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Interaction of the phospholipid scramblase 1 with HIV-1 Tat results in the repression of Tat-dependent transcription

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ABSTRACT

Human phospholipid scramblase 1 (PLSCR1) is an interferon (IFN)-stimulated gene and possesses an IFN-mediated antiviral function. We show here that PLSCR1 directly interacts with human immunodeficiency virus type-1 (HIV-1) Tat. This interaction occurs both *in vitro* and *in vivo* through amino acids 160–250 of PLSCR1. Overexpression of PLSCR1 efficiently represses the Tat-dependent transactivation of the HIV-1 long terminal repeat (LTR) and reduces the nuclear translocation of Tat. In addition, shRNA-mediated suppression of endogenous PLSCR1 expression enhances the levels of *gag* mRNA in an HIV-1-infected T-cell line. These findings indicate that PLSCR1 negatively regulates the Tat-dependent transactivation of the HIV-1 LTR during HIV-1 infection.

1. Introduction

Human phospholipid scramblase 1 (PLSCR1) expression is robustly induced in response to interferon (IFN) treatment and viral infection [1,2]. In addition, PLSCR1 is necessary for the IFN-dependent induction of IFN-stimulated gene expression and antiviral activity, and PLSCR1 is suggested to play an important role in the IFN-mediated antiviral response [2–4]. Recently, we identified that PLSCR1 specifically interacts with human T-cell leukemia virus type-1 (HTLV-1) Tax and represses Tax-mediated transactivation [5]. The interaction of PLSCR1 with viral transcription factors may play an important role for PLSCR1-mediated antiviral activity.

Human immunodeficiency virus type-1 (HIV-1) encodes a small transactivator protein called Tat, which is essential for activating transcription from the HIV-1 long terminal repeat (LTR) [6]. Tat is synthesized as a protein of 72 to 101 amino acids, depending on the viral strain and the alternative splicing of the mRNA. The form of Tat that contains the first exon, which includes the first 72 amino acids, is sufficient to activate transcription from the LTR [7]. Tat is essential for efficient transcription of the provirus and for HIV-1 replication [8]. To stimulate efficient transcriptional elongation, Tat recruits positive transcription elongation factor b (P-TEFb) to the 5′ end of the nascent viral transcript through the interaction with the cyclin T1 subunit of P-TEFb. This viral transcript forms a stem–loop RNA structure called the trans-acting response (TAR) RNA element [6]. Once recruited to the TAR RNA element, P-TEFb phosphorylates RNA polymerase II and stimulates the transition from abortive to productive elongation by RNA polymerase II [6].

In this report, we show that through its amino acids 160–250, PLSCR1 directly and specifically interacted with Tat. Overexpression of PLSCR1 repressed the Tat-dependent transactivation of the HIV-1 LTR and reduced the nuclear translocation of Tat. In addition, shRNA-mediated suppression of endogenous PLSCR1 expression enhanced the levels of *gag* mRNA in an HIV-1-infected T-cell line. These findings indicate that PLSCR1 negatively regulates the Tat-dependent transactivation of the HIV-1 LTR during HIV-1 infection by reducing the nuclear localization of Tat.

2. Materials and methods

2.1. Materials

The HIV-1 Tat cDNA encoding pSV2Tat72 and the HIV-1 3′LTR encoding pBlue3′LTR-Luc were kindly provided by the National Institute of Health AIDS Research and Reference Reagent Program. An antibody to histone H1 was purchased from Upstate. Other materials were obtained as previously described [5].

2.2. Construction of plasmids

PLSCR1 and its mutant expression plasmids were constructed as previously described [5]. The Tat cDNA, which encodes amino acids 1–72, was amplified by PCR from pSV2Tat72 and cloned into pcDNA3 with two HA epitopes and one FLAG epitope at the C-terminus to produce Tat-HA2 and Tat-FG, respectively. Tat cDNA encoding amino acids 2–72 was amplified by PCR and cloned into

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pET32a(+) to produce pET-S-Tat. The HIV-1 3′LTR was amplified by PCR from pBlue3′LTR-Luc and cloned into TAL-pGL4.10 [9] to produce 3′LTR-TAL-pGL4.10.

2.3. Bacterial protein expression and pull-down assay

Bacterial protein expression and pull-down assay were performed as previously described [5]. Immunoblotting (IB) was performed using the indicated antibodies as previously described [5].

2.4. Cell culture and transfection

COS-1 cells were maintained as previously described [5]. COS-1 cells (6×10^5, 5×10^4 and 9×10^3) were plated in 60 mm cell culture plates for pull-down assays and subcellular fractionation, each well of a 24-well plate for a luciferase assay and an 8-well chamber slide for immunofluorescence analysis, respectively, and the indicated amounts of DNA were transfected using GeneJuice (Novagen). HIV-1-infected MOLT-4 (MOLT/HIV) cells [10] were maintained in RPMI-1640 supplemented with 10% FBS. MOLT/HIV cells were electroporated using Ingenio Electroporation Solution (Mirus) as previously described [5].

