstrated lower SAMHD1 expression in CD4+ cell lines and CD4+ T-cells from SS patients compared to those from healthy donors. The levels of SAMHD1 protein and miR-181b expression in these cell lines showed a significant inverse correlation. This expression pattern was re-capitulated in CD4+ T-cells from SS patients compared to healthy donors. Furthermore, over-expression miR-181b in primary CD4+ T-cells from healthy donors significantly decreased the levels of SAMHD1 protein, and miR-181 inhibition in a CD4+ T cell line significantly increased SAMHD1 protein. Neither treatment altered mRNA expression compared to controls, suggesting that the mechanism of SAMHD1 down-regulation by miR-181 is through translational suppression.

2. Materials and methods

2.1. Cell lines and primary CD4+ T-cells from healthy donors and SS patients

The CTCL patient-derived HH, HuT78, HuT102 cell lines were described in previous studies [46] and leukemia patient-derived CD4+ T-cell lines MT1, MT2, SLB-1, and C8166 were kindly provided by Dr. Patrick Green (Ohio State University, Columbus, OH). All cell lines except SLB-1 were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS). SLB-1 cells were maintained in DMEM containing 10% FBS. De-identified peripheral blood mononuclear cells (PBMC) from SS patients, containing predominantly neoplastic CD4+ T-cells (ranging from 81.8% to 96.6% of PBMC), were obtained from the Leukemia Tissue Bank and used with approval from the Institutional Review Board of the Ohio State University. The study was conducted according to the Declaration of Helsinki guidelines and all participants gave their written informed consent. Healthy blood donors' leukocyte samples (buffy coats) were purchased from the Red Cross, Columbus, OH. PBMC were isolated from the buffy coats using Ficoll gradient centrifugation (Sigma Aldrich, St. Louis, MO) followed by CD4+ T-cell enrichment with RosetteSep Isolation kit for human T-cells (StemCell Technologies, Vancouver, British Columbia). Primary CD4+ T-cells from healthy donors and PBMC from SS patients were maintained in RPMI 1640 medium containing 10% FBS.

2.2. Immunoblotting analysis

Cells were disrupted in lysis buffer (Tris-HCl with 1× protease inhibitor (Sigma Aldrich, St. Louis, MO). Protein concentration was quantified by Bicinchoninic acid assay (Thermo Fisher, Waltham, MA). Immunoblotting was performed as described [24] using anti-SAMHD1 (ProSci Inc, Poway, CA) and anti-GAPDH (Bio-Rad, Hercules, CA). Images were obtained using the Amersham Imager (GE Healthcare, Pittsburgh, PA). Relative protein expression level was quantified using ImageJ software based on the densitometry of the protein bands.

2.3. Quantitative RT-PCR

For *SAMHD1* mRNA quantification, total cellular RNA samples were isolated using RNeasy Mini Kit (Qiagen, Venlo, Netherlands). The mRNA levels of *SAMHD1* was determined by quantitative real time RT-PCR as previously described [24]. *GAPDH* mRNA was used as an internal control. For miR-181b quantification, total RNAs were isolated as described above. cDNAs were generated from 10 ng total RNA by reverse transcription using TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) and the T100 Thermal Cycler (Bio-Rad, Hercules, CA). The expression of miR-181 was determined by real-time PCR (TaqMan MicroRNA Assays, Bio-Rad, Hercules, CA). U6 RNA was used as an internal control.

2.3. Over-expression or inhibition of miR-181 in CD4+ T-cells

Nucleofection was performed with the nucleofector device (Lonza, Basel, Switzerland) using Amaxa nucleofector kit specific for primary CD4+ T cells. CD4+ T-cells (5×10^6) from healthy donors were nucleofected with 300 nM pre-MiR-181b-5p miRNA precursor (Ambion, Thermo Fisher, Waltham, MA). Cells were cultured and then collected at 24 and 48 hours post-nucleofection for analysis of miR181b and SAMHD1 mRNA and protein expression. Similarly, MT2 cells (5×10^6) were nucleofected using Amaxa nucleofector kit specific for T-cell lines with 30 nM miR-181 family inhibitors, including miR-181a-5p, miR-181b-5p, miR-181c-5p, and miR-181d-5p miRCURY LNA DNA oligonucleotide (Exiqon, Denmark). Cells were cultured and then collected at 24 and 48 hours-post nucleofection per kit instruction for analysis of SAMHD1 mRNA and protein expression.

2.4. Statistical analysis

Statistical analysis was performed using Mann-Whitney test, Student's unpaired T-test, one-way ANOVA with Dunnett's post-test, and Pearson's correlation as indicated in the figure legends. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Down-regulated SAMHD1 expression in CTCL- and leukemia-derived CD4+ T-cell lines compared to normal CD4+ T-cells

Our previous studies have shown consistently high levels of endogenous SAMHD1 protein expression in primary resting CD4+ T-cells from multiple healthy donors [24, 47]. Four CD4+ T-cell lines including Jurkat cells have been shown to have lower or undetectable

SAMHD1 protein expression compared to monocytic THP-1 cells [24]. To confirm these observations with additional transformed CD4⁺ T-cell lines derived from leukemia patients (MT1, MT2, SLB-1, and C8166), as well as those derived from CTCL patients (HH, HuT78, and HuT102), we quantified the levels of SAMHD1 mRNA and protein expression in these 7 cell lines previously unexamined for SAMHD1 expression. Except HuT78 cells, the other 6 cell lines had nearly undetectable levels of SAMHD1 protein by immunoblotting as compared to high level of endogenous SAMHD1 expression in resting CD4⁺ T-cells from a healthy donor (Fig. 1A). Of 7 healthy donors examined, SAMHD1 protein level was consistently and similarly high (Fig. 3A). SAMHD1 protein expression profile corresponded to low mRNA expression in these cell lines as determined by quantitative PCR (Fig. 1B). HuT78 cells had low, but detectable protein expression (Fig. 1A), and only slightly reduced mRNA level as compared to the normal CD4⁺ T-cells (Fig. 1B). These data suggest that down-regulation of SAMHD1 expression is a consistent phenotype in CTCL- or leukemia-derived CD4⁺ T-cell lines.

