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Association of HTLV Tax proteins with TAK1-binding protein 2 and RelA in calreticulin-containing cytoplasmic structures participates in Tax-mediated NF-κB activation

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ABSTRACT

HTLV-1 is more pathogenic than HTLV-2 despite having a similar genome and closely related transactivating oncoproteins. Both Tax-1 protein from HTLV-1 and Tax-2 from HTLV-2 activate the NF-κB pathway. The mechanisms involved in Tax-1 deregulation of this signalling pathway have been thoroughly investigated, but little is known about regulation by Tax-2. We have compared the interaction of Tax-1 and Tax-2 with two key NF-κB signalling factors: TAK1-binding protein 2 (TAB2), an adaptor involved in the activation of TAK1 kinase, and RelA, the active subunit of the canonical RelA/p50 NF-κB transcription factor. Tax-2 formed stable complexes with both RelA and TAB2. These two NF-κB factors colocalized with Tax proteins in dotted cytoplasmic structures targeted by calreticulin, a multi-process calcium-buffering chaperone. Co-expression of RelA and/or TAB2 markedly increased Tax-mediated NF-κB activation. These findings provide new insights into the role of RelA, TAB2 and Tax in the deregulation of the NF-κB pathway.

Introduction

Human T-lymphotropic viruses type 1 (HTLV-1) and type 2 (HTLV-2) are closely related human retroviruses. HTLV-1, the etiologic agent of adult T-cell leukaemia/lymphoma (ATLL) and HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Matsuoka and Jeang, 2007), is tropic for $CD4^+$ T-cells. HTLV-2 preferentially infects $CD8^+$ T-cells (Feuer and Green, 2005), but has no known pathologic role, other than an association with rare cases of subacute myelopathy resembling HAM/TSP (Araujo and Hall, 2004).

HTLV-1 and HTLV-2B Tax proteins (Tax-1 and Tax-2B) share an amino acid similarity of 85%. The Tax proteins play a pivotal role in the induction of T-cell transformation (Grassmann et al., 1992; Robek and Ratner, 1999), but their phenotypes are different. Tax-1 has a higher transforming activity than Tax-2 (Endo et al., 2002; Feuer and Green, 2005), induces micronuclei formation (Semmes et al., 1996), arrests the cell cycle of human $CD34^+$ cells (Tripp et al., 2005), and regulates viral transcription by interacting with different members of the activating transcription factors/cAMP responsive elements binding protein family (Lemasson et al., 2002). Tax-1 activates transcription of numerous cellular genes involved in immune regulation and cell proliferation via interaction with serum response factors (SRF), AP-1 and nuclear factor κB (NF-κB) pathways (Hall and Fujii, 2005; Kashanchi and Brady, 2005; Lewis et al., 2002). Tax-1 intracellular localization and transcriptional activity is strictly controlled by post-translational modifications (Lamsoul et al., 2005). We have recently demonstrated (Turci et al., 2009) that Tax-2B is modified by ubiquitination and sumoylation and distributed in punctuate nuclear structures that include the RelA subunit of NF-κB, as previously reported for Tax-1 (Lamsoul et al., 2005).

Both Tax-1 and Tax-2B activate gene expression via the NF-κB pathway to similar levels (Huang et al, 2009; Turci et al., 2009). The activation of NF-κB pathway is essential for Tax-mediated transformation of human T-cell lines by HTLV-1 and by HTLV-2 (Sun and Yamaoka, 2005). The so-called canonical and non-canonical NF-κB signalling pathways that mediate transcriptional activation are

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deregulated by Tax-1 interaction with several factors. These include the RelA subunit of NF-κB and the IκB kinase complex (e.g. IKKα, IKKβ and NEMO/IKKγ) of the canonical signalling cascade (for a review, see Boxus et al., 2008), and p100, a precursor of the non-canonical pathway (Higuchi et al., 2007). Tax-1-mediated activation of gene expression via the NF-κB pathway has been extensively studied. Tax-1 interacts with the Rel homology domain of the RelA subunit of NF-κB (Suzuki et al., 1994) and colocalizes with NF-κB subunits p50 and RelA, the splicing factors Sm and SC-35 and the large subunit of RNA polymerase II within nuclear bodies (Bex et al., 1997; Lamsoul et al., 2005). Tax-1 also induces persistent overexpression of TAB2 (TGFβ activating kinase 1-binding protein 2), a scaffold protein involved in the signal transduction between TGFβ activating kinase 1 (TAK1) and other kinases including mitogen activated protein kinase (MAPK), Jun N-terminal kinase (JNK) and IκB kinase complex (IKK) (Kanayama et al., 2004, Suzuki et al., 2007). The association of TAB2 with Tax-1 was reported to be critical for Tax-1-mediated activation of TAK1 kinase (Yu et al., 2008; Wu and Sun, 2007). However, whether this association favours Tax-mediated NF-κB activation is still controversial (Suzuki et al., 2007, 2010).

Little is known about how Tax-2B interacts with cellular factors of the NF-κB pathway. However, it is known that Tax-2 interacts with NEMO/IKKγ and with NEMO-related protein/optineurin (Huang et al., 2009; Journo et al., 2009; Meertens et al., 2004a,b) but does not recognize p100 (Higuchi et al., 2007).

Our goal in this study was to investigate the ability of Tax-1 and Tax-2B to interact with the RelA and TAB2 components of the NF-κB canonical pathway, as well as identifying their intracellular localization in Tax-expressing cells. Our focus was to identify the punctate cytoplasmic structures containing Tax complexes in association with TAB2 and RelA and assess the potential role of these complexes on NF-κB activation.

