INTRODUCTION

Human T cell lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) are closely related human delta retroviruses. Although currently there are four known types of HTLV retroviruses (HTLV-1, HTLV-2, HTLV-3, and HTLV-4), HTLV-1 is the most pathogenic of all and the first oncogenic retrovirus discovered in humans. HTLV-1 infects 15–20 million individuals worldwide. It is transmitted horizontally (sexual transmission), vertically (mother to child), and by blood transfusion (Kaplan et al., 1996). HTLV-1 infection is endemic in Japan, Africa, South America, the Caribbean, Melanesia, and certain areas in the Middle East and Eastern Europe (reviewed in Gessain and Mahieux, 2005). HTLV-1 infection is also endemic in the spread of certain diseases. Numerous studies have provided accumulating evidence on the involvement of the viral transactivators Tax-1 versus Tax-2 in T cell transformation. Tax-1 is a potent transcriptional activator of both viral and cellular genes. Tax-1 post-translational modifications and specifically ubiquitylation and SUMOylation have been implicated in nuclear factor-kappaB (NF-κB) activation and may contribute to its transformation capacity. Although Tax-2 has similar protein structure compared to Tax-1, the two proteins display differences both in their protein–protein interaction and activation of signal transduction pathways. Recent studies on Tax-2 have suggested ubiquitylation and SUMOylation independent mechanisms of NF-κB activation. In this present review, structural and functional differences between Tax-1 and Tax-2 will be summarized. Specifically, we will address their subcellular localization, nuclear trafficking and their effect on cellular regulatory proteins. A special attention will be given to Tax-1/Tax-2 post-translational modification such as ubiquitylation, SUMOylation, phosphorylation, acetylation, NF-κB activation, and protein–protein interactions involved in oncogenicity both in vitro and in vivo.

Keywords: HTLV-1, HTLV-2, Tax-1, Tax-2, NF-κB
Tax-1 and Tax-2 share overall sequence homology (Figure 1A), but have distinctive differences both at the structural and functional levels (Higuchi and Fujii, 2009; Bertazzoni et al., 2011). Tax-1 is a 353aa (amino acid) residue protein, which is highly conserved in all HTLV-1 serotypes. Of the four serotypes of HTLV-2, Tax-2 subtype A and B are the best characterized (Sheehy et al., 2006) and Tax-2B is the subtype which is represented in Figure 1. Tax-2B has 356 amino acid residues, whereas Tax-2A possesses a 25 amino acid truncation at the C-terminus. Tax-1 and Tax-2B share 85% amino acid sequence similarity and have several common domains (Figure 1A).

The N-terminal region of both Tax-1 and Tax-2 contain CREB (cyclic AMP responsive element binding)-activating domain and a zinc finger domain (Ross et al., 1997; Feuer and Green, 2005; Figure 1B). The CREB domain is required for activation of the viral promoter (Giebler et al., 1997; Boxus et al., 2008). Depending on the cell type, Tax-1 mutants deficient for CREB activation are incompetent for transformation or induction of aneuploidy (Akagi et al., 1997; de la Fuente et al., 2006; Geiger et al., 2008). The zinc finger domain is required for association with a variety of transcription factors including the p62 nucleoporin and mutations in this motif abolishes Tax-1 interaction with p62 and nuclear import (Tsuji et al., 2007). Within the first 60 amino acids of Tax-1, there is a nuclear localization signal NLS (Gitlin et al., 1991; Smith and Greene, 1992) whereas the first 42 amino acid sequence of Tax-2 contain a nuclear localization determinant (Turci et al., 2006).
required for its nuclear functionality (Figure 1B). Furthermore, Tax-2 has an additional cytoplasmic localization domain about 10 amino acids long, situated at amino acid position 89–113 which has been shown to be responsible for its divergent localization compared to Tax-1 (Meertens et al., 2004).

