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DDX3 RNA helicase is required for HIV-1 Tat function

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ABSTRACT

Host RNA helicase has been involved in human immunodeficiency virus type 1 (HIV-1) replication, since HIV-1 does not encode an RNA helicase. Indeed, DDX1 and DDX3 DEAD-box RNA helicases are known to be required for efficient HIV-1 Rev-dependent RNA export. However, it remains unclear whether DDX RNA helicases modulate the HIV-1 Tat function. In this study, we demonstrate, for the first time, that DDX3 is required for the HIV-1 Tat function. Notably, DDX3 colocalized and interacted with HIV-1 Tat in cytoplasmic foci. Indeed, DDX3 localized in the cytoplasmic foci P-bodies or stress granules under stress condition after the treatment with arsenite. Importantly, only DDX3 enhanced the Tat function, while various distinct DEAD-box RNA helicases including DDX1, DDX3, DDX5, DDX17, DDX21, and DDX56, stimulated the HIV-1 Rev-dependent RNA export function, indicating a specific role of DDX3 in Tat function. Indeed, the ATPase-dependent RNA helicase activity of DDX3 seemed to be required for the Tat function as well as the colocalization with Tat. Furthermore, the combination of DDX3 with other distinct DDX RNA helicases cooperated to stimulate the Rev but not Tat function. Thus, DDX3 seems to interact with the HIV-1 Tat and facilitate the Tat function.

1. Introduction

The gene expression of human immunodeficiency virus type 1 (HIV-1) is regulated transcriptionally by Tat through its binding to a nascent viral *trans*-activation responsive (TAR) RNA [1,2], and post-transcriptionally by Rev through its association with Rev-responsive element (RRE) RNA in the *env* gene [3–5]. Tat binds to TAR RNA and recruits transcription factors, such as p300/CREB-binding protein (CBP), p300/CBP-associated factor (PCAF), chromatin remodeling factors Brahma (BRM), Brahma-related gene 1 (BRG1), integrase interactor 1 (INI1), and positive transcription elongation factor (P-TEFb), a complex of cyclin T1 and cyclin-dependent kinase 9 (CDK9), to stimulate both transcription initiation from the HIV-1 long terminal repeat (LTR) and transcription elongation [1,2,6,7]. CDK9 hyperphosphorylates the C-terminal domain (CTD) of RNA polymerase II and activates transcription elongation.

DEAD-box RNA helicases are involved in various RNA metabolic processes, including transcription, translation, RNA splicing, RNA transport, and RNA degradation in the ATPase-dependent manner [8–10]. Host RNA helicases may be involved in HIV-1 replication [11,12], since HIV-1 does not encode an RNA helicase. In fact, DDX1 and DDX3 have been implicated in the replication of HIV-1 replication [13–15]. Both DDX1 and DDX3 interact with HIV-1 Rev and enhance Rev-dependent HIV-1 nuclear export [13–15]. In addition, we recently demonstrated that several DDX, including DDX1, DDX3, DDX5, DDX17, DDX21, and DDX56 interact with HIV-1 Rev and enhance the Rev-dependent nuclear export [16]. However, the role of these DDX DEAD-box RNA helicases in HIV-1 Tat function is still unknown. To address this issue, we first examined the interaction of these DDX RNA helicases with HIV-1 Tat and the potential role of DDX in the Tat function.

2. Material and methods

2.1. Cell culture

293FT cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS).

2.2. Plasmids

We used pcDNA3-HA, pcDNA3-FLAG, pHLV-1-LTR-Luc [17], pHA-DDX3 [13,18,19], pcRev, pDM628 [14,15,20], pcDNA3-Tat101-FLAG [21], pcDNA3-HA-DDX1, pcDNA3-HA-DDX5, pcDNA3-HA-DDX17, pcDNA3-HA-DDX21, and pcDNA3-HA-DDX56 [16].

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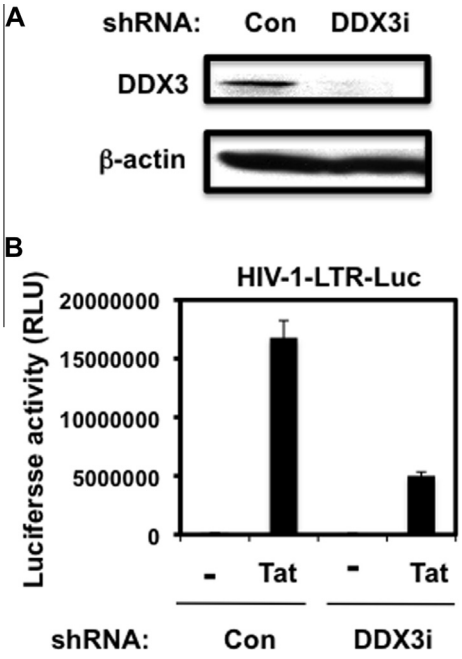

Fig. 1. Requirement of DDX3 for HIV-1 Tat function. (A) Inhibition of DDX3 expression by shRNA-producing lentiviral vector. The results of Western blot analysis of cellular lysates with anti-DDX3 or anti-β-actin antibody in the cells expressing shRNA targeted to DDX3 (DDX3i) as well as in the cells transduced with a control lentiviral vector (shCon) are shown. (B) HIV-1 Tat-mediated transcription in the DDX3 knockdown cells. Cells (2×10^4 cells) were cotransfected with pHIV-1-LTR-Luc [7,17,20] (100 ng) and/or pcDNA3-Tat101-FLAG [21] (100 ng). 24 h after transfection, luciferase activity in the cellular lysates was measured. Results are from three independent experiments.