3.2. Elevated miR-181 expression in transformed CD4⁺ T-cell lines compared to normal CD4⁺ T-cells

Since miR-181 was found to bind the 3' UTR of *SAMHD1* mRNA and over-expression of miR-181a down-modulates its expression in THP-1 and Jurkat T-cells, while inhibition of miR181a increases the levels of SAMHD1 mRNA and protein expression in these cell lines [27], we hypothesized that miR-mediated regulation might contribute to SAMHD1 down-regulation in CTCL- or leukemia-derived CD4⁺ T-cells. Consistent and significantly higher levels of all four miR-181a-d family members were detected in all cell lines as compared to relatively low miR-181 levels in CD4⁺ T-cells from a healthy donor (Fig. 2A–D). To determine whether miR-181 expression correlated with SAMHD1 protein expression in these cell lines, we performed a Pearson's correlation analysis. This analysis revealed a significant inverse correlation between the expression of SAMHD1 protein and miR-181b level in HH, HuT78, HuT102, MT1, and MT2 cell lines (Fig. 2E; $R^2=0.8483$, $p=0.026$). Additionally, there was no significant correlation between expression of *SAMHD1* mRNA and any miR-181 family members for these cell lines (data not shown). This data suggests that miR-181-mediated translational repression of SAMHD1 contributes to the significantly reduced protein expression consistently observed in these CTCL- or leukemia-derived cell lines.

3.3. Low levels of SAMHD1 expression in CD4⁺ T-cells from SS patients compared to healthy donors' cells

To determine whether SAMHD1 protein expression was consistently down-regulated in CD4⁺ T-cells from SS patients relative to healthy donors, we obtained CD4⁺ T-cells from 15 SS patients and 7 healthy donors. Our previous study showed significantly lower SAMHD1 protein expression in PBMCs and CD4⁺ T-cells from three SS patients by flow cytometry as compared to cells from 4 healthy donors [25]. Here, we used immunoblotting to confirm that SAMHD1 protein down-regulation is consistent in a larger SS patient cohort. Comparing CD4⁺ T-cells from 7 healthy donors, SAMHD1 protein levels in CD4⁺ T-cells from 15 SS patients were significantly reduced (Fig. 3A and 3B). All patients included in the cohort were clinically diagnosed with SS, and classified as stage IVA or IVB (Table 1). All but one

patient (#669) were undergoing treatment at the time of sample collection. The most common treatment regimens were Bexarotene, a retinoid (5/15), systemic interferon alpha (3/15), a combination of the above (2/15), and Denileukin Difitox, a directed toxin for interleukin-2 receptor (2/5). Remaining patients (1 each) were administered Prednisone and Vorinostat (SAHA). SAMHD1 protein level did not correlate with SS disease stage or treatment protocol (Table 1). Furthermore, we evaluated *SAMHD1* mRNA level in 4 SS patients compared to 5 healthy donors and show that there is no difference in transcript expression (Fig. 3C). This finding suggests that translational regulation may affect SAMHD1 protein level in SS patient cells.

3.4. Increased miR-181 expression in CD4+ T-cells from SS patients compared to those from healthy donors

To determine if the miR-181 cluster was also consistently over-expressed in our SS patient cohort as in some cell lines examined in this study and as reported in CTCL patients [45], we quantified all miR-181 family members in CD4+ T-cells of SS patients and healthy donors. All 4 miR-181 members (a–d) were elevated in CD4+ T-cells from 14 SS patients as compared to those from 5 healthy donors (Fig. 3D). The expression levels were significantly elevated for miR-181a, b, and d ($p = 0.0018$), but not for miR-181c. Together with the levels of SAMHD1 protein expression (Fig. 3A and 3B), these results show a trend of inverse correlation between the levels of miR-181 (a, b and d) and SAMHD1 protein expression in CD4+ T-cells from SS patients. Recent evaluation of the miR expression profile in CTCL showed significant overall increase in miR-181b, however there was inter-patient variability [45], similar to our patient cohort. Furthermore, the level of miR-181 expression did not show any clear association with disease stage or treatment protocol (Table 1).

3.5. Reduced SAMHD1 protein level by miR-181b over-expression in CD4+ T-cells from healthy donors

The finding that miR-181 family members are consistently elevated in CTCL- or leukemia-derived CD4+ T cell lines as well as in SS patients, and that miR-181b negatively correlates with SAMHD1 protein expression in cell lines, led us to investigate the role of miR-181b in the regulation of SAMHD1 protein expression in normal primary CD4+ T cells. In our experiment, miR-181b was the most highly expressed family member in SS patients (Fig. 3C), and was thus selected to use for over-expression studies in primary CD4+ T-cells from healthy donors. Furthermore, primary CD4+ T-cells from healthy donors have high levels of endogenous SAMHD1 expression and low miR-181b levels compared to SS patient cells (Fig. 3), allowing us to over-express miR-181b and to detect potential down-regulation of SAMHD1 expression. Nucleofection of a precursor pre-miR-181b into primary CD4+ T-cells significantly increased miR-181b levels over 130-fold at 24 and 48 hours post-nucleofection (Fig. 4A). Surprisingly, at 24 and 48 hours post-nucleofection, *SAMHD1* mRNA levels were unchanged (Fig. 4B), yet at 48 hours post-nucleofection, SAMHD1 protein expression was significantly reduced by approximately 40% in the cells over-expressing miR-181b relative to control cells (Fig. 4C and 4D). These results suggest that miR-181b down-modulates SAMHD1 protein expression in primary CD4+ T-cells, likely by a mechanism of translational repression.

