Modulation of LINE-1 retrotransposition by a human SAMHD1 polymorphism

Tommy E. White, Albert Einstein College of Medicine
Alberto Brandariz-Nuñez, Albert Einstein College of Medicine
Kyudong Han, Dankook University
Sara L. Sawyer, University of Colorado
Baek Kim, Emory University
Felipe Diaz-Griffero, Albert Einstein College of Medicine

Journal Title: Virology Reports
Volume: Volume 6
Publisher: Elsevier | 2016-12-01, Pages 53-60
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.virep.2016.06.001
Permanent URL: https://pid.emory.edu/ark:/25593/s6pnw

Final published version: http://dx.doi.org/10.1016/j.virep.2016.06.001

Copyright information:
© 2016 The Authors.
Accessed September 27, 2019 8:02 AM EDT
Modulation of LINE-1 Retrotransposition by a Human SAMHD1 Polymorphism

Tommy E. White1, Alberto Brandariz-Nuñez1, Kyudong Han2, Sara L. Sawyer3, Baek Kim4, and Felipe Diaz-Griffero∗,1
1Department of Microbiology and Immunology, Albert Einstein College of Medicine Bronx, NY 10461
2Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan, Republic of Korea
3Department of Molecular, Cellular, and Developmental Biology, University of Colorado Boulder, Boulder, Colorado, 80309, USA
4Department of Pediatrics, Emory University, Atlanta, GA 30322

Abstract

The HIV-1 restriction factor SAMHD1 has the ability to negatively modulate retrotransposition of the long interspersed element 1 (LINE-1). By exploring the ability of human SAMHD1 polymorphisms to inhibit LINE-1, we found that the single nucleotide polymorphism S33A present in the Korean population lose the ability to inhibit LINE-1 retrotransposition. Because SAMHD1 residue S33 is phosphorylated in human cycling and non-cycling cells, we demonstrated that SAMHD1 requires to be either phosphorylated on position 33 or to contain a bulky residue in order to inhibit LINE-1 retrotransposition. Therefore this unique mutation uncouples functions in this important restriction factor.

Keywords

SAMHD1; LINE-1; SNPs; retrotransposition; restriction; HIV-1

INTRODUCTION

SAMHD1 is a human restriction factor that prevents efficient infection of macrophages, dendritic cells and resting CD4+ T cells by HIV-1 (Baldauf et al., 2012) (Berger et al., 2011; Descours et al., 2012; Hrecka et al., 2011; Laguette et al., 2011). In addition, SAMHD1 has
the ability to block LINE-1 retrotransposition (Hu et al., 2015; White et al., 2014; Zhao et al., 2013). Although several human SAMHD1 polymorphisms have been studied (Coon et al., 2012; White et al., 2014), none of the analyzed human SAMHD1 polymorphism have shown to exhibit a defect on the known functions of SAMHD1.

The database of single nucleotide polymorphisms (SNP) at NCBI (dbSNP) reports two human polymorphisms in Korean individuals at codon 33 in the SAMHD1 open reading frame (Kim et al., 2009; Park et al., 2010). Most alleles of SAMHD1 encode a serine at this position (S33)(White et al., 2014), which is a residue that is permanently phosphorylated in the SAMHD1 protein (Pauls et al., 2014; Welbourn et al., 2013; White et al., 2013b). Here we explored the role of S33 in the ability of SAMHD1 to modulate the retrotransposition of the long interspersed element 1 (LINE-1). For this purpose, we generated a set of SAMHD1 variants by changing S33 to different residues and explored the ability of the different variants to modulate LINE-1 retrotransposition. Furthermore, we tested the different SAMHD1 variants for oligomerization, RNA binding, subcellular localization, Vpx-mediated degradation, HIV-1 restriction, and the ability to decrease the cellular levels of dNTPs.