The central region of Tax-1 includes two leucine zipper-like regions (LZR), which are known to be essential for protein dimerization and DNA interaction (Jin and Jeang, 1997; Basbous et al., 2003; Bonnet et al., 2008). The first LZR is located at amino acid position 116-145 and is responsible for the non-canonical nuclear factor-kappaB (NF-kB) activation and protein dimerization whereas the second LZR is located at amino acid position 225–232 and is responsible for p100 processing and p52 nuclear translocation involved in NF-kB2 activation (Xiao et al., 2003; Higuchi et al., 2007; Shi et al., 2009; Figure 1B). Importantly, Tax-2 lacks these two LZR regions. Both Tax-1 and Tax-2 have nuclear export signal (NES) located at amino acid position 189–202 (Alfantis et al., 2003; Chevalier et al., 2005; Figure 1B). Furthermore, Tax-1 and Tax-2 have at the C-terminal region CREB/activating transcription factor (ATF)-activating domain, essential for transactivation of the CREB/ATF and for NF-kB/Rel signaling pathways (Ross et al., 1997; Figure 1B).

Tax-1 interacts in vitro with a number of proteins of the CREB/ATF family of transcription factors: CREB, CREM (cyclic AMP responsive element modulator), ATF1, ATF2, ATF3, ATF4 like regions (LZR), which are known to be essential for protein dimerization and DNA interaction (Jin and Jeang, 1997; Basbous et al., 2003; Bonnet et al., 2008). The first LZR is located at amino acid position 116-145 and is responsible for non-canonical nuclear factor-kappaB (NF-kB) activation and protein dimerization whereas the second LZR is located at amino acid position 225–232 and is responsible for p100 processing and p52 nuclear translocation involved in NF-kB2 activation (Xiao et al., 2003; Higuchi et al., 2007; Shi et al., 2009; Figure 1B). Importantly, Tax-2 lacks these two LZR regions. Both Tax-1 and Tax-2 have nuclear export signal (NES) located at amino acid position 189–202 (Alfantis et al., 2003; Chevalier et al., 2005; Figure 1B). Furthermore, Tax-1 and Tax-2 have at the C-terminal region CREB/activating transcription factor (ATF)-activating domain, essential for transactivation of the CREB/ATF and for NF-kB/Rel signaling pathways (Ross et al., 1997; Figure 1B).

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Finally, Tax-1 interacts with histone methyltransferase (HMTage) SMYD3 which affects its nucleo-cytoplasmic shuttling and regulates NF-κB activation (Yamamoto et al., 2011). Interaction of Tax-1 with the four and a half LIM domain protein 3 (FHL3) also affects Tax-1 sub cellular localization and transactivation capacity (McAule et al., 2013).

MODULATION OF CELLULAR PATHWAYS BY Tax-1 AND Tax-2

Tax-1 interacts with various components of the cell signaling system which control cell transformation, proliferation, intracellular protein distribution, cell migration, and virological synapses (Azran et al., 2004; Jeang et al., 2004; Grassmann et al., 2005; Boxus et al., 2008). More than 100 proteins have been reported to interact with Tax-1 (Boxus et al., 2008). Tax-2, however, interacts with a limited number of partners and most of them belong to the NF-κB family of proteins. It is important to note that Tax-1 and to a lesser extent Tax-2 interaction is undergoing a dramatic expansion with additional interaction partners being discovered continuously.