3.6. Increased SAMHD1 protein level by miR-181 (a-d) inhibition in transformed CD4+ T-cell line

To confirm the role of miR-181-mediated down-regulation of SAMHD1 protein expression in cells, we sought to use a specific miR-181 family inhibitor to sequester and inactivate the mature miR family members in a CD4+ T-cell line. We selected MT2 cells because they expressed a high level of endogenous miR-181 (a–d), but barely detectable SAMHD1 protein (Fig. 1A and 2B). Nucleofection of miR-181 family inhibitor into MT2 cells did not affect *SAMHD1* mRNA levels at 24 or 48 hours post-nucleofection (Fig. 5A). Validating our previous finding, the expression level of SAMHD1 was increased at 48 hours post-nucleofection by 5-fold (Fig. 5B). These data further suggest miR-181-mediated down-regulation of SAMHD1 protein expression in CD4+ T-cells. Inhibitor sequences were specific for each miR-181 family member (Fig. 5C).

4. Discussion

Epigenetic regulation of protein expression is a common feature in the progression of SS patients and is characterized by loss of tumor suppressor gene expression [1]. We show here that SAMHD1 protein expression is significantly reduced in CTCL- and leukemia-derived CD4+ T-cell lines as well as in neoplastic CD4+ T-cells from SS patients compared to normal CD4+ T-cells. Using primary CD4+ T-cells from healthy donors to overexpress miR-181b, we observed a reduction in SAMHD1 protein, but not mRNA expression, suggesting that miR-181 is a post-transcriptional regulator of SAMHD1 expression in normal CD4+ T-cells. We subsequently confirmed this regulatory role by using miR-181 family inhibitor to increase SAMHD1 protein expression while not affecting mRNA expression.

MiR-mediated translational repression, as well as transcriptional mechanisms of regulation such as promoter DNA methylation and histone acetylation [24, 25], likely cooperate to result in low SAMHD1 protein expression in CD4+ T-cells from SS patients. Jin *et al.* demonstrated that by binding the 3' UTR of the *SAMHD1* mRNA, miR-181a down-regulated SAMHD1 protein and mRNA level in THP-1 and Jurkat cell lines [27]. They concluded that regulation of SAMHD1 by miR-181 was by mRNA degradation. However, in our study, there is an inverse correlation between miR-181b and SAMHD1 protein expression in cell lines, but no such relationship for *SAMHD1* mRNA levels. Similarly, miR-181b overexpression reduces SAMHD1 protein levels in primary CD4+ T-cells, while the opposite result is achieved with miR-181 inhibition in MT2 cells, but in neither case does miR-181 affect *SAMHD1* mRNA levels. Thus, our results suggest that the translational suppression of SAMHD1 protein expression by miR181 can be a mechanism in CD4+ T-cell lines and, importantly, also in primary CD4+ T-cells. Regulation of gene expression by miRs is complex, and investigations into these mechanisms continue to be valuable, particularly as it relates to CTCL development [1, 42, 44, 45].

MiR-181 family plays a significant role in T-cell differentiation and development in the thymus [23, 27, 35, 36, 41]. MiR-181a was recently shown to be consistently overexpressed in T-cell leukemia and lymphoma [39]. Over-expression of miR-181a also correlated with chemotherapeutic resistance, tumor progression, and advanced disease in these cancers [31,

38, 44, 45]. The anti-apoptotic protein Bcl-2 is a target of miR-181a/b, which contributes to tumor resistance to apoptosis in CLL [31]. Other targets of miR-181b include the oncogenes *Tcl-1* and *Mcl-1*, with low expression of miR-181b in these CLL patients correlating with poor prognosis [31, 39, 48]. In CTCL, miRs may also contribute to chemotherapy-induced apoptosis, aberrant cytokine expression, and impaired immune responses [49]. MiR-181a and miR-181b in particular were recently found to be significantly upregulated in CTCL patients, with implications in prognosis for patients with this profile [45].

Down-regulation of SAMHD1 expression in SS patient cells correlates with promoter DNA methylation of the *SAMHD1* gene [24], and we present here correlation with miR-181 levels. The pathologic implications of lost SAMHD1 expression in cancer cells are under investigation. SAMHD1 may be a potential candidate tumor suppressor, as its function as a unique dNTPase places it at the intersection of cell cycle regulation, cellular proliferation, and mutagenesis [13, 14, 50, 51]. Heterozygous loss-of-function mutations in SAMHD1 are common in colon cancer and these mutants were determined to affect dNTP pools and increase mutation rates in colon cancer cells [52]. Mutations in the *SAMHD1* gene have been identified as likely founder events in CLL [18], and a cohort of relapsed CLL patients had an 11% incidence of *SAMHD1* mutations, as compared to a 3% incidence in the pre-treatment group [17]. Somatic mutations in *SAMHD1* have also been identified in other hematopoietic and solid neoplasms [13, 17, 53–60]. A homozygous germline mutation in SAMHD1 was recently identified in a patient with an aggressive type of CTCL [61]. Finally, deletions in the SAMHD1 locus were also identified in 12% of SS patients, and novel mutations in SAMHD1 were identified in 3% of SS patients [19].