RESULTS

Ability of the human SAMHD1 polymorphism S33A to inhibit LINE-1 retrotransposition

By the use of mass spectrometry, others and we have previously observed that SAMHD1 is phosphorylated on S33 (Pauls et al., 2014; Welbourn et al., 2013; White et al., 2013b). Interestingly, the nucleotides that codify S33 exhibit at least two described single nucleotide polymorphisms, in Korean individuals, that changes S33 to tyrosine or alanine (White et al., 2014). To understand the role of S33 in the ability of SAMHD1 to negatively modulate retrotransposition of the long interspersed element 1 (LINE-1) (Zhao et al., 2013), we used a reporter assay to measure LINE-1 retrotransposition in human HEK293T cells (Goodier et al., 2013). For this purpose, we used the LINE-1 episomal construct 99-PUR-RPS-EGFP (L1RP-EGFP), which contains an EGFP reporter gene interrupted by an intron in the opposite transcriptional orientation (Figure 1A). The EGFP cassette is inserted into the 3′UTR of a retrotransposition-component L1 (L1RP). EGFP is expressed only when the intron of the LINE-1 transcript is removed by splicing, and the resulting transcript is reverse transcribed and subsequently integrated into the genomic DNA. After integration, the EGFP gene will be expressed from its CMV promoter (Figure 1A). To control for background levels of retrotransposition in human cells, we used the same L1RP-EGFP construct containing two missense mutations on ORF1(JM111) that abolish the retrotransposition activity of LINE-1 (Figure 1A) (Moran et al., 1996). As shown in Figure 1B, SAMHD1 variants S33D (phosphomimetic) and S33Y (bulky group) negatively modulate LINE-1 retrotransposition when compared to wild-type SAMHD1. Interestingly, the SAMHD1 variant S33A did not inhibit LINE-1 retrotransposition (Figure 1B). As a negative control, we used the SAMHD1 variant H123P, which does not inhibit LINE-1 retrotransposition (Zhao et al., 2013). As a positive control, we used the SAMHD1 enzymatic variant HD206AA, which has lost the dNTPase activity (Goldstone et al., 2011). These results suggested that phosphorylation on S33 is required for inhibition of LINE-1 since S33D, a
phosphomimetic change, and wild type SAMHD1 inhibits LINE-1 retrotransposition. When S33 was replaced by a bulky group such as tyrosine, the protein was still able to inhibit LINE-1 retrotransposition suggesting that a phosphorylation or a bulky group in position 33 is important for the ability of SAMHD1 to inhibit LINE-1 retrotransposition.

**Oligomerization, RNA Binding and Localization of SAMHD1 variants**

We have previously demonstrated the ability of SAMHD1 to oligomerize in mammalian cells (White et al., 2013a), which is in agreement with biochemical data suggesting that purified SAMHD1 is a tetramer (Brandariz-Nunez et al., 2013; Ji et al., 2013; Yan et al., 2013; Zhu et al., 2013). For this purpose, we tested the ability of the different human SAMHD1-FLAG variants to oligomerize with wild type SAMHD1-HA. As shown in Figure 2A, all the tested SAMHD1 variants retained their ability to oligomerize. As a control, we tested the oligomerization ability of the SAMHD1-Y146S/Y154S variant, which is defective for oligomerization (Brandariz-Nunez et al., 2013). These results indicated that all tested SAMHD1 variants are not defective for oligomerization.

Others and we have demonstrated the ability of SAMHD1 to bind RNA (Goncalves et al., 2012; White et al., 2013b). Next we tested the ability of SAMHD1 variants to bind the interferon-stimulatory DNA sequence containing a phosphorothioate backbone (ISD-PS), which is an RNA analog. As shown in Figure 2B, all tested human SAMHD1 variants interacted with ISD-PS when compared to wild-type SAMHD1.