PI3K AND AKT PATHWAY

Phospho-inositol triphosphate kinase (PI3K) and its downstream kinase AKT (protein kinase B) are activated in T cells by many cytokines including interleukin 2 (IL-2), and provide cell survival and growth signals (Cantley, 2002). PI3K activation results in phosphorylation of AKT at Ser473 which in turn activates a broad range of regulatory proteins and transcription factors such as AP1 (Zhang et al., 2007). In both HTLV-I transformed and ATL cells, the transcription factor AP1 and hence the PI3K/AKT pressor, through the ubiquitin–proteasome pathway (Oteiza and Mechti, 2011). Conversely, a recent study demonstrated that Tax-2 interacts with various members of the small GTPase Rho family G proteins such as p27/Kip1 accumulation, and further found that Tax-2 induced ERK6 also known as p38β (Pimenta and Pascal, 2007). Tax-1 binds the MAP3K MEKK1 to stimulate IKK-β kinase activity and NF-κB activation (Yin et al., 1998). TGF-β-activating kinase 1 (TAK1) is the other MAP3K which interacts with Tax-1 and phospho-phytoses IKK-β and M KK6 (MAP2K6) serine/threonine kinase, thereby activating NF-κB and JNK (Adhikari et al., 2007). Tax-2 interaction with the MAPK signaling pathway leading to its constitutive activation have also been recently reported (Ren et al., 2012).

TGFβ SIGNALING PATHWAY

Transforming growth factor β (TGFβ) inhibits T cell growth in mid-G1 but can also promote tumorigenesis (Pennision and Pasche, 2007). TGFβ binds to a heterodimeric complex composed of type I (TβRI) and type II (TβRII) serine/threonine kinase receptors and activates downstream targets such as Smad proteins. These include receptor-activated R-Smad (Smad1–2, 3–5–8) and the common mediator Co-Smad (Smad4). Smad4 containing complexes then translocate to the nucleus and activate transcription of genes under the control of a Smad-binding element (Waterston and Davies, 1993).

Adult T cell leukemia/lymphoma cells produce high levels of TGFβ in the sera of HTLV-1-infected patients due to constitutive activation of AP-1 in the PI3K/AKT pathway (Kim et al., 1990). Tax-1 binds the N-terminus of Smad2, Smad3, and Smad4 proteins, which inhibits their association with Smad-binding elements and competes with Smads for recruitment of CBP/P300. This inhibition will also result in promoting resistance of HTLV-1-infected cells to TGFβ (Mori et al., 2001; Arnulf et al., 2002; Lee et al., 2002). So far, interaction of Tax-2 with Smads has not been reported.

G PROTEINS AND CYTOSKELETAL ORGANIZATION

The guanine nucleotide-binding proteins GTPases (G proteins) are molecular switches that cycle between active (GTP-bound) and inactive (GDP-bound) states. GTPases are activated in response to external stimuli such as growth factors, stress, or cytokines. Following activation, they regulate a variety of cellular and biochemical functions such as cytoskeleton organization, regulation of gene expression, and enzymatic activities (Jaffe and Hall, 2005).

Tax-1 binds to proteins involved in cytoskeleton structure and dynamics such as α-interneurin, cytoferatin, actin, gelsolin, annexin, and γ-tubulin (Trinh et al., 1997; Reddy et al., 1998; Wu et al., 2004; Koury et al., 2008) and through these interactions it might connect Rho GTPases to their targets and affects cytoskeletal organization. Tax-1 binds the Gβ subunit of the G-protein-coupled receptor (GPCR) affecting the SDF-1-dependent activation of CXCR4 GPCR chemokine receptor resulting in MAPK pathway over-activation and increased cell chemotaxis (Ohshima, 2007). Additionally, Tax-1 expression at the microtubule assembly center and the Golgi in the cell to cell contact region has been shown to contribute to the intracellular signal which synergizes with...
ICAM-1 (intracellular adhesion molecule) to induce T cell microtubule polarization at the virological synapse (Nejmeddine et al., 2005, 2009). Tax-2, however, has not yet been reported to associate with proteins involved in cytoskeletal rearrangement. It is of importance to mention again that Tax-2 lacks a PDZ domain (Figure 1). This PDZ domain might contribute to Tax-1 binding to proteins involved in microtubule and cytoskeleton organization, which in turn may play an important role in pathogenicity and transformation capacity (Endo et al., 2002; Ishioaka et al., 2006).