Loss of function mutations in SAMHD1 are also a monogenic cause of 17% of cases of Aicardi-Gouttierres syndrome (AGS), a juvenile sterile encephalitis thought to be caused by dysregulated type I interferon expression [62]. Fibroblasts from AGS patients lacking functional SAMHD1 protein are characterized by elevated basal DNA damage signaling and global genomic instability [11]. SAMHD1 has also been shown to inhibit retroelement activity in dividing cells [15, 16]. These findings suggest an important role for SAMHD1 in the maintenance of genomic stability, with implications for its role in cancer development.

Our results demonstrate the role of miR-181 in down-regulation of SAMHD1 expression in CTCL- and leukemia-derived CD4⁺ T-cell lines and neoplastic CD4⁺ T-cells from SS patients. Additionally, we show a direct relationship of miR-181-mediated down-regulation of SAMHD1 protein expression in primary CD4⁺ T-cells and a CD4⁺ T-cell line, which is likely through a mechanism of translational repression.

Acknowledgments

The authors are grateful to Dr. Patrick Green (Ohio State University) for providing cell lines and helpful discussions of the Wu lab members. The authors also thank Leah Grinshpun for assisting with miR-181 detection and Dr. Estelle Cormet-Boyaka for critically reading the manuscript. This work was supported by an NIH grant (CA181997) to LW. RK is supported by an NIH T32 training grant (OD010429). LW is also supported in part by NIH grants (AI074658, AI120209, and AI127667) and the Public Health Preparedness for Infectious Diseases Program of the Ohio State University.

List of abbreviations

SAMHD1	Sterile alpha motif and HD domain containing protein 1
CTCL	Cutaneous T-cell lymphoma
SS	Sézary syndrome
miR	MicroRNA
CLL	Chronic lymphocytic leukemia
AGS	Aicardi-Gouttieres syndrome.

References

1. Wong HK. Novel biomarkers, dysregulated epigenetics, and therapy in cutaneous T-cell lymphoma. *Discov Med*. 2013; 16:71–78. [PubMed: 23998443]
2. Wong HK, Mishra A, Hake T, Porcu P. Evolving insights in the pathogenesis and therapy of cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome). *Br J Haematol*. 2011; 155:150–166. [PubMed: 21883142]
3. Wilcox RA. Cutaneous T-cell lymphoma: 2014 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2014; 89:837–851. [PubMed: 25042790]
4. Goldstone DC, Ennis-Adeniran V, Hedden JJ, Groom HC, Rice GI, Christodoulou E, Walker PA, Kelly G, Haire LF, Yap MW, et al. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature*. 2011; 480:379–382. [PubMed: 22056990]
5. Laguette N, Sobhian B, Casartelli N, Ringear M, Chable-Bessia C, Ségéral E, Yatim A, Emiliani S, Schwartz O, Benkirane M. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature*. 2011; 474:654–657. [PubMed: 21613998]
6. Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, Florens L, Washburn MP, Skowronski J. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature*. 2011; 474:658–661. [PubMed: 21720370]
7. Powell RD, Holland PJ, Hollis T, Perrino FW. Aicardi-Gouttieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. *J Biol Chem*. 2011; 286:43596–43600. [PubMed: 22069334]
8. Chen Z, Zhu M, Pan X, Zhu Y, Yan H, Jiang T, Shen Y, Dong X, Zheng N, Lu J, Ying S. Inhibition of Hepatitis B virus replication by SAMHD1. *Biochem Biophys Res Commun*. 2014; 450:1462–1468. [PubMed: 25019997]
9. Lahouassa H, Daddacha W, Hofmann H, Ayinde D, Logue EC, Dragin L, Bloch N, Maudet C, Bertrand M, Gramberg T, et al. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nat Immunol*. 2012; 13:223–228. [PubMed: 22327569]
10. Hollenbaugh JA, Gee P, Baker J, Daly MB, Amie SM, Tate J, Kasai N, Kanemura Y, Kim DH, Ward BM, et al. Host factor SAMHD1 restricts DNA viruses in non-dividing myeloid cells. *PLoS Pathog*. 2013; 9:e1003481. [PubMed: 23825958]
11. Kretschmer S, Wolf C, König N, Staroske W, Guck J, Häusler M, Luksch H, Nguyen LA, Kim B, Alexopoulou D, et al. SAMHD1 prevents autoimmunity by maintaining genome stability. *Ann Rheum Dis*. 2015; 74:e17. [PubMed: 24445253]
12. Rice GI, Bond J, Asipu A, Brunette RL, Manfield IW, Carr IM, Fuller JC, Jackson RM, Lamb T, Briggs TA, et al. Mutations involved in Aicardi-Gouttières syndrome implicate SAMHD1 as regulator of the innate immune response. *Nat Genet*. 2009; 41:829–832. [PubMed: 19525956]
13. Kohnken R, Kodigepalli KM, Wu L. Regulation of deoxynucleotide metabolism in cancer: novel mechanisms and therapeutic implications. *Mol Cancer*. 2015; 14:176. [PubMed: 26416562]

