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Phosphorylation of mouse SAMHD1 regulates its restriction of human immunodeficiency virus type 1 infection, but not murine leukemia virus infection

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ABSTRACT

Human SAMHD1 (hSAMHD1) restricts HIV-1 infection in non-dividing cells by depleting intracellular dNTPs to limit viral reverse transcription. Phosphorylation of hSAMHD1 at threonine (T) 592 by cyclin-dependent kinase (CDK) 1 and CDK2 negatively regulates HIV-1 restriction. Mouse SAMHD1 (mSAMHD1) restricts HIV-1 infection in non-dividing cells, but whether its phosphorylation regulates retroviral restriction is unknown. Here we identified six phospho-sites of mSAMHD1, including T634 that is homologous to T592 of hSAMHD1 and phosphorylated by CDK1 and CDK2. We found that wild-type (WT) mSAMHD1 and a phospho-ablative mutant, but not a phospho-mimetic mutant, restricted HIV-1 infection in differentiated U937 cells. Murine leukemia virus (MLV) infection of dividing NIH3T3 cells was modestly restricted by mSAMHD1 WT and phospho-mutants, but not by a dNTPase-defective mutant. Our results suggest that phosphorylation of mSAMHD1 at T634 by CDK1/2 negatively regulates its HIV-1 restriction in differentiated cells, but does not affect its MLV restriction in dividing cells.

Introduction

Sterile α motif (SAM) and HD domain containing protein 1 (SAMHD1) is a deoxynucleotide triphosphohydrolase (dNTPase) (Goldstone et al., 2011; Powell et al., 2011) and functions as an HIV-1 restriction factor in non-dividing myeloid cells and resting $CD4^+$ T cells (Baldauf et al., 2012; Hrecka et al., 2011; Laguette et al., 2011a). Human SAMHD1 (hSAMHD1) inhibits infection of a wide range of retroviruses including HIV-1 and murine leukemia virus (MLV) (Gramberg et al., 2013), and DNA viruses, such as herpes simplex virus type 1 in human macrophages (Kim et al., 2013). The dNTPase activity of SAMHD1 enables it to decrease intracellular dNTPs to below the levels required for retroviral replication (Lahouassa et al., 2012), a general mechanism by which hSAMHD1 impedes viral infection (Wu, 2013).

SAMHD1 is a conserved protein in humans and mice (Yang et al., 2014). The mouse *SAMHD1* gene contains two start codons, and it is possible that both start codons are used for translation *in vivo*. Alternative splicing of the mSAMHD1 pre-mRNA results in two isoforms of the protein (isoform 1 and 2), which share 72% and 74% protein sequence identities with hSAMHD1. Both mSAMHD1 isoforms possess dNTPase activity (Lahouassa et al., 2012; Powell et al., 2011; Zhang et al., 2014), and isoform 1 mRNA is more abundantly expressed than isoform 2 mRNA in various mouse tissues (Zhang et al., 2014). Compared to hSAMHD1 wild-type (WT) or phospho-ablative mutants, phospho-mimetic mutants at residue T592 lose their HIV-1 restriction phenotype in non-dividing cells (Cribier et al., 2013; Pauls et al., 2014; St Gelais et al., 2014; Welbourn et al., 2013; White et al., 2013b). It has been proposed that T592 phosphorylation of hSAMHD1 negatively regulates its RNase activity in cells and may affect HIV-1 restriction (Ryoo et al., 2014). These studies suggest that T592 phosphorylation of hSAMHD1 plays a role in regulating its HIV-1 restriction function. However, there is no evidence linking the phosphorylation of mSAMHD1 to its retroviral restriction

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phenotype. It is also unknown whether mSAMHD1 and hSAMHD1 restrict HIV-1 and MLV through the same mechanism.

Here, we aim to elucidate the contribution of mSAMHD1 phosphorylation to its retroviral restriction function in cells. We identified that T634 is a phosphosite of mSAMHD1 isoform 1 (refer to as mSAMHD1 unless isoform 2 is indicated) and demonstrated that CDK1 and CDK2 phosphorylate mSAMHD1 at T634 in dividing cells. We examined the effect of T634 phosphorylation on mSAMHD1-mediated restriction of HIV-1 or MLV in human and mouse cell lines stably expressing mSAMHD1 WT, phospho-ablative, phospho-mimetic or dNTPase-defective mutants. We found that T634 phosphorylation of mSAMHD1 regulates its HIV-1 restriction in differentiated human U937 cells. In dividing mouse NIH3T3 cells, overexpression of mSAMHD1 or hSAMHD1 modestly restricts MLV infection, which is independent of T634 phosphorylation of mSAMHD1. Our results demonstrate that phosphorylation of mSAMHD1 modulates its restriction of HIV-1 infection in non-dividing cells, but not MLV infection in dividing cells, suggesting different mechanisms of regulating retroviral restriction by hSAMHD1 and mSAMHD1.

Results

Identification of phosphorylation sites of mSAMHD1 protein

Mouse SAMHD1 was identified as a phosphoprotein in previous large-scale analyses of phosphorylated proteins (Sweet et al., 2009; Villen et al., 2007; Zanivan et al., 2008). The C-terminal protein sequences of human and mouse SAMHD1 are highly conserved (Yang et al., 2014), and contain the residue T592 in hSAMHD1 and a predicted phosphosite at position T634 in mSAMHD1 isoform 1 with the first start codon aligning with the start codon of hSAMHD1 (Cribier et al., 2013; Villen et al., 2007). In non-dividing cells, WT hSAMHD1 and a phospho-ablative mutant (T592A) restrict HIV-1 infection, while phospho-mimetic mutants of hSAMHD1 lose HIV-1 restriction (Cribier et al., 2013; Welbourn et al., 2013; White et al., 2013b), suggesting that phosphorylation negatively affects the HIV-1 restriction function of hSAMHD1. We thus questioned whether phosphorylation at T634 could be a negative regulatory mechanism for mSAMHD1-mediated restriction of retroviral infection. Given that mSAMHD1 isoform 1 is more abundantly expressed compared to isoform 2, in multiple tissues (Zhang et al., 2014), we focused our study on mSAMHD1 isoform 1.