**ACTIVATION OF CREB SIGNALING**

As mentioned previously, both Tax-1 and Tax-2, respectively, act as transcriptional activators of the HTLV long terminal repeat (LTR). Tax-1 and Tax-2 modulate CREB and ATF function (Jeang et al., 1988; Adya and Giam, 1995; Bodo et al., 1995; Braunweiler et al., 1995; Yin et al., 1995b; Bantignies et al., 1996; Tie et al., 1996; Yin and Gaynor, 1996; Rex et al., 1998). Tax-1/Tax-2 activation of the CREB/ATF pathway is critical for efficient viral gene expression and replication (Zhao and Giam, 1992; Wagner and Green, 1995; Adya et al., 1994; Anderson and Dynan, 1994; Yin et al., 1995a; Bantignies et al., 1996). A number of mutants in both Tax-1 and Tax-2 have been described that selectively abrogate the ability of Tax to activate transcription through the CREB/ATF signaling pathway (Smith and Greene, 1990; Semmes and Jeang, 1992; Ross et al., 1997). Tax-1 activates a variety of cellular genes through its interactions with CREB/ATF proteins, for example those encoding IL-17 or c-fos (Alexandre and Verrier, 1991; Dodon et al., 2004). On the other hand, Tax-1 also represses expression of genes like ICAM-1 and c-myc by targeting CREB/ATF factors (Nicot et al., 2000; Kühler and Jeang, 2001). Furthermore, Tax-1 has been shown to repress Smad-dependent TGFβ signaling through interaction with CBP/p300 (Mori et al., 2001). Tax-1 has also been shown to abrogate p53-induced cell cycle arrest and apoptosis through its CREB/ATF functional domain (Mulloy et al., 1998). Some bioinformatic analysis of wild type and CREB-deficient Tax-1 protein revealed several cellular genes controlled by CRE elements activated by Tax-1 (de la Fuente et al., 2006) such as Sgt1 (suppressor of G2 allele of SKP1) and p97 (Vcp, valosin containing protein) which have functions in spindle formation and disassembly, respectively.

Both Tax-1 and Tax-2 interact with a series of CREB/ATF factors and mediate expression of viral and cellular genes through CRE elements. However, the specific binding of each CREB/ATF member still needs to be studied, although some in vitro analyses suggest Tax-1 interaction with a number of proteins of the CREB/ATF family of transcription factors: CREB, CREM, ATF1, ATF2, ATF3, ATF4 (also named CREB2), and XBP1 (Zhao and Giam, 1992; Franklin et al., 1993; Bantignies et al., 1996; Reddy et al., 1997).

**REPRESSION OF P53 SIGNALING**

P53 is a DNA-binding transcription factor, which plays an important role as a tumor suppressor and is primarily involved in cell cycle regulation, apoptosis, and DNA repair (Vousden and Lu, 2002; Zenz et al., 2008). The P53 gene is very often mutated in human tumors and hematologic malignancies (Xu-Monette et al., 2012). Several in vitro studies in different cell types have shown that Tax-1 represses p53 activity through different mechanisms including NF-κB activation and/or the CREB pathway (Ariumi et al., 2000, 2005; Pise-Masison et al., 2000; Leong et al., 2004, 2005). Recently, Wip-1 phosphatase protein was shown to interact with Tax-1 and inhibits p53 (Zane et al., 2012). In this study authors have used Tax transgenic mice and found significant differences in Tax-1-driven inactivation of p53 versus p53 inactivation due to genetic mutations. Several studies explored Tax-2 contribution to p53 inactivation. In HTLV-2 subtype A- and B-infected cells, both Tax-2B and to a lesser extent Tax-2A were shown to inhibit p53 in T cells (Mahieux et al., 2000a).