14. Mathews CK. Deoxyribonucleotide metabolism, mutagenesis and cancer. *Nat Rev Cancer*. 2015; 15:528–539. [PubMed: 26299592]
15. Zhao K, Du J, Han X, Goodier JL, Li P, Zhou X, Wei W, Evans SL, Li L, Zhang W, et al. Modulation of LINE-1 and Alu/SVA retrotransposition by Aicardi-Goutières syndrome-related SAMHD1. *Cell Rep*. 2013; 4:1108–1115. [PubMed: 24035396]
16. Hu S, Li J, Xu F, Mei S, Le Duff Y, Yin L, Pang X, Cen S, Jin Q, Liang C, Guo F. SAMHD1 Inhibits LINE-1 Retrotransposition by Promoting Stress Granule Formation. *PLoS Genet*. 2015; 11:e1005367. [PubMed: 26134849]
17. Clifford R, Louis T, Robbe P, Ackroyd S, Burns A, Timbs AT, Wright Colopy G, Dreau H, Sigaux F, Judde JG, et al. SAMHD1 is mutated recurrently in chronic lymphocytic leukemia and is involved in response to DNA damage. *Blood*. 2014; 123:1021–1031. [PubMed: 24335234]
18. Guèze R, Robbe P, Clifford R, de Guibert S, Pereira B, Timbs A, Dilhuydy MS, Cables M, Ysebaert L, Burns A, et al. Presence of multiple recurrent mutations confers poor trial outcome of relapsed/refractory CLL. *Blood*. 2015; 126:2110–2117. [PubMed: 26316624]
19. Kiel MJ, Sahasrabudhe AA, Rolland DC, Velusamy T, Chung F, Schaller M, Bailey NG, Betz BL, Miranda RN, Porcu P, et al. Genomic analyses reveal recurrent mutations in epigenetic modifiers and the JAK-STAT pathway in Sézary syndrome. *Nat Commun*. 2015; 6:8470. [PubMed: 26415585]
20. Cribier A, Descours B, Valadão AL, Laguet N, Benkirane M. Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. *Cell Rep*. 2013; 3:1036–1043. [PubMed: 23602554]
21. White TE, Brandariz-Núñez A, Valle-Casuso JC, Amie S, Nguyen LA, Kim B, Tuzova M, Diaz-Griffero F. The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. *Cell Host Microbe*. 2013; 13:441–451. [PubMed: 23601106]
22. St Gelais C, de Silva S, Hach JC, White TE, Diaz-Griffero F, Yount JS, Wu L. Identification of cellular proteins interacting with the retroviral restriction factor SAMHD1. *J Virol*. 2014; 88:5834–5844. [PubMed: 24623419]
23. Yan J, Hao C, DeLucia M, Swanson S, Florens L, Washburn MP, Ahn J, Skowronski J. CyclinA2-Cyclin-dependent Kinase Regulates SAMHD1 Protein Phosphohydrolase Domain. *J Biol Chem*. 2015; 290:13279–13292. [PubMed: 25847232]
24. de Silva S, Hoy H, Hake TS, Wong HK, Porcu P, Wu L. Promoter methylation regulates SAMHD1 gene expression in human CD4+ T cells. *J Biol Chem*. 2013; 288:9284–9292. [PubMed: 23426363]
25. de Silva S, Wang F, Hake TS, Porcu P, Wong HK, Wu L. Downregulation of SAMHD1 expression correlates with promoter DNA methylation in Sezary syndrome patients. *J Invest Dermatol*. 2014; 134:562–565. [PubMed: 23884314]
26. Wang JL, Lu FZ, Shen XY, Wu Y, Zhao LT. SAMHD1 is down regulated in lung cancer by methylation and inhibits tumor cell proliferation. *Biochem Biophys Res Commun*. 2014; 455:229–233. [PubMed: 25449277]
27. Jin C, Peng X, Liu F, Cheng L, Lu X, Yao H, Wu H, Wu N. MicroRNA-181 expression regulates specific post-transcriptional level of SAMHD1 expression in vitro. *Biochem Biophys Res Commun*. 2014; 452:760–767. [PubMed: 25201733]
28. Pilakka-Kanthikeel S, Raymond A, Atluri VS, Sagar V, Saxena SK, Diaz P, Chevelon S, Concepcion M, Nair M. Sterile alpha motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1)-facilitated HIV restriction in astrocytes is regulated by miRNA-181a. *J Neuroinflammation*. 2015; 12:66. [PubMed: 25890101]
29. Jin C, Peng X, Liu F, Cheng L, Xie T, Lu X, Wu H, Wu N. Interferon-induced SAMHD1 expression in astrocytes and microglia is mediated by miR-181a. *AIDS*. 2016
30. Wienholds E, Plasterk RH. MicroRNA function in animal development. *FEBS Lett*. 2005; 579:5911–5922. [PubMed: 16111679]
31. Zhu DX, Zhu W, Fang C, Fan L, Zou ZJ, Wang YH, Liu P, Hong M, Miao KR, Xu W, Li JY. miR-181a/b significantly enhances drug sensitivity in chronic lymphocytic leukemia cells via