SAMHD1 is a nuclear protein that exhibits a nuclear localization signal on residues 11KRPR14 (Brandariz-Nunez et al., 2012; Hofmann et al., 2012; Rice et al., 2009). Next we tested the subcellular localization of SAMHD1 variants in human HeLa cells by immunofluorescence, as previously described (Brandariz-Nunez et al., 2012). For this purpose, we transiently transfected the different human SAMHD1-FLAG polymorphisms in HeLa cells and performed immunofluorescence staining using anti-FLAG antibodies. As control, we stained the cellular nuclei using DAPI. As shown in Figure 2C, all tested human SAMHD1 polymorphisms localized to the nucleus. These experiments suggested that the different SAMHD1 variants are not affected for their ability to localize in the nuclear compartment.

**Susceptibility of SAMHD1 variants to Vpx mediated degradation**

Because the viral protein Vpx from different viruses induces the degradation of SAMHD1, we tested the ability of Vpx from HIV-2ROD (VpxROD) to degrade the different SAMHD1 variants, as previously described (White et al., 2013b). For this purpose, we cotransfected the different SAMHD1 variants together with VpxROD and measure the expression level of SAMHD1 (Figure 2D). As a control, we cotransfected the different human SAMHD1 polymorphisms with Vpx SIVrcm (Vpxrcm), which is unable to induce the degradation of human SAMHD1 (Brandariz-Nunez et al., 2012). As shown in Figure 2D, all the tested human SAMHD1 variants were degraded by VpxROD. These results indicated that the tested SAMHD1 variants are not defective for their ability to be degraded by Vpx.
Ability of SAMHD1 variants to block HIV-1 infection

To evaluate the ability of SAMHD1 variants to block HIV-1 infection, we first stably expressed SAMHD1 variants in the human monocytic cell line U937, as previously described (Brandariz-Nunez et al., 2012; Brandariz-Nunez et al., 2013; White et al., 2013a; White et al., 2013b). U937 stably expressing the different SAMHD1-FLAG variants were differentiated by treatment with Phorbol 12-myristate 13-acetate (PMA) (Schwende et al., 1996), and the expression level of human SAMHD1-FLAG polymorphisms was determined by western blotting using anti-FLAG antibodies (Figure 3A).

Next, we tested the ability of the different SAMHD1 variants to restrict HIV-1 infection. For this purpose, we challenged PMA-treated U937 cells stably expressing the different SAMHD1 variants using an HIV-1 virus expressing GFP as a reporter of infection (HIV-1-GFP). Interestingly, all tested human SAMHD1 polymorphisms showed potent restriction of HIV-1 (Figure 3B). As a control, we challenged cells expressing the SAMHD1-HD206AA variant, which does not block HIV-1 infection, by using increasing amounts of HIV-1-GFP (Hrecka et al., 2011; Laguette et al., 2011). These results indicated that SAMHD1 variants do not change the ability of SAMHD1 to block HIV-1 infection.

Levels of cellular dNTPs in U937 cells stably expressing the different human SAMHD1 variants

Previous observations have suggested that SAMHD1 decreases the intracellular pool of deoxynucleotide triphosphates (dNTPs) (Goldstone et al., 2011; Kim et al., 2012; Lahouassa et al., 2012; Powell et al., 2011; White et al., 2013a). To test whether the indicated human SAMHD1 proteins are affected on their ability to decrease the cellular levels of dNTPs, we measured the levels of dATP, dTTP and dGTP in U937 cells stably expressing the different human SAMHD1 polymorphisms. As shown in Figure 4, all tested human SAMHD1 variants were able to decrease the cellular levels of dNTPs.