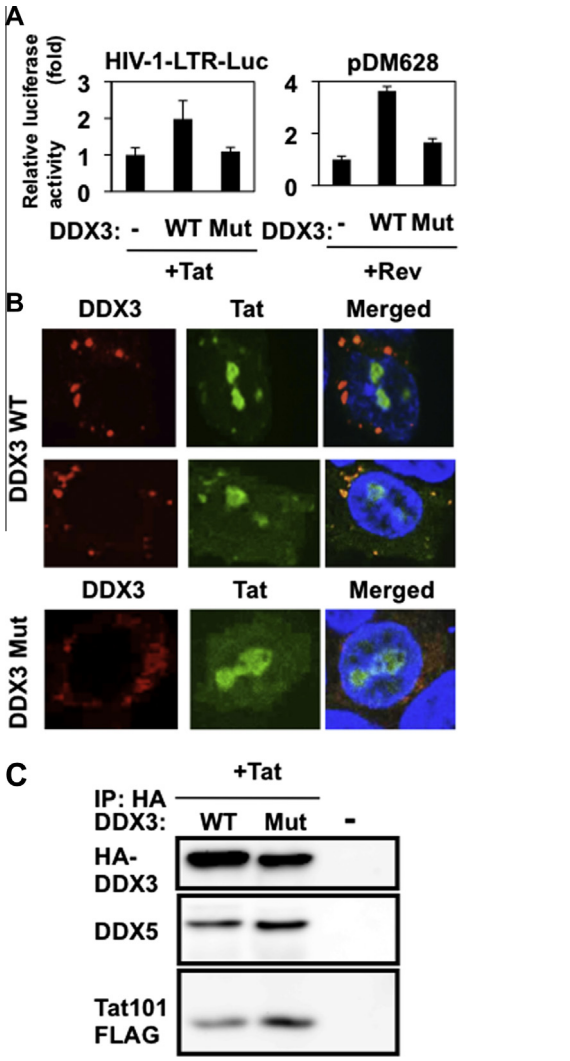

Fig. 2. DDX3 interacts with HIV-1 Tat in cytoplasmic foci. (A) The ATPase-dependent RNA helicase activity of DDX3 is required for the Tat function. 293FT cells (2×10^4 cells) were cotransfected with pHIV-1-LTR-Luc (100 ng), pHA-DDX3 WT (wild-type) or pHA-DDX3 Mut (ATPase-defective mutant) [13], and/or pcDNA3-Tat101-FLAG (100 ng). 24 h after transfection, luciferase activity in the cellular lysates was measured. (B) DDX3 colocalizes with Tat. 293FT cells cotransfected with pHA-DDX3 WT or pHA-DDX3 Mut (200 ng) and pcDNA3-Tat101-FLAG (200 ng) were examined by confocal laser scanning microscopy. Cells were stained with anti-DDX3 (LS-C64576) and Cy3-conjugated anti-mouse IgG antibody followed by staining with FITC-conjugated anti-FLAG antibody, and then visualized with Cy3 (DDX3) or FITC (Tat). Nuclei were stained with DAPI. Images were visualized by using confocal laser scanning microscopy. The right panels exhibit the two-color overlay images (Merged). (C) 293FT cells were cotransfected with pcDNA3-Tat101-FLAG (2 μg) and either pHA-DDX3 WT or pHA-DDX3 Mut (2 μg). The cell lysates were immunoprecipitated with an anti-HA antibody, followed by immunoblot analysis using anti-HA, anti-DDX5, or anti-FLAG antibody. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. Lentiviral vector production

pLV-DDX3i and the vesicular stomatitis virus (VSV)-G-pseudotyped HIV-1-based vector system has been described previously [18,22,23]. The lentiviral vector particles were produced by transient transfection of the second-generation packaging construct pCMV-ΔR8.91 [22,23] and the VSV-G-envelope-expressing plasmid pMDG2 as well as pRDI292 into 293FT cells with FuGENE 6 (Promega, Madison, WI, USA).

2.4. Luciferase assay

Plasmids were transfected into 293FT cells (2×10^4 cells) using the FuGENE 6 transfection reagent. Luciferase assays were performed 24 h after transfection using luciferase assay reagent according to the manufacturer's instructions (Promega). All transfections utilized equal total amounts of plasmid DNA quantities owing to the addition of empty vector into the transfection mixture. Results were obtained through three independent transfections. A lumat LB9507 luminometer (Berthold, Bad Wildbad, Germany) was used to detect the luciferase activity.

2.5. Western blot analysis

Cells were lysed in buffer containing 50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 4 mM EDTA, 1% Nonidet P (NP)-40, 0.1% sodium dodecyl sulfate (SDS), 1 mM dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride (PMSF). Supernatants from these lysates were subjected to SDS–polyacrylamide gel electrophoresis, followed by immunoblot analysis using anti-HA (HA-7; Sigma, Saint Louis, MI, USA), anti-DDX3 (54257 [NT] and 5428 [IN]; Anaspec, San Jose, CA, USA), anti-DDX5 (A300-523A; Bethyl Laboratories, Montgomery, TX, USA) anti-β-actin (A5441, Sigma), or anti-FLAG antibody (M2, Sigma).

2.6. Immunoprecipitation

Cells were lysed in buffer containing 10 mM Tris–HCl (pH 8.0), 150 mM NaCl, 4 mM EDTA, 0.1% NP-40, 10 mM NaF, 1 mM DTT and 1 mM PMSF. Lysates were pre-cleared with 30 μl of protein-G-Sepharose (GE Healthcare Bio-Sciences, Uppsala, Sweden). Pre-cleared supernatants were incubated with 5 μg of anti-HA