2.5. Pull-down assay using transfected cell lysates

48 h after transfection, cells were harvested and pull-downs were performed using anti-FLAG M2 beads and anti-myc prebound beads to precipitate FLAG epitope-tagged Tat and three myc epitope-tagged PLSCR1, respectively, as previously described [5,11].

2.6. Luciferase assay

The luciferase assay was performed using the Dual-Luciferase Reporter Assay System (Promega) as previously described [9]. The PCR reaction contained 1 μl of 5-fold dilution of each cDNA reaction mix and primers for human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) [11] or HIV-1 *gag* (5′-AAGGGGAAGTGACATAGCAG-3′ and 5′-GGACCAACAAGGTTTCTGTC-3′). To collect PCR products at the linear range, the number of PCR cycles was optimized for each primer set. PCR was performed using Hybripol DNA polymerase (Bioline) and the following protocol: for G3PDH, 22 cycles of 94 °C for 30 s, 64 °C for 30 s, 72 °C for 20 s; for HIV-1 *gag*, 25 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 15 s.

2.7. Immunofluorescence analysis

The immunofluorescence analysis was performed as previously described [9].

2.8. Subcellular fractionation

The subcellular fractionation was performed as previously described [5].

3. Results

3.1. PLSCR1 interacts with HIV-1 Tat both in vitro and in vivo

We previously reported that PLSCR1 is a cellular target of the hypothetical EBV protein RK-BARF0 [12]. Other target proteins of RK-BARF0, such as human I-mfa domain-containing protein, Guranulins, and Notch, are known to interact with HIV-1 Tat [7,8,13,14]. Thus, it is possible that RK-BARF0 and Tat target the same cellular proteins. To determine if an interaction exists between PLSCR1 and Tat, amino acids 2–72 of Tat was tagged with trxA and S epitopes (pET-S-Tat) and full-length PLSCR1 encoding 1–318 amino acids was tagged with three myc epitopes (pTE-3M-PLSCR1) (Fig. 1A). These constructs were expressed in *Escherichia coli*. Cell lysates were mixed and pull-downs were performed using S-protein beads to precipitate Tat. Immunoblot analysis of the Tat-containing complexes revealed that Tat efficiently co-precipitated with PLSCR1 (Fig. 1B, lane 8), whereas the empty vector-transformed bacterial lysates precipitated only a trace amount of PLSCR1 (Fig. 1B, lane 6). This result indicated that PLSCR1 directly interacts with Tat in vitro.

To confirm this interaction *in vivo*, amino acids 1–72 of Tat tagged with the FLAG epitope (Tat-FG) was expressed in COS-1 cells in the presence of IFN-α-2b. Immunoblot analysis of total cell lysates indicated that the basal expression levels of PLSCR1 were significantly lower in the absence of IFN in COS-1 cells; however, PLSCR1 expression was markedly induced in the presence of IFN-α-2b and the levels of IFN-α-2b-induced expression of PLSCR1 were identical in the presence and absence of Tat (Fig. 1C, lanes 2 and 3), which is consistent with previous observations [5]. Interestingly, immunoblot analysis of the Tat-precipitated complexes revealed that PLSCR1 was efficiently co-precipitated with Tat, but no PLSCR1 was detected in empty vector-transfected complexes in the presence of IFN-α-2b (Fig. 1C, lanes 5 and 6). This result indicated that IFN-induced endogenous PLSCR1 interacts with Tat *in vivo*.

3.2. Amino acids 160–250 of PLSCR1 contains two binding regions for Tat

PLSCR1 contains several functional domains including the three predicted intrinsically disordered (ID) regions [5] and the amino-terminal ID region, which is involved in the interaction with target proteins [5,15]. To identify which regions of PLSCR1 are involved in this interaction, full-length PLSCR1 (3 M-PLSCR1) or truncated mutants encoding amino acids 1–163 (3M-PLSCR1(1–163)), 158–318 (3M-PLSCR1(158–318)), or 160–250 (3M-PLSCR1(160–250)) were tagged with three myc epitopes and co-expressed in COS-1 cells with amino acids 1–72 of the Tat tagged with two HA epitopes (Tat-HA2). Immunoblot analysis of the total cell lysates indicated that all constructs were expressed at similar levels (Fig. 2A, lanes 2–6). However, immunoblot analysis of the PLSCR1-containing complexes revealed that PLSCR1, PLSCR1(158–318) and PLSCR1(160–250) efficiently co-precipitated with Tat, while PLSCR1(1–163) did not (Fig. 2A, lanes 9–12). These observations demonstrated that amino acids 160–250 of PLSCR1 are required for this interaction *in vivo*.

