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The prolyl isomerase Pin1 stabilizes the human T-cell leukemia virus type 1 (HTLV-1) Tax oncoprotein and promotes malignant transformation

Soo-Jin Jeong, Akihide Ryo *, Naoki Yamamoto *

AIDS Research Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

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ABSTRACT

The HTLV Tax protein is crucial for viral replication and malignant transformation. We investigated the possible role of peptidyl prolyl isomerase Pin1 in the positive regulation of the human T-cell leukemia virus type 1 Tax. Pin1 is highly expressed in adult T-cell leukemia (ATL) cells expressing Tax protein and forced expression of Pin1 in turn increases the Tax protein expression. Pin1 prolonged the protein half-life of Tax by suppressing the ubiquitination and subsequent lysosomal degradation of Tax. Pin1 interacts with phosphorylated Tax on its Ser160-Pro motif at the mitotic phase. Finally, we found that Pin1 plays a supporting role in Tax-mediated cell transformation. Our current study demonstrates an important role for Pin1 in the post-translational regulation of Tax and suggests that the targeting of Pin1 may offer a new insight into the pathogenesis of HTLV-1 related diseases.

Introduction

The peptidyl-prolyl *cis/trans* isomerase (PPIase) Pin1 binds only to phospho-Ser/Thr-Pro motifs on its substrate proteins, thereby catalyzing the *cis/trans* isomerization of the peptide bond and acting as a post-phosphorylation catalyst in the regulation of protein function [1]. Pin1 is highly expressed in various cancers and its deregulation of Pin1 may play a pivotal role in these diseases [1,2]. For instance, it has been reported that Pin1 positively regulates both colorectal and mammary tumorigenesis by increasing β-catenin and cyclin D1 expression [3]. Also, our recent studies have demonstrated that Pin1 acts as a putative anti-apoptotic molecule by the negative regulating Daxx in malignant tumor cells [4].

Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of the aggressive and fatal malignancy of CD4+ lymphocytes known as adult T-cell leukemia (ATL) [5]. During the carrier state in HTLV-1-mediated malignancies such as ATL, the oncoprotein Tax has been shown to play an essential role in the cell proliferation and oncogenesis [6]. Tax is a 40-kDa viral regulatory protein and plays critical roles in the activation of various cellular genes and for viral gene expression, replication and transformation [7]. Tax is a phosphoprotein with a predominately nuclear subcellular localization that accomplishes multiple functions via protein–protein interactions [8].

In our current study, we have investigated the regulation of Tax by Pin1 through phosphorylation-dependent prolyl isomerization. Our results demonstrate that Pin1 physically interacts with Tax, especially during the mitotic phase there by inhibiting both the ubiquitination and the lysosomal degradation of Tax. We also demonstrate that the phosphorylation of Tax on Ser160-Pro motif by mitotic kinase(s) is crucial for both its interaction with Pin1 and its prolonged stabilization. Of importance, a soft agar colony transformation assay using CTLL-2 cells demonstrated that Pin1 increases the transformation activity of Tax whereas the targeted inhibition of Pin1 significantly suppressed the Tax-mediated cell transformation. Hence, our current data provide the evidence that Pin1 plays a critical role in the post-translational regulation of HTLV-1 Tax.

Materials and methods

Cells. HTLV-1-transformed cells and T-lymphocytes were maintained in RPMI supplemented with 10% fetal calf serum, 2 μM L-glutamine and penicillin/streptomycin. 293T cells were maintained in DMEM supplemented with 10% fetal calf serum, 2 μM L-glutamine and penicillin/streptomycin. Mouse T-lymphocyte CTLL-2 cells were kindly provided by Dr. Masahiro Fujii (Niigata University, Japan) and maintained in RPMI supplemented with 10% fetal calf serum, 1 nM IL-2, 50 nM 2-ME, 2 μM L-glutamine and penicillin/streptomycin.

Transfection and luciferase assay. Transient transfections were carried out using Effectene Transfection Reagent (Qiagen). Luciferase assays have been described previously [9].

RT-PCR. Total RNA was isolated using the RNeasy Mini kit (Qiagen). RT-PCR was performed using the Takara one-step RT-PCR kit, following the manufacturers' instructions.

* Corresponding authors. Fax: +81 3 5285 1165 (N. Yamamoto).
E-mail addresses: aryo@nih.go.jp (A. Ryo), nyama@nih.go.jp (N. Yamamoto).

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Protein degradation assay. Protein degradation assays were performed as described previously [10]. Briefly, cycloheximide (100 μg/ml) was added to the medium 24 h after cell transfection, and the cells were harvested at different time points.

In vitro and in vivo protein binding assays. GST pull-down and co-immunoprecipitation assay were carried out as described previously [4].

Cell cycle analyses. Cell cycle was analyzed for DNA content using FACSCalibur (Becton Dickinson Bioscience). Cells were stained with propidium idodide (50 μg/ml) after RNase treatment as described previously [11].

Colony formation assay. Colony formation assays were performed with CTLL-2 cells infected with retrovirus carrying pMRX-ires-blst-Tax and/or pMRX-ires-puro-Pin1, and pMRX-ires-blst-Tax and/or pSuper-retro-puro-Pin1 siRNA in soft agar media using a CytoSelect 96-well cell transformation assay kit (Cell Biolabs) according to the manufacturer's instructions.