- targeting multiple anti-apoptosis genes. *Carcinogenesis*. 2012; 33:1294–1301. [PubMed: 22610076]
32. Liu J, Wu CP, Lu BF, Jiang JT. Mechanism of T cell regulation by microRNAs. *Cancer Biol Med*. 2013; 10:131–137. [PubMed: 24379987]
 33. Mraz M, Pospisilova S, Malinova K, Slapak I, Mayer J. MicroRNAs in chronic lymphocytic leukemia pathogenesis and disease subtypes. *Leuk Lymphoma*. 2009; 50:506–509. [PubMed: 19347736]
 34. Wilczynska A, Bushell M. The complexity of miRNA-mediated repression. *Cell Death Differ*. 2015; 22:22–33. [PubMed: 25190144]
 35. Belkaya S, van Oers NS. Transgenic expression of microRNA-181d augments the stress-sensitivity of CD4(+)CD8(+) thymocytes. *PLoS One*. 2014; 9:e85274. [PubMed: 24416377]
 36. Weng H, Lal K, Yang FF, Chen J. The pathological role and prognostic impact of miR-181 in acute myeloid leukemia. *Cancer Genet*. 2015; 208:225–229. [PubMed: 25686674]
 37. Su R, Lin HS, Zhang XH, Yin XL, Ning HM, Liu B, Zhai PF, Gong JN, Shen C, Song L, et al. MiR-181 family: regulators of myeloid differentiation and acute myeloid leukemia as well as potential therapeutic targets. *Oncogene*. 2015; 34:3226–3239. [PubMed: 25174404]
 38. Verduci L, Azzalin G, Gioiosa S, Carissimi C, Laudadio I, Fulci V, Macino G. microRNA-181a enhances cell proliferation in acute lymphoblastic leukemia by targeting EGR1. *Leuk Res*. 2015; 39:479–485. [PubMed: 25740602]
 39. Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu CG, Rassenti L, et al. Tc11 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. *Cancer Res*. 2006; 66:11590–11593. [PubMed: 17178851]
 40. Sun X, Luo S, He Y, Shao Y, Liu C, Chen Q, Cui S, Liu H. Screening of the miRNAs related to breast cancer and identification of its target genes. *Eur J Gynaecol Oncol*. 2014; 35:696–700. [PubMed: 25566594]
 41. Palin AC, Ramachandran V, Acharya S, Lewis DB. Human neonatal naive CD4+ T cells have enhanced activation-dependent signaling regulated by the microRNA miR-181a. *J Immunol*. 2013; 190:2682–2691. [PubMed: 23408835]
 42. Ballabio E, Mitchell T, van Kester MS, Taylor S, Dunlop HM, Chi J, Tosi I, Vermeer MH, Tramonti D, Saunders NJ, et al. MicroRNA expression in Sezary syndrome: identification, function, and diagnostic potential. *Blood*. 2010; 116:1105–1113. [PubMed: 20448109]
 43. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med*. 2005; 353:1793–1801. [PubMed: 16251535]
 44. Ralfkiaer U, Hagedorn PH, Bangsgaard N, Løvendorf MB, Ahler CB, Svensson L, Kopp KL, Vennegaard MT, Lauenborg B, Zibert JR, et al. Diagnostic microRNA profiling in cutaneous T-cell lymphoma (CTCL). *Blood*. 2011; 118:5891–5900. [PubMed: 21865341]
 45. Sandoval J, Díaz-Lagares A, Salgado R, Servitje O, Climent F, Ortiz-Romero PL, Pérez-Ferriols A, Garcia-Muret MP, Estrach T, Garcia M, et al. MicroRNA expression profiling and DNA methylation signature for deregulated microRNA in cutaneous T-cell lymphoma. *J Invest Dermatol*. 2015; 135:1128–1137. [PubMed: 25405321]
 46. Gibson HM, Mishra A, Chan DV, Hake TS, Porcu P, Wong HK. Impaired proteasome function activates GATA3 in T cells and upregulates CTLA-4: relevance for Sézary syndrome. *J Invest Dermatol*. 2013; 133:249–257. [PubMed: 22951729]
 47. St Gelais C, de Silva S, Amie SM, Coleman CM, Hoy H, Hollenbaugh JA, Kim B, Wu L. 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*. 2012; 9:105. [PubMed: 23231760]
 48. Visone R, Veronese A, Rassenti LZ, Balatti V, Pearl DK, Acunzo M, Volinia S, Taccioli C, Kipps TJ, Croce CM. miR-181b is a biomarker of disease progression in chronic lymphocytic leukemia. *Blood*. 2011; 118:3072–3079. [PubMed: 21636858]
 49. Persson JL. miRNA in mycosis fungoides and skin inflammation. *APMIS*. 2013; 121:1017–1019. [PubMed: 24106870]