Amino acids 160–250 of PLSCR1 have been suggested to contain a short ID region (amino acids 166–176) [5]. To identify whether this ID region of PLSCR1 is involved in its interaction with Tat, plasmids were expressed in *E. coli* containing the following PLSCR1 mutants tagged with three myc epitopes: amino acids 160–250 (3M-PLSCR1(160–250)), 160–205 (3M-PLSCR1(160–205)) and 200–250 (3M-PLSCR1(200–250)). Lysates from bacteria expressing S-Tat and 3M-PLSCR1(160–250), 3M-PLSCR1(160–205) or 3M-PLSCR1(200–250) were mixed, and pull-downs were performed using S-protein beads to precipitate Tat. Immunoblot analysis of the Tat-containing complexes revealed that all PLSCR1 mutants were co-precipitated with Tat (Fig. 2B, lanes 12–14). However, the precipitated level of PLSCR1(200–250) was significantly lower than that of PLSCR1(160–250) and PLSCR1(160–205) (Fig. 2B, lanes 12–14). This observation indicated that amino acids 160–250 of PLSCR1 contain two binding regions for Tat and amino acids 160–205 of PLSCR1, which have been suggested to contain a short ID region, must be the favorable Tat binding site.

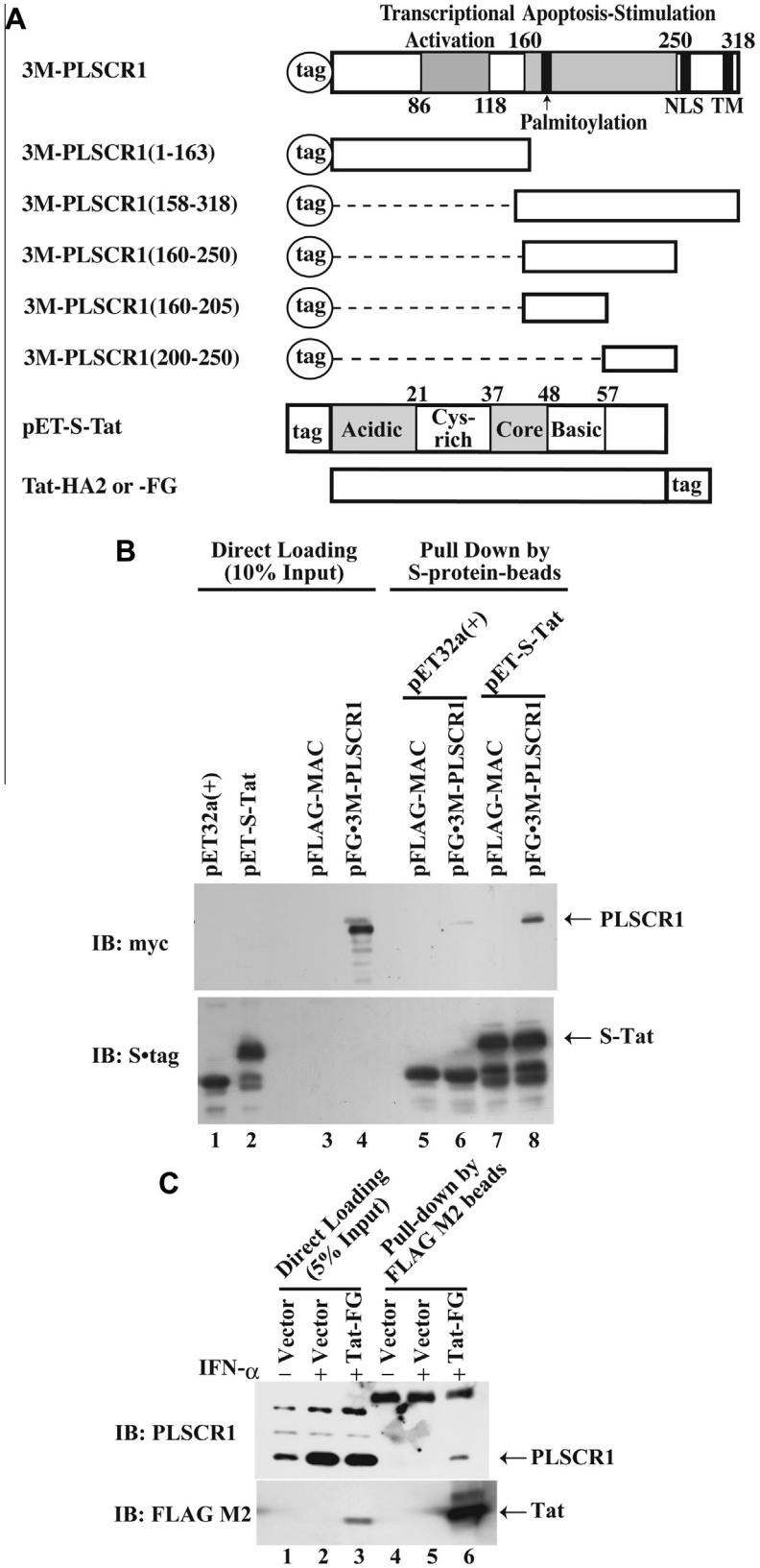

Fig. 1. PLSCR1 directly interacts with HIV-1 Tat *in vitro* and *in vivo*. (A) Schematic representation of PLSCR1, Tat and mutant expression constructs containing epitope-tags. The structural motifs of PLSCR1 are previously described [5] and Tat is indicated as follows: Acidic, acidic domain; Cys-rich, cysteine-rich region; Core, core domain; Basic, basic domain. (B) Bacterial lysates were mixed and incubated with S-protein beads to precipitate Tat as follows: 20 μg of pET32a(+) or pET-S-Tat lysates and 20 μg of pTriEx-3-Hyg or pTE-3 M-PLSCR1 lysates. Following pull-down, 2 μg of the bacterial lysates and the precipitated complexes were divided into two portions and subjected to SDS–PAGE. Immunoblotting (IB) was performed with an anti-S•tag antibody for Tat or with an anti-myc antibody for PLSCR1. (C) COS-1 cells were transfected with 5 μg of pcDNA3 or Tat-FG. Twenty-four hours after transfection, cells were treated with 2000 units/ml of IFN-α-2b for 16 h. A total of 500 μg of total cell lysate was incubated with anti-FLAG M2 beads to precipitate Tat. Following pull-down, 10 μg of total cell lysates and the precipitated complexes were subjected to SDS–PAGE. IB was performed with an anti-PLSCR1 antibody for PLSCR1 or with an anti-FLAG M2 antibody for Tat.

