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Modulation of LINE-1 retrotransposition by a human SAMHD1 polymorphism

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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.

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.

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2. Results

2.1. 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 ($L1_{RP}$-EGFP), which contains an EGFP reporter gene interrupted by an intron in the opposite transcriptional orientation (Fig. 1A). The EGFP cassette is inserted into the 3'UTR of a retrotransposition-component L1 ($L1_{RP}$). 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 (Fig. 1A). To control for background levels of retrotransposition in human cells, we used the same $L1_{RP}$-EGFP construct containing two missense mutations on ORF1(JM111) that abolish the retrotransposition activity of LINE-1 (Fig. 1A) (Moran et al., 1996). As shown in Fig. 1B, SAMHD1 variants S33D (phosphomimetic) and S33Y (bulky

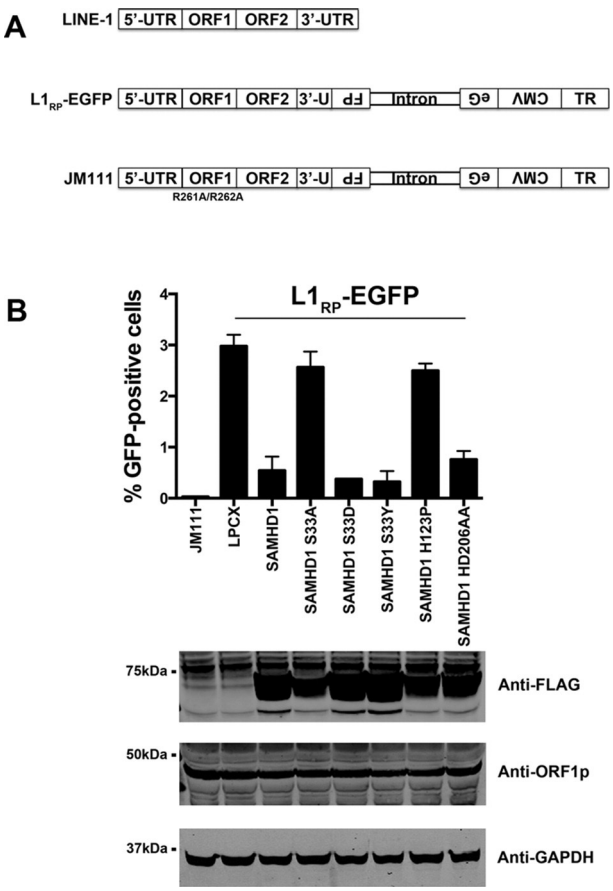

Fig. 1. Modulation of LINE-1 retrotransposition by human SAMHD1 polymorphisms. (A) Schematic representation of the different episonal vectors used in the LINE-1 enhanced-GFP(EGFP)-based reporter assay. The reporter cassetes shown here are part of a circular episomal retrotransposition system. LINE-1, 99-PUR-RPS-EGFP ($L1_{RP}$-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 ($L1_{RP}$-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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)