50. Ballana E, Esté JA. SAMHD1: At the Crossroads of Cell Proliferation, Immune Responses, and Virus Restriction. *Trends Microbiol.* 2015; 23:680–692. [PubMed: 26439297]
51. Bonifati S, Daly MB, St Gelais C, Kim SH, Hollenbaugh JA, Shepard C, Kennedy EM, Kim DH, Schinazi RF, Kim B, Wu L. SAMHD1 controls cell cycle status, apoptosis and HIV-1 infection in monocytic THP-1 cells. *Virology.* 2016; 495:92–100. [PubMed: 27183329]
52. Rentoft M, Lindell K, Tran P, Chabes AL, Buckland RJ, Watt DL, Marjavaara L, Nilsson AK, Melin B, Trygg J, et al. Heterozygous colon cancer-associated mutations of SAMHD1 have functional significance. *Proc Natl Acad Sci U S A.* 2016; 113:4723–4728. [PubMed: 27071091]
53. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science.* 2008; 321:1801–1806. [PubMed: 18772397]
54. Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, Lawrence MS, Sougnez C, Stewart C, Sivachenko A, Wang L, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell.* 2013; 152:714–726. [PubMed: 23415222]
55. Liu J, Lee W, Jiang Z, Chen Z, Jhunjhunwala S, Haverty PM, Gnad F, Guan Y, Gilbert HN, Stinson J, et al. Genome and transcriptome sequencing of lung cancers reveal diverse mutational and splicing events. *Genome Res.* 2012; 22:2315–2327. [PubMed: 23033341]
56. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science.* 2008; 321:1807–1812. [PubMed: 18772396]
57. Schuh A, Becq J, Humphray S, Alexa A, Burns A, Clifford R, Feller SM, Grocock R, Henderson S, Khrebukova I, et al. Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns. *Blood.* 2012; 120:4191–4196. [PubMed: 22915640]
58. Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, et al. The consensus coding sequences of human breast and colorectal cancers. *Science.* 2006; 314:268–274. [PubMed: 16959974]
59. Walker BA, Wardell CP, Melchor L, Hulkki S, Potter NE, Johnson DC, Fenwick K, Kozarewa I, Gonzalez D, Lord CJ, et al. Intracлонаl heterogeneity and distinct molecular mechanisms characterize the development of t(4;14) and t(11;14) myeloma. *Blood.* 2012; 120:1077–1086. [PubMed: 22573403]
60. Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB, Chaudhuri S, Guan Y, Janakiraman V, Jaiswal BS, et al. Recurrent R-spondin fusions in colon cancer. *Nature.* 2012; 488:660–664. [PubMed: 22895193]
61. Merati M, Buehler DJ, Cooper KD, Honda KS, Wang H, Gerstenblith MR. Aggressive CD8(+) epidermotropic cutaneous T-cell lymphoma associated with homozygous mutation in SAMHD1. *JAAD Case Rep.* 2015; 1:227–229. [PubMed: 27051737]
62. Crow YJ. Aicardi-Goutières syndrome. *Handb Clin Neurol.* 2013; 113:1629–1635. [PubMed: 23622384]
63. Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome): part II. Prognosis, management, and future directions. *J Am Acad Dermatol.* 2014; 70:223.e221–217. quiz 240–222. [PubMed: 24438970]
64. Olek-Hrab K, Silny W. Diagnostics in mycosis fungoides and Sezary syndrome. *Rep Pract Oncol Radiother.* 2014; 19:72–76. [PubMed: 24936324]

Figure 1. SAMHD1 protein and mRNA expression in CD4+ T-cells from a healthy donor and transformed CD4+ T-cell lines

(A) Immunoblotting analysis of endogenous SAMHD1 expression in CD4+ T-cells from a healthy donor (HD) and seven CD4+ T-cell lines derived from leukemia patients (MT1, MT2, SLB-1, and C8166) or CTCL patients (HH, HuT78, and HuT102). This blot is representative from four independent experiments with four healthy donors. GAPDH was used as a loading control. (B) Quantitative real-time RT-PCR of endogenous *SAMHD1* mRNA level in CD4+ T-cells from a healthy donor and seven CD4+ T-cell lines. This plot is representative from four independent experiments. GAPDH was used as a normalization control. Delta-delta CT analysis was performed to quantify *SAMHD1* mRNA level of cell lines relative to that of healthy donor cells. Statistical significance of *SAMHD1* mRNA levels was achieved (***: $p < 0.0001$) for all cell lines except HuT78 cells compared to healthy donor cells by One-way ANOVA.

Figure 2. MiR-181a-d expression in CD4⁺ T-cells from healthy donor and CTCL- or leukemia-derived CD4⁺ T-cell lines

(A–D) Real-time PCR detection of the levels of miR-181a-d, respectively, in CD4⁺ T-cells from a healthy donor (HD) and seven transformed CD4⁺ T-cell lines. These plots are representative of three independent experiments with three healthy donors. U6 was used as an internal control. Delta delta CT analysis was performed quantifying miR-181 level of cell lines relative to the normal CD4⁺ T-cells set as 1 following normalization to U6. Statistical significance was achieved comparing cell lines to normal cells (*: $p < 0.05$; ***: $p < 0.0007$)

by Student's unpaired T-test. **(E)** Correlation analysis of miR-181b and SAMHD1 protein levels in HH, HuT78, HuT102, MT1, and MT2 cell lines. Pearson's $R^2 = 0.8483$, $p = 0.026$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3. Down-regulation of SAMHD1 protein expression and up-regulation of microRNA-181 levels in CD4+ T-cells from SS patients relative to healthy donors

(A) Immunoblotting analysis of endogenous SAMHD1 protein expression in CD4+ T-cells from 7 healthy donors (HD) and 15 SS patients. These blots are representative of two independent experiments. GAPDH was used as a loading control. (B) Relative protein expression was quantified based on the protein bands in (a) using ImageJ to normalize SAMHD1 expression to GAPDH. Statistical significance was achieved ($p=0.0049$) comparing SS patient SAMHD1 expression levels to that of healthy donors by Student's unpaired T-test. (C) Quantification of SAMHD1 mRNA by qPCR. This plot is representative of three independent experiments. GAPDH mRNA was used as an internal control. Delta delta CT analysis was performed to quantify relative mRNA expression levels of CD4+ T-cells from SS patients and healthy donors. (D) Quantification of microRNA-181 a, b, c, and

d in CD4+ T-cells from 5 healthy donors (HD) and 14 SS patients by real-time RT-PCR. This plot is representative of three independent experiments. U6 RNA was used as an internal control. Delta CT analysis was performed to quantify relative miR expression levels of CD4+ T-cells from SS patients and healthy donors by normalizing to U6. Within each box, the median value is represented by the horizontal line, and the first and third quartiles are the ends of the box. The maximum and minimum data points are the ends of the whiskers. Statistical significance was achieved comparing SS patients to healthy donors for three miR-181 family members (181a: $p=0.009$; 181b: $p=0.0018$; and 181d: $p=0.0121$) by Student's unpaired T-test with Mann-Whitney post-test.