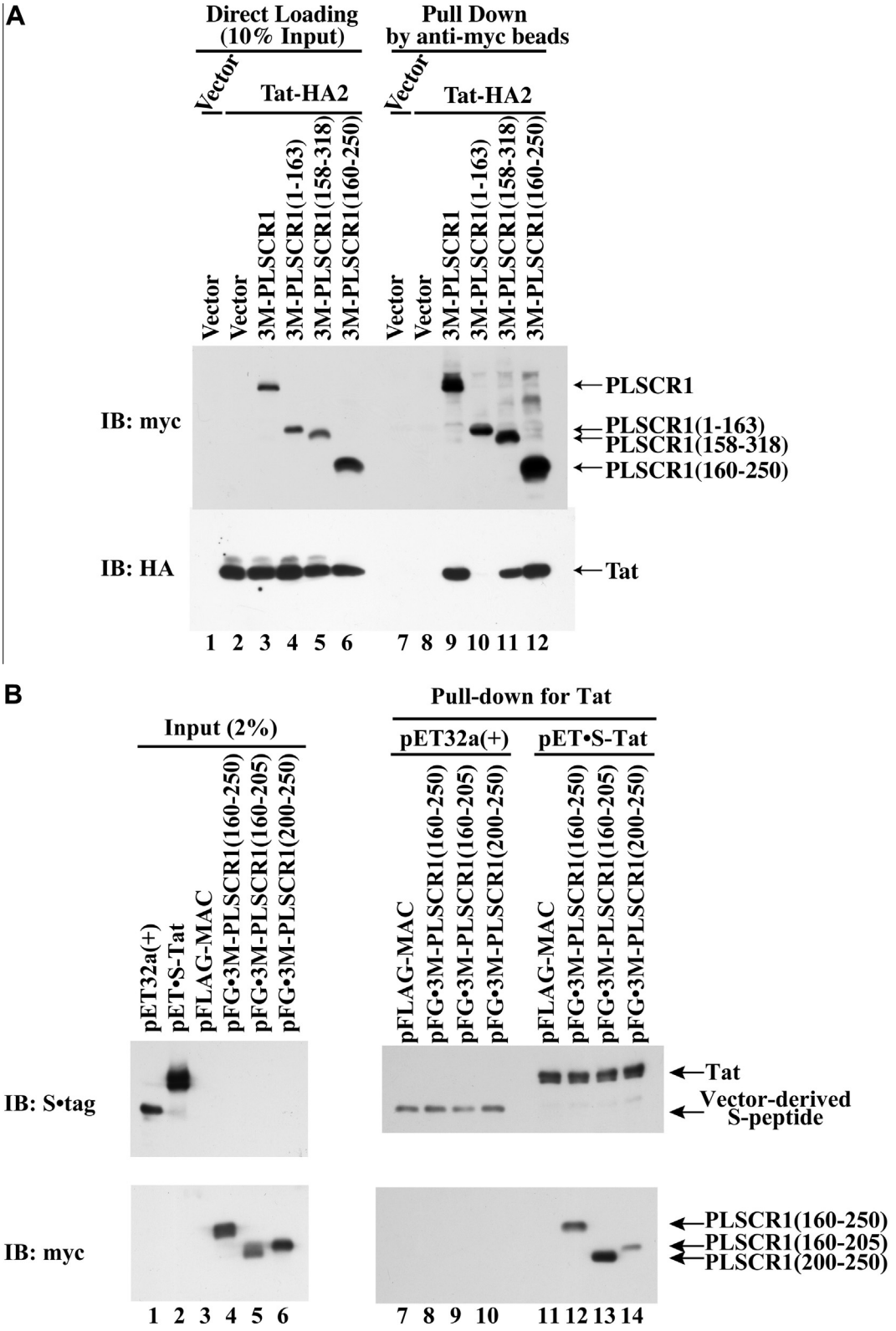

Fig. 2. PLSCR1 interacts with HIV-1 Tat through its 160–250 amino acid region. (A) COS-1 cells were transfected with 1 μg of pcDNA3 or Tat-HA2 and 3 μg of pcDNA3, 3 M-PLSCR1, 3M-PLSCR1(1–163), 3M-PLSCR1(158–318) or 3M-PLSCR1(160–250). A total of 300 μg of total cell lysate was incubated with anti-myc antibody-prebound beads to precipitate PLSCR1 and mutants. Following pull-down, 30 μg of total cell lysate and the precipitated complexes were subjected to SDS–PAGE. IB was performed with an anti-HA antibody for Tat or with an anti-myc antibody for PLSCR1 and mutants. (B) Bacterial lysates were mixed and incubated with S-protein beads to precipitate Tat as follows: 20 μg of pET32a(+) or pET-S-Tat lysates and 20 μg of pTriEx-3-Hyg, pTE-3M-PLSCR1(160–250), pTE-3M-PLSCR1(160–205) or pTE-3M-PLSCR1(200–250) lysates. Following pull-down, 2 μg of the bacterial lysates and the precipitated complexes were subjected to SDS–PAGE. IB was performed with an anti-S•tag antibody for Tat or with an anti-myc antibody for the PLSCR1 mutants.

3.3. PLSCR1 represses the Tat-dependent transactivation of the HIV-1 LTR

To elucidate the functional relevance of the PLSCR1•Tat complex on Tat-mediated transactivation of the HIV-1 LTR, we first determined the activity of a luciferase-reporter plasmid under the control of the HIV-1 3′LTR (3′LTR-TAL-pGL4.10) in the presence of Tat-HA2 and 3M-PLSCR1 in COS-1 cells. In the presence of Tat, luciferase activity was increased approximately 23-fold in the absence of PLSCR1 expression (Fig. 3A, lane 4). PLSCR1 expression efficiently decreased this Tat-mediated transactivation in a dose-dependent manner (Fig. 3A, lanes 5 and 6). To identify whether PLSCR1-binding to Tat is sufficient for this repression, we next determined the luciferase activity of 3'LTR-TAL-pGL4.10 in COS-1 cells containing empty vector or Tat-HA2 and either 3M-PLSCR1 or 3M-PLSCR1(160–250). In the presence of Tat, the luciferase activity was increased approximately 4.5-fold (Fig. 3B, lane 5). PLSCR1 efficiently decreased this Tat-mediated transactivation to approximately 50% (Fig. 3B, lanes 7 and 8). However, PLSCR1(160–250) did