To identify phosphosites of mSAMHD1, tandem mass spectrometry (MS/MS) was performed with phosphorylation modification analysis after immunoprecipitation of full-length mSAMHD1 isoform 1 (St Gelais et al., 2014). A total of 678 peptides were assigned to mSAMHD1 providing 72% coverage of the protein. Six phosphorylated residues were identified including threonines 52, 56, 310, and 634, and serines 55 and 140 (Table 1). Fig. 1 shows a representative MS/MS spectrum for the peptide that allowed identification of T634 phosphorylation of mSAMHD1 isoform 1. There were 36 peptides identified covering this region, of which 17 corresponded to phosphorylated T634, providing strong confidence in phospho-modification of this residue. Interestingly, based on sequence alignment of mSAMHD1 and hSAMHD1 (Fig. 2A), T634 is homologous to the known T592 phosphosite of hSAMHD1 that negatively regulates hSAMHD1-mediated HIV-1 restriction (Cribier et al., 2013; St Gelais et al., 2014; Welbourn et al., 2013; White et al., 2013b).

Table 1
Mapping mouse SAMHD1 phosphorylation sites by mass spectrometry.

	% Residues mapped [a]	Phosphorylation sites [b]
Serines (S)	72% (26/36)	S55, S140
Threonines (T)	83% (20/24)	T52, T56, T310, T634
Total residues	72% (476/658)	Indicated above

[a] The percentages of covered residues over total residue in MS/MS data.
[b] Residue positions of phosphorylated serines (S) and threonines (T) were identified by MS/MS analysis.

To confirm the phosphorylation status of mSAMHD1 isoform 1 at T634 in cells, we generated a phospho-specific SAMHD1 antibody, whose epitope targets the consensus C-terminal sequences of hSAMHD1 and mSAMHD1 encompassing the phospho-sites T592 and T634. Thus, the antibody can specifically detect T592 phosphorylated hSAMHD1 and T634 phosphorylated mSAMHD1. We generated two mSAMHD1 isoform 1 mutants substituting threonine with a phospho-ablative alanine residue (T634A) or phospho-mimetic aspartic acid residue (T634D). Transient overexpression of mSAMHD1 WT, T634A or T634D in HEK293T cells was detected by immunoblotting. Total hSAMHD1 was detected at 72 kDa, while mSAMHD1 WT and mutants were detected at 76 kDa, as expected (Fig. 2B). Phosphorylation of hSAMHD1 at T592 and mSAMHD1 isoform 1 at T634 was detected using the specific phospho-SAMHD1 antibody. As expected, phospho-ablative and phospho-mimetic mutants of mSAMHD1 isoform 1 had no detectable signal when using the specific phospho-SAMHD1 antibody (Fig. 2B). Moreover, to test whether the endogenous mSAMHD1 is also phosphorylated, we performed immunoblotting with lysates from mouse fibroblast NIH3T3 cells and mouse embryonic fibroblasts (MEFs). Specific SAMHD1 bands were detected in MEFs using a SAMHD1 antibody and a phospho-SAMHD1 antibody (Fig. 2C), suggesting that endogenous mSAMHD1 is also phosphorylated at T634 in primary mouse fibroblasts. In contrast, a very low level of endogenous mSAMHD1, but not the T634 phosphorylated protein, was detected in NIH3T3 cells (Fig. 2C), which might be due to detection limit of the phospho-SAMHD1 antibody.

CDK1 and CDK2 contribute to T634 phosphorylation of mSAMHD1 in HEK293T cells

The CDK1/CDK2 and cyclin A2 complex phosphorylates hSAMHD1 at position T592 in dividing cells (Cribier et al., 2013; Pauls et al., 2014; St Gelais et al., 2014; White et al., 2013b). Our previous results showed that both hSAMHD1 and mSAMHD1 interact with CDK1 and CDK2 in HEK293T cells (St Gelais et al., 2014). Similar to T592 in hSAMHD1, T634 of mSAMHD1 is located within a predicted CDK-binding motif, TPXK (Cribier et al., 2013) (Fig. 2A); therefore, it is possible that CDK1 and CDK2 could be responsible for the phosphorylation of mSAMHD1 at T634.

To determine whether CDK1 and CDK2 contribute to phosphorylation of mSAMHD1 at T634, we first inhibited the kinase activities of CDK1 and CDK2 using specific inhibitors. HEK293T cells were pretreated with specific inhibitors for 6 h, and transfected to transiently express mSAMHD1 WT. Cell lysates were collected 24 h post-transfection and used for immunoblotting to determine the levels of total and phospho-SAMHD1. To evaluate changes in the level of phospho-mSAMHD1, the relative level of phospho/total mSAMHD1 was calculated after GAPDH normalization based on densitometry analysis of these protein bands. Compared to control cells treated with DMSO, individual and combined treatments with inhibitors to CDK1 and CDK2 reduced the relative levels of phospho-mSAMHD1 by 62% ($P < 0.01$), 34% ($P < 0.05$), and 66% ($P < 0.01$), respectively (Fig. 3A and B). These results suggest that both CDK1 and CDK2 phosphorylate mSAMHD1 at T634 in dividing human cells.

To exclude the potential off-target effect of using specific inhibitors to CDK1 and CDK2, we also used well-characterized