Results

Tax-1 and Tax-2B form complexes with RelA and TAB2 in living cells

To determine whether Tax-2B, like Tax-1, formed stable complexes with RelA and TAB2, we first compared the ability of both to assemble complexes with RelA. Lysates of 293T cells co-expressing RelA with either Tax-1 or V5 tagged Tax-2B (Tax-2B-V5) were co-immunoprecipitated with an anti-RelA antibody. The immunoprecipitated complexes were western blotted with anti-Tax-1 or anti-V5 antibodies. Tax-1 and Tax-2B-V5 were detected in the complexes immunoprecipitated (IP) by the anti-RelA antibody in cells expressing Tax in the absence (Fig. 1A, IP panels, lanes 3 and 7) or presence (Fig. 1A, IP panels, lanes 4 and 8) of overexpressed RelA. As expected, co-immunoprecipitation of Tax-1 or Tax-2B was more obvious in cells overexpressing RelA than in cells only expressing endogenous RelA. Neither lysates of untransfected cells (Fig. 1A, IP panels, lanes 1 and 5) nor cells expressing RelA alone (Fig. 1A, IP panels, lanes 2 and 6) gave detectable binding. The presence of RelA, as well as the Tax proteins, in whole cell lysates was detected by western blotting using anti-RelA, anti-Tax-1 or anti-V5 antibodies (Fig. 1A, input, middle and lower panels). Both Tax-1 and Tax-2B formed stable complexes within the cell with the RelA subunit of NF-κB.

We next analyzed the ability of Tax-1 and Tax-2B to form complexes with TAB2 by co-immunoprecipitation experiments. Either Tax-1 or Tax-2B could be detected within the complexes immunoprecipitated by an anti-Flag antibody in 293T cells co-expressing TAB2-Flag and Tax-1 or Tax-2B-V5 (Fig. 1B, IP panels, lanes 2 and 6, respectively). Neither lysates of untransfected cells (Fig. 1B, IP panels, lanes 1 and 5) nor lysates of cells expressing Tax-1, Tax-2B-V5 or TAB2-Flag alone (Fig. 1B, IP panels, lanes 3, 4 and 7, 8) gave detectable signal. The presence of TAB2-Flag and Tax proteins in the whole cell lysates was detected by western blotting using anti-Flag, anti-Tax-1 or

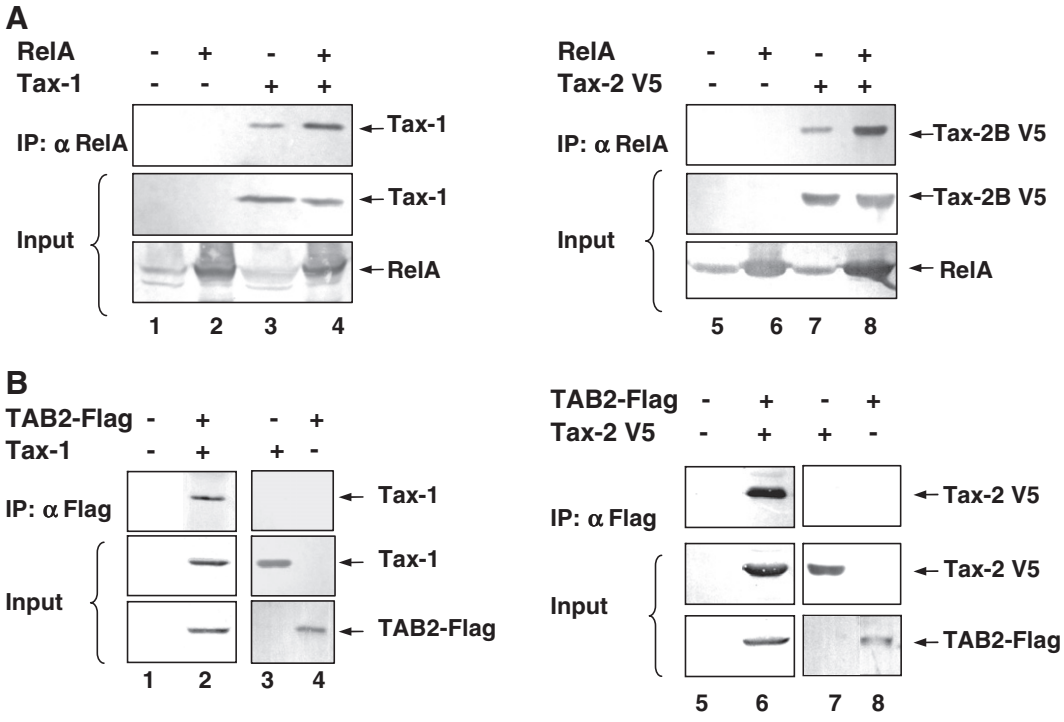

Fig. 1. Tax-1 and Tax-2B proteins form *in vivo* complexes with RelA and TAB2. (A) 293T cells were co-transfected with either the vector control or vectors expressing Tax-1 or Tax-2B V5 and with or without a vector expressing RelA, as indicated. Cell extracts were immunoprecipitated (IP) with anti-RelA and the immunoprecipitated complexes were analyzed with anti-Tax-1 or anti-V5 antibodies. Whole cell extracts (Input) were also analyzed for Tax or RelA proteins. (B) 293T cells were transfected with the vector control or vectors expressing Tax-1 or Tax-2B V5 and with or without a vector expressing TAB2-Flag. Cell extracts were immunoprecipitated (IP) with an anti-Flag antibody and the immunoprecipitated complexes were analyzed with anti-Tax-1 or anti-V5 antibodies. Whole cell extracts (Input) were also analyzed to determine the presence of the Tax and TAB2 proteins.