not repress the luciferase activity even when an excess amount of protein was expressed by transfecting 10 times more DNA (Fig. 3B, lanes 5–8). These observations revealed that the Tat-binding region of PLSCR1 (amino acids 160–250) is not sufficient to repress the Tat-dependent transactivation of the HIV-1 LTR; rather, this repression requires full-length PLSCR1.

To confirm this PLSCR1-mediated repression of the Tat-dependent transcription from the HIV-1 LTR, we next asked whether PLSCR1 repressed Tat-mediated transcription in a HIV-1-infected T-cell line. HIV-1-infected MOLT-4 (MOLT/HIV) cells were transfected with a PLSCR1-specific shRNA plasmid or a non-target shRNA plasmid [5]. Immunoblot analysis of total cell lysates revealed that PLSCR1 was highly expressed in non-target shRNA-transfected cells and PLSCR1-specific shRNA transfection significantly repressed PLSCR1 expression (Fig. 3C). Semi-quantitative RT-PCR showed that the levels of G3PDH mRNA were almost identical in the non-target shRNA- and PLSCR1-specific shRNA-transfected cells (Fig. 3C). However, the levels of HIV-1 *gag* mRNA were efficiently increased by 2.4-fold in the PLSCR1-specific shRNA-transfected cells. These data also indicated that PLSCR1 expression repressed the HIV-1 transcription in an HIV-1-infected T-cell line.

3.4. PLSCR1 stabilizes Tat in the cytoplasm

PLSCR1 is localized at the plasma membrane and in various internal membrane pools; however, PLSCR1 also localizes to the nucleus in response to cytokine stimulation [16]. Tat has been reported to primarily localize in the nucleus [8]. To further investigate this protein distribution, we assessed the intracellular location of Tat and PLSCR1 using immunofluorescence analysis. Consistent with previous observations, Tat was primarily detected in the nucleus when PLSCR1 was absent [8]; however, PLSCR1 and PLSCR1(160–250) were found throughout the cytoplasm and nucleus (Fig. 4A) [5]. Interestingly, in cells co-expressing Tat and PLSCR1, Tat was efficiently detected throughout the cytoplasm and nucleus, and this cytoplasmic stabilized Tat was co-localized with PLSCR1 (Fig. 4B). However, the nuclear localization pattern