In ATL-derived cell lines, P53 has been shown to be very often inactive and sometimes mutated despite its high expression levels and this activation has been shown to be dependent on Tax-1 induced NF-κB activation through phosphorylation of p53 Ser-15 and Ser-392 (Pise-Masison et al., 2000). Studies by Ariumi et al. (2000) have shown that the phosphorylation of p53 on Ser-15 is not a major cause of the Tax-mediated inactivation of p53. However, Tax with a mutation in the coactivator CREB-binding site (K88A), which activates NF-κB but not the CREB pathway, could not repress the p53 transcriptional function. A study dedicated to Tax-2 inhibition of p53 was performed by (Mahieux et al., 2000a) where abundant levels of p53 protein were detected in both HTLV-2A and -2B virus-infected cell lines and p53 was shown to be inactive. Furthermore, they showed that although Tax-2A and Tax-2B inactive p53, the Tax-2A protein appeared to inhibit p53 function less efficiently than either Tax-1 or Tax-2B. Jurkat cells that constitutively express Tax-1 and Tax-2 showed reduced cellular replication, and Tax-1 inhibition of cellular replication was higher in comparison to Tax-2 (Sieburg et al., 2004).

**ACTIVATION OF THE NF-κB PATHWAY**

**Generalities on NF-κB**

Nuclear factor-kappaB is a family of transcription factors that play a crucial role in proliferation, apoptosis, oncogenesis, and immune response. To date, five members of NF-κB have been described: p65 (RelA), c-Rel, RelB, p50/p105, and p52/p100. The precursor proteins p105 and p100 are processed proteolytically to the mature p50 and p52 forms, respectively (Ghosh and Hayden, 2008). All five members share a common Rel homology domain, which is a conserved domain of 300 amino acids that contains a DNA-binding domain, a dimerization domain, a region of interaction with inhibitory proteins IκB, and a NLS (Baeuerle and Henkel, 1994; Baldwin, 1996). These proteins are capable of homo- or heterodimerization using all possible combinations, except for RelB which dimerizes only with p50 or p52 (Ryseck et al., 1992).

In resting cells, NF-κB dimers are trapped in the cytoplasm by inhibitory proteins called IκBs such as p105, p100, IκBα, IκBβ, and IκBγ which mask the nuclear localization signal of NF-κB factors through physical interaction (Siebenlist et al., 1988, 1994; Perkins, 2007). NF-κB activation involves phosphorylation of IκB inhibitors by the IκK, which triggers their ubiquitylation and subsequent proteasomal degradation, resulting in nuclear translocation of NF-κB dimers (Karim and Ben-Neriah, 2000; Perkins, 2007).

Nuclear factor-kappaB is activated by a wide variety of signals through two distinct pathways: the canonical and the non-canonical pathways. The canonical pathway is activated by...
phosphorylation by NF-κB proteins leading to their ubiquitylation and phosphorylation of the Iκα kinases to the IKK complex and forces the phosphorylation of IKK-upstream kinases such as MAPK/ERK kinase kinase 1 (MEKK1), and Sun, 1999; Jin et al., 1999; Kfoury et al., 2005) and activates way, Tax-1 associates with the IKK-α way on the other hand primarily involves IKK-β and IKK-ε, and IKK-degradation at multiple levels, thereby allowing nuclear translocation of NF-κB independently of external stimuli. In the non-canonical pathway, Tax-1 interacts with IKK-γ (NEMO) and p100, induces p100 processing and nuclear translocation of the p52/RelB dimer (Figure 2A). It therefore appears that IKK-γ is an important Tax-1-binding partner for activation of both pathways (Xiao et al., 2001; Hijikata et al., 2007).

**Tax-2 activation of the NF-κB pathway**

Many studies have shown the ability of Tax-2 to activate the canonical NF-κB pathway to a level comparable to Tax-1 (Hijikata et al., 2007). The major difference between Tax-1 and Tax-2 lies in the inability of Tax-2 to process p100 (Hijikata et al., 2007; Figure 2B). The LZR at amino acid 225–232 of Tax-1, which is missing in Tax-2, is responsible for p100 processing and p52 nuclear translocation (Shoji et al., 2009). To date, there is no evidence of the ability of Tax