Figure 4. Reduced SAMHD1 protein expression by miR-181b over-expression in resting CD4+ T-cells from healthy donors

(A) Real-time RT-PCR detection of miR-181b levels in CD4+ T-cells from healthy donor at 24 and 48 hours post-nucleofection with precursor miR-181b or controls. This plot represents combined data from two healthy donors, with three independent experiments each. U6 RNA was used as an internal control. Delta-delta CT analysis was performed to quantify relative miR-181b levels in nucleofected cells compared to control cells. Statistical significance was achieved by comparing miR-181b-nucleofected cells to control (scrambled)-nucleofected cells (24 hrs: $p=0.0004$; 48 hrs: $p=0.0001$) by Student's unpaired T-test with Mann-Whitney post-test. (B) Real-time RT-PCR quantification of *SAMHD1* mRNA levels in CD4+ T-cells from healthy donors at 24 and 48 hours post-nucleofection with precursor miR-181b. This plot is combined from results of two healthy donors' cells, with three independent experiments each. GAPDH was used as a normalization control. Delta-delta CT analysis relative to mock nucleofected cells was performed and there was no statistically significant difference at either time point. (C) Immunoblotting analysis of endogenous SAMHD1 protein expression in CD4+ T-cells from a healthy donor at 24 and 48 hours post-nucleofection with precursor miR-181b. This blot is representative of three independent experiments for two healthy donor's cells. GAPDH was used as a loading control. (D) Quantification of protein expression by Image J from (C) and combining data from both donors and plotting protein level of nucleofected cells relative to control cells set as 1 for each time point. Statistical significance was achieved at 48 hours post-nucleofection comparing miR-181b-nucleofected cells to control cells ($p=0.0197$) by Student's unpaired T-test with Mann-Whitney post-test.

Figure 5. Increased SAMHD1 protein expression by miR-181(a-d) inhibition in MT2 cells
(A) Real-time RT-PCR quantification of *SAMHD1* mRNA levels in MT2 CD4⁺ T-cells at 24 and 48 hours post-nucleofection with miR-181 family inhibitor. This plot represents two independent experiments. GAPDH was used as a normalization control. Delta-delta CT analysis relative to mock nucleofected cells was performed and there was no statistically significant difference. **(B)** Immunoblotting analysis of endogenous SAMHD1 protein expression in MT2 CD4⁺ T-cells at 24 and 48 hours post-nucleofection with miR-181 family inhibitor. This blot is representative of two independent experiments. GAPDH was used as a loading control. Quantification of protein expression relative to mock nucleofected cells by Image J. **(C)** Sequence alignment of the 3' UTR of *SAMHD1*, mature miR-181a-d, and miR-181 family inhibitors. The miR-181b and miR-181d inhibitors have mixed DNA bases; R= A and G.

Clinical information of 15 Sézary syndrome patients and relative SAMHD1 protein levels in their cells.

Table 1

Patient number ¹	Disease stage ²	Absolute lymphocyte count (cells/uL)	CD4+ cells (%) ³	Sézary cells (%) ⁴	Relative SAMHD1 protein levels ⁵	Relative miR-181b levels ⁶
#834	T4NXXM0B2	5,700	96	91	0.625	18.00
#29	T4NXXM0B2	8,300	92	90	0.448	3.00
#928	T4NXXM0B2	6,600	98	95	0.662	2.00
#1259	T4N3M0B2	12,800	91	90	0.057	160.00
#269	T4NXXM0B2	4,500	97	96	0.046	22.00
#11	T4N3M0B2	6,550	97	97	0.026	1.98
#144	T4NXXM0B2	10,200	88	88	0.009	2.78
#210	T4N3M0B2	7,200	91	90	0.436	5.78
#214	T4N3M0B2	3,700	99	98	0.017	16.30
#558	T4N0M0B2	11,000	89	88	0.769	2.41
#563	T4N3M1B2	4,100	98	97	0.027	2.59
#669	T4N3M1B2	10,300	92	90	0.017	10.31
#670	T4N0M0B2	3,900	99	97	0.071	N/A ⁷
#1282	T4N3M0B2	4,500	98	93	0.420	43.78
#1233	T3N3M0B2	110,000	99	95	0.163	15.98

¹ Patients were treated with bexarotene, interferon, a combination of both, denileukin diftitoxin, vorinostat, or prednisone at the time of peripheral blood collection. One patient (#669) was not on a treatment protocol.

² Clinical tumor staging based on TNMB system, which is based upon an evaluation of the skin (T), lymph nodes (N), visceral involvement (M), and blood (B) [63, 64].

³ CD4-positive cells (%) indicate percentage of CD4-positive cells of the patient's absolute lymphocyte count by flow cytometry analysis.

⁴ Sézary cells (%) indicate percentage of CD4-positive and CD26-negative T-cells (percentage of the patient's absolute lymphocyte count by flow cytometry analysis).

⁵ Relative levels of SAMHD1 protein in PBMCs from 15 SS patients (mean±SD is 0.25±0.27) compared to the average level of 7 healthy donors (1.07±0.22).

⁶ Relative levels of miR-181b in PBMCs from 14 SS patients (mean±SD is 21.92±41.41) compared to the average level of 5 healthy donors (2.94±1.21).

⁷ N/A, sample not available.