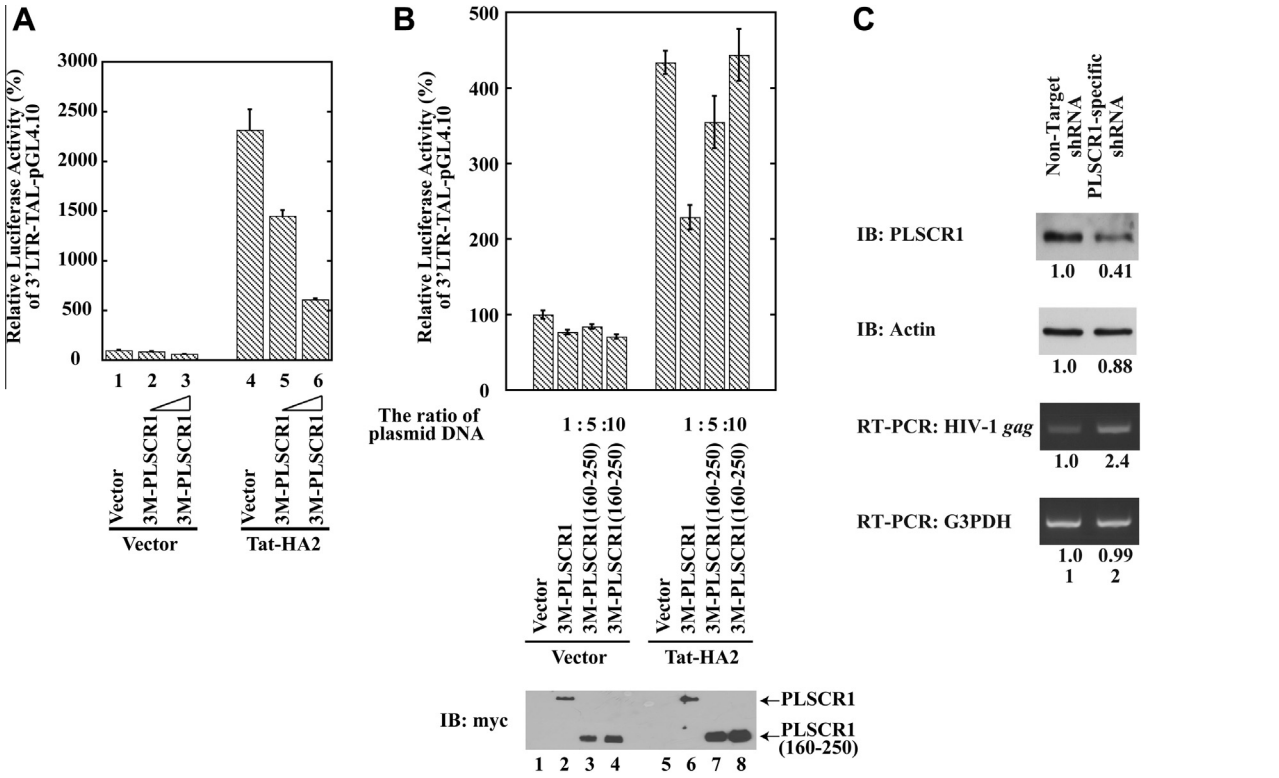

Fig. 3. Overexpression of PLSCR1 represses the Tat-mediated transactivation of the HIV-1 LTR. (A) COS-1 cells were transfected with 100 ng of 3′LTR-TAL-pGL4.10, 50 ng of pTAL-pGL4.70, 2.5 ng of pcDNA3 or Tat-HA2 and 20 or 100 ng of pcDNA3 or 3M-PLSCR1. The total amount of plasmid transfected was equalized by the addition of pcDNA3. After 48 h of transfection, cells were lysed and luciferase activity was determined. The firefly luciferase/*Renilla* luciferase activity ratio of cells transfected with vector only was set as 100%. The data represent the average relative values from three experiments, and the error bars indicate standard deviations. (B) COS-1 cells were transfected with 100 ng of 3′LTR-TAL-pGL4.10, 50 ng of pTAL-pGL4.70, 1.0 ng of pcDNA3 or Tat-HA2 and 50 ng of 3M-PLSCR1 or 250 or 500 ng of 3M-PLSCR1(160–250). The total amount of plasmid transfected was equalized by the addition of pcDNA3. Following 48 h of transfection, luciferase activity was determined as in panel A. The expression levels of transfected PLSCR1 and mutant were monitored by IB using 1/10 of the volume of the total cell lysates. (C) HIV-1-infected MOLT-4 cells were electroporated with 10 μg of non-target shRNA control (SHC002) or PLSCR1-specific shRNA (TRCN56271). 4 μg of total cell lysates were prepared and subjected to SDS–PAGE. IB was performed with an anti-PLSCR1 antibody for PLSCR1 and an anti-actin antibody for actin. RT-PCR products were subjected to agarose gel electrophoresis, and DNA bands were visualized using SYBR Safe DNA gel stain (Invitrogen). The intensity of the IB and RT-PCR products were quantified using ImageJ software.