DISCUSSION

Although LINE-1 sequences comprise 17% of the human genome, the vast majority of LINE-1 elements (>99%) have accumulated mutations that render them transpositionally inactive (Beck et al., 2011; Hancks and Kazazian, 2012). Remarkably, only ~80–100 copies of LINE-1 are potentially active in the human genome (Beck et al., 2011; Hancks and Kazazian, 2012). Endogenous LINE-1 retrotransposition can result in genetic disorders which can be caused by LINE-1 mediated gene disruption, nucleotide deletions, duplications, and chromosomal instability through heterologous recombination (Hancks and Kazazian, 2012; Kazazian and Goodier, 2002). For example, LINE-1 activation in humans has been correlated with liver, colon and breast oncogenesis (Heikenwalder and Protzer, 2014; Patnala et al., 2014). Therefore, expression of SAMHD1 as a secondary level of protection against active LINE-1 elements in human cells is warranted. SAMHD1 is likely to represent one more among the many different intracellular mechanisms against the mobilization of LINE-1 elements such as cytidine deaminases, CpG methylation of promoters, TREX1, small RNA-based mechanisms and others (Hancks and Kazazian, 2012).
Interestingly, the ability of SAMHD1 to block HIV-1 infection and LINE-1 retrotransposition exhibit separate requirements. The ability of SAMHD1 to block HIV-1 infection requires SAMHD1’s enzymatic activity (Laguette et al., 2011; Lahouassa et al., 2012; Seamon et al., 2015). By contrast, the ability of SAMHD1 to block LINE-1 retrotransposition does not require SAMHD1’s enzymatic activity (Zhao et al., 2013). Here, we revealed another clue that will be important for teasing apart the mechanistic divide between these two restriction scenarios. One possibility is that SAMHD1 uses its sterile alpha motif (SAM) to interact with the RNA of complexes undergoing retrotransposition blocking the process by steric hindrance, which is in agreement with the fact that SAMHD1 variants that do not contain a SAM domain lost their ability to block LINE-1 retrotransposition (Zhao et al., 2013).

Although we sequenced SAMHD1 alleles from 80 Korean individuals, we were unable to document the previously described single nucleotide polymorphisms that results in the S33A change (Kim et al., 2009; Park et al., 2010). This might be due to a low frequency of this particular alleles. Either way this mutant provide a valuable tool for the separation of HIV-1 restriction from LINE-1 inhibition by SAMHD1.

Acknowledgments

We are grateful to Dr. Kyudong Han for helping us accessing the sequence database in Korea, and the sequencing of SAMHD1 in 80 Korean individuals. We are thankful to the NIH/AIDS repository program for providing valuable reagents such as antibodies and drugs. We would like to thanks Dr. John Goodier for sharing his protocols and antibodies against LINE-1 ORFp with us. This work was funded by NIH R01 AI087390, R21 AI102824, and R56 AI108432 grants to F.D.-G. Sara Sawyer was supported by an NIH grant R01-GM-093086 to S.S.

References


Figure 1. Modulation of LINE-1 retrotransposition by human SAMHD1 polymorphisms

(A) Schematic representation of the different episomal vectors used in the LINE-1 enhanced-GFP(EGFP)-based reporter assay. The reporter cassettes shown here are part of a circular episomal retrotransposition system. LINE-1, 99-PUR-RPS-EGFP (L1RP-EGFP) and JM111 (JM111) are shown. The 99-PUR-RPS-EGFP contains combined promoters from both CMV and the 5′-UTR of the LINE-1. An antisense cassette of EGFP containing an inserted intron sequence was cloned near the 3′ end of the L1 3′ UTR. JM111, the negative control for retrotransposition, contains the double mutation R261A/R262A in ORF1, which has been shown to abolish the retrotransposition activity of LINE-1. The occurrence of retrotransposition is detected by expression of EGFP in human HEK293T cells. 5′-UTR: 5′-untranslated region. 3′-UTR: 3′-untranslated region. ORF1: open reading frame 1. ORF2: open reading frame 2. eGFP: enhanced green fluorescent protein. CMV: cytomegalovirus promoter. (B) The ability of the indicated SAMHD1 variants to inhibit retrotransposition was measured by cotransfecting the LINE-1 reporter vector, 99 PUR RPS EGFP (L1RP-EGFP), together with the different FLAG-tagged SAMHD1 variants into HEK293T cells. Twenty four hours after transfection expression of FLAG-tagged SAMHD1 variants, ORF1p and GAPDH was measured using specific antibodies. Five days post-transfection, the occurrence of retrotransposition was determined by expression of EGFP measured by flow cytometry. As control, we have cotransfected the empty vector LPCX in HEK 293T cells as described (White et al., 2014). The defective LINE-1 construct, JM111, was used for determining the background levels of EGFP. The SAMHD1 mutant H123P was used as a positive control. Experiments were performed in triplicates and a standard deviation is shown.
Figure 2. Characterization of SAMHD1 polymorphisms