Results

Pin1 increases HTLV-1 Tax-mediated transactivation

To examine whether Pin1 influences Tax-mediated transcriptional activation, 293T cells were transiently transfected with luciferase reporter constructs, HTLV-1 LTR- or NF-κB-Luc in the absence or presence of Tax and/or Pin1. As expected, the expression of Tax increased the activation of both the HTLV-1 LTR and NF-κB (Fig. 1A and B, lane 2). In contrast, the expression of Pin1 had no significant effect upon the viral LTR and NF-κB (lane 3). Interestingly, the co-expression of Tax and Pin1 produced a significant increase in the reporter activity for both HTLV-1 LTR- and NF-κB-Luc, indicating that Pin1 increases Tax-mediated transcriptional activation (lane 4). These data were further confirmed in HTLV-1-transformed Hut 102 cells expressing endogenous Tax (Fig. 1C). Hut 102 cells infected with a retroviral vector carrying Pin1 siRNA decreased the HTLV-1 LTR luciferase activity compared with cells infected with control siRNA. These data thus demonstrate that Pin1 enhances Tax-mediated transactivation in HTLV-1-transformed cells.

Pin1 overexpression facilitates the protein stability of HTLV-1 Tax

Next, we sought to examine the expression of Pin1 in HTLV-1-transformed cells and T-lymphocytes (Fig. S1A). Pin1 was found to be highly expressed in Tax-expressing HTLV-1-transformed cells M8166, Hut 102, MT-1, MT-2 and MT-4 compared with Tax-non-expressing HTLV-1-transformed cells ATL-43Tb(−) and T-lymphocytes Jurkat, Molt-4 and CEM cells. To further confirm this contention, we performed Western blot analysis and RT-PCR using 293T/Pin1 siRNA cells transfected with Pin1 and Tax. Pin1 significantly increased the protein expression of Tax in a dose-dependent manner (Fig. S1B) but the Tax mRNA levels showed no significant change (Fig. S1C). These results suggest that Pin1 regulates the expression of the Tax protein via a post-translational mechanism.

To analyze whether Pin1 can stabilize the Tax protein, we performed a protein degradation assay by treating cells with cycloheximide (CHX). First, 293T cells stably expressing either control or Pin1 siRNA cells were transiently co-transfected with Tax and HA-LacZ as an internal control (Fig. 2A). Cycloheximide was then added 24 h post-transfection. Cells were collected at 0, 2, 4, 6 or 8 h and Western blot analyses were performed for Tax, Pin1 and HA-LacZ. This experiment revealed that the protein stability of Tax was significantly reduced in 293T/Pin1 siRNA cells compared with control cells, indicating that Pin1 indeed enhances the protein stability of Tax. Consistent with this result, the stability of Tax was found to be restored by Pin1 overexpression in 293T/Pin1 siRNA cells (Fig. 2B).

Two different pathways, proteasome- and lysosome-dependent, are principally responsible for intracellular protein degradation [12]. To address whether Tax degradation is mediated by either pathway, we performed parallel experiments using either the proteasome inhibitor MG-132 or the lysosome inhibitor NH_4Cl (Fig. S2A). Treatment with NH_4Cl significantly inhibited the rapid degradation of Tax in 293T/Pin1 siRNA cells, whereas the treat-

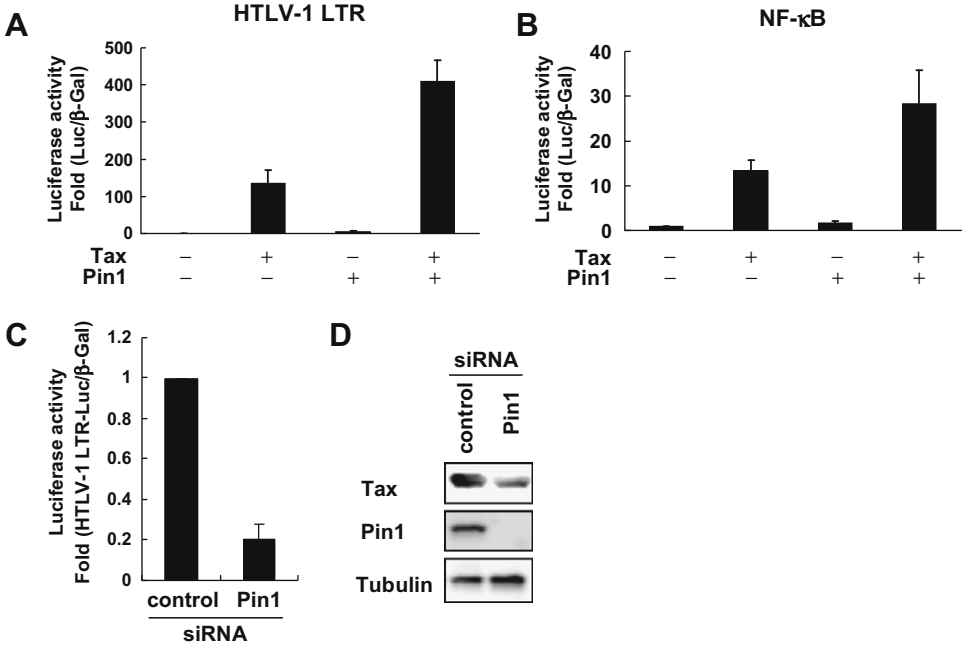

Fig. 1. Pin1 enhances HTLV-1 Tax-mediated transcriptional activation. (A,B) 293T cells were transiently transfected with plasmids expressing Tax, Pin1 and the reporter constructs HTLV-1 LTR- (A) or NF-κB-Luc (B). (C) Hut 102 cells were infected with either control or Pin1 siRNA molecules using the pSuper-Retro-puro-vector and then transiently transfected with HTLV-1 LTR-Luc. Forty-eight hours post-transfection, cells were collected and luciferase activities were measured. Luciferase values were adjusted for transfection efficiency using RSV β-galactosidase. (D) Western blot analysis of Hut 102 cells was performed for Tax, Pin1 and tubulin.