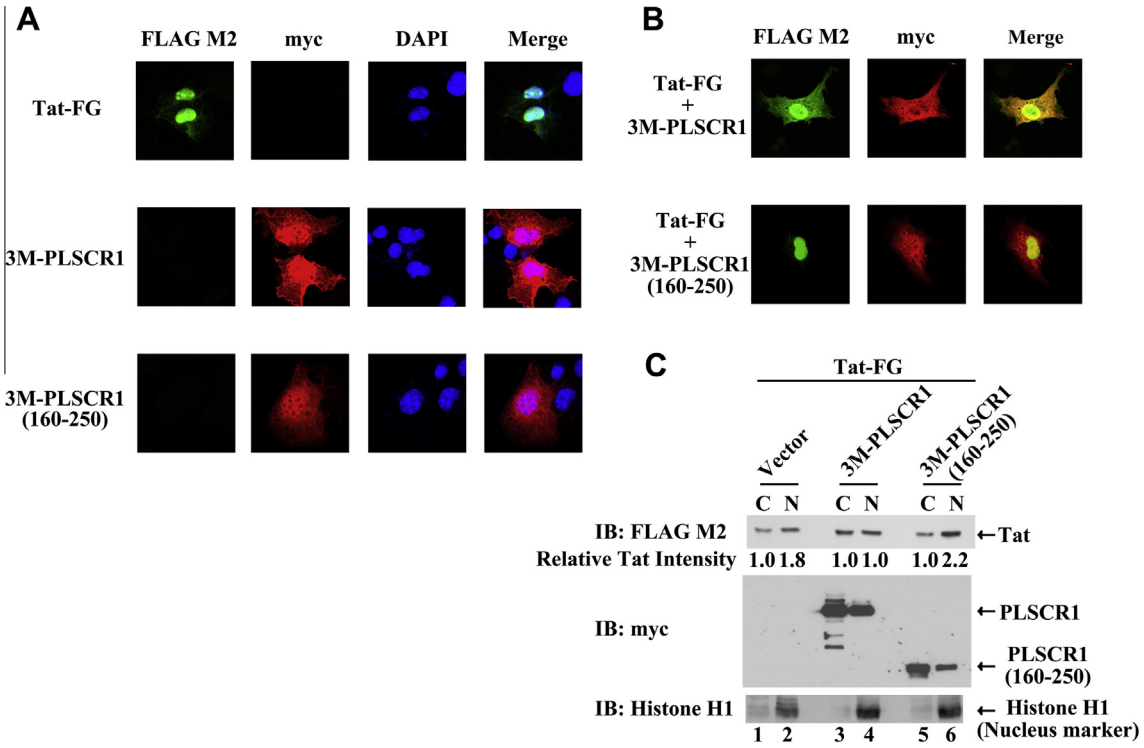

Fig. 4. PLSCR1 reduces the nuclear localization of Tat. (A) COS-1 cells were transfected with 50 ng of Tat-FG or 150 ng of 3M-PLSCR1 or 3M-PLSCR1 (160–250). The cells were stained using an anti-FLAG M2 antibody for Tat (green) and an anti-myc antibody for PLSCR1 or its mutant (red). The cell nuclei were stained with DAPI. DAPI and anti-FLAG M2 antibody or anti-myc antibody images were merged using Olympus FLUOVIEW software. (B) COS-1 cells were transfected with 50 ng of Tat-FG and 150 ng of 3M-PLSCR1 or 3M-PLSCR1 (160–250). Staining and acquisition of the images were performed as in panel A. Yellow indicates co-localization of the two proteins. (C) COS-1 cells were transfected with 0.5 μg of Tat-FG and 0.5 μg of 3M-PLSCR1 or 2 μg of 3M-PLSCR1(160–250). The total amount of plasmid transfected was equalized by the addition of pcDNA3. Subcellular fractionation was carried out as described in Section 2. An equal volume of the cytoplasmic and nuclear fractions was subjected to SDS–PAGE. IB was performed with an anti-FLAG M2 antibody for Tat, an anti-myc antibody for PLSCR1 or its mutant, and an anti-histone H1 for histone H1 (as a nuclear marker). The levels of Tat in the cytoplasmic fraction were set as 1.0 for each transfected samples. The intensity of IB products were quantified using ImageJ software. C, cytoplasmic fraction. N, nuclear fraction.

of Tat was not affected by the presence of PLSCR1(160–250) (Fig. 4B). This observation suggested that the full-length PLSCR1 stabilizes Tat in the cytoplasm.