(A) Oligomerization of the different human SAMHD1 polymorphisms. Human 293T cells were cotransfected with a plasmid expressing wild type SAMHD1-HA with either plasmids expressing wild type or the indicated SAMHD1-FLAG variant. Cells were lysed 24 h after transfection and analyzed by Western blotting using anti-HA and anti-FLAG antibodies (Input). Subsequently, lysates were immunoprecipitated by using anti-FLAG agarose beads, as described (White et al., 2013b). Anti-FLAG agarose beads were eluted using FLAG peptide, and elutions were analyzed by Western blotting using anti-HA and anti-FLAG antibodies (IP). Similar results were obtained in three independent experiments and representative data is shown. WB, Western blot; IP, Immunoprecipitation; WT, wild type.

(B) Ability of SAMHD1 variants to bind nucleic acid. To this end, we tested the ability of SAMHD1 to bind the interferon-stimulatory DNA sequence containing a phosphorothioate backbone (ISD-PS), which is an RNA analog. Human 293T cells were transfected with plasmid expressing the indicated Human SAMHD1 polymorphisms. Lysed cells 24 h after transfection (Input) were incubated with StrepTactin Superflow affinity resin containing (+) or not (−) immobilized ISD-PS. Similarly, eluted proteins from the resin were visualized by
Western blotting using anti-FLAG antibodies (Bound). Similar results were obtained in three independent experiments and a representative experiment is shown. ISD-PS, interferon-stimulatory DNA sequence containing a phosphorothioate backbone; WB, Western blot; WT, wild type. (C) Intracellular distribution of human SAMHD1 variants in HeLa cells. HeLa cells expressing the indicated human SAMHD1 polymorphism were fixed and immunostained using antibodies against FLAG (red). Cellular nuclei were stained by using DAPI (blue). Similar results were obtained in three independent experiments and a representative experiment is shown. WT, wild type. (D) Vpx-dependent degradation of SAMHD1 variants. HeLa cells were cotransfected with plasmids expressing the indicated SAMHD1 variants and HA-tagged Vpx from HIV-2_ROD (Vpx_ROD) or SIV_RCM (Vpx_rcm). Thirty-six hours post-transfection cells were harvested, and the expression levels of SAMHD1 and Vpx were analyzed by Western blotting using anti-FLAG and anti-HA antibodies. As a loading control, cell extracts were Western blotted using antibodies against GAPDH. Similar results were obtained in three independent experiments and a representative experiment is shown.
Figure 3. Ability of human SAMHD1 polymorphisms to restrict HIV-1 infection

PMA-treated human monocytic U937 cells stably expressing the indicated human SAMHD1 polymorphisms (A) were challenged with increasing amounts of HIV-1-GFP (B). As a control, U937 cells stably transduced with the empty vector LPCX were challenged with HIV-1-GFP. Similar results were obtained in three independent experiments and a representative experiment is shown. WT, wild type.
Figure 4. Cellular dATP, dTTP and dGTP levels in U937 cells stably expressing the different SAMHD1 variants

Quantification of dATP, dTTP and dGTP levels on PMA-treated U937 cells expressing the indicated SAMHD1 variants was performed by a primer extension assay as previously described (White et al., 2013a). Similar results were obtained in three independent experiments and standard deviation is shown. WT, wild type.