To confirm this PLSCR1-mediated cytoplasmic stabilization of Tat using subcellular fractionation, COS-1 cells were transfected with Tat-FG and either 3M-PLSCR1 or 3M-PLSCR1(160–250). Immunoblot analysis of the cytoplasmic and nuclear fractions indicated that histone H1 was present exclusively in the nuclear fractions, which demonstrated the accuracy of the fractionation (Fig. 4C, bottom panel). PLSCR1 and PLSCR1(160–250) were efficiently detected in the cytoplasmic and nuclear fractions, and similar levels of the expression of both proteins were observed (Fig. 4C, middle panel). The levels of Tat in the nuclear fraction were observed to be approximately 2-fold higher than that in the cytoplasmic fraction in the absence of PLSCR1 (Fig. 4C, top panel). Interestingly, the expression of full-length PLSCR1 expression resulted in a shift in the localization of Tat from the nucleus to the cytoplasm at equal levels (Fig. 4C, top panel). However, PLSCR1(160–250) expression did not affect the levels of Tat in the cytoplasmic and nuclear fractions (Fig. 4C, top panel). This observation is consistent with the results from our immunofluorescence analysis, demonstrating that only full-length PLSCR1 stabilizes Tat in the cytoplasm. These observations confirmed that PLSCR1 interacts with Tat *in vivo* and that full-length PLSCR1 is required for the cytoplasmic stabilization of Tat.

4. Discussion

This work revealed for the first time that PLSCR1 interacts directly with HIV-1 Tat *in vitro* and *in vivo* (Fig. 1) and those amino acids 160–205 and 200–250 of PLSCR1 are involved in this interaction (Fig. 2). Interestingly, the 160–205 amino acid region of PLSCR1, which contains a short ID region (amino acids 166–176) [5], exhibited a greater affinity to Tat than that of the 200–250 amino acid region (Fig. 2). The ID region confers conformational flexibility and enables a protein to functionally interact with many target proteins [17]. In fact, the 160–250 amino acid region of PLSCR1 is an interaction region for the anti-apoptotic protein onzin and HTLV-1 Tax [5,18], and this Tat-binding region of PLSCR1 may be involved in its interaction with other target proteins. In addition, our mutational analysis demonstrated that the cysteine-rich and core domains of Tat are necessary for the interaction with PLSCR1 (Fig. S1), and these PLSCR1-binding regions of Tat also contain an ID region (Fig. S1). This ID region of Tat may alter its conformation to allow it to interact with distinct regions of PLSCR1. The ID regions of both proteins must play key roles in this protein–protein interaction.

Because the transcriptional regulation of HIV-1 is primarily regulated by Tat [6], the functional consequences of the PLSCR1•Tat interaction on the Tat-dependent transactivation of the HIV-1 LTR were determined *in vivo*. In this study, reporter analysis demonstrated that overexpression of PLSCR1 efficiently repressed the Tat-dependent transactivation of the HIV-1 LTR (Fig. 3). Additionally, shRNA-mediated down-regulation of endogenous PLSCR1 significantly increased the levels of HIV-1 transcripts in HIV-1-infected T-cell lines (Fig. 3). Interestingly, the Tat-binding region of PLSCR1 is not sufficient for this repression, but full-length PLSCR1 is required (Fig. 3). The Tat-dependent transactivation of the HIV-1 LTR requires Tat•P-TEFb complex formation. However, *in vitro* pull-down analysis revealed that PLSCR1 did not affect

Tat•cyclin T1 complex stability and that PLSCR1 was incorporated into the Tat•cyclin T1 complex (Fig. S2). This observation suggested that Tat•P-TEFb complex formation is not a target of the PLSCR1-mediated repression of Tat-dependent transactivation. Tat•P-TEFb complex formation must occur in the nucleus, and nuclear localization is critical for Tat-mediated transactivation [6]. The nuclear transport of Tat is an active and signal-mediated process controlled by the import factor importin β, which selectively binds the NLS of Tat to mediate the translocation of Tat to the nucleus [19]. Our immunofluorescence staining and subcellular fractionation experiments demonstrated that Tat was predominantly located in the nuclear fraction when expressed on its own or co-expressed with the Tat-binding region of PLSCR1 (Fig. 4). However, co-expression of full-length PLSCR1 and Tat resulted in the stabilization of Tat in the cytoplasmic fraction (Fig. 4). Furthermore, the NLS of Tat is contained within its basic region [19], and the basic region of Tat is not a target for its interaction with PLSCR1 (Fig. S1). These observations suggested that the Tat-binding region of PLSCR1 may not be sufficient to mask the NLS of Tat. Therefore, other regions of PLSCR1 must be required to sterically interfere with its molecular recognition of importin β and its nuclear import. These results strongly suggest that the full-length PLSCR1-mediated cytoplasmic stabilization of Tat plays a major role in the repression of the Tat-dependent transactivation of HIV-1 LTR.

IFN-γ inhibits HIV replication and the Tat-dependent transactivation of the HIV LTR in macrophages [20]. Because PLSCR1 expression is enhanced by IFN-γ treatment and PLSCR1 plays an important role for the IFN-γ-dependent repression of hepatitis C virus replication [1,4], PLSCR1 may play a central role in the IFN-γ-mediated repression of HIV-1 replication through a direct interaction with Tat. PLSCR1-mediated repression of Tat-dependent transactivation may also be critical for IFN-mediated anti-HIV-1 function during HIV-1 infection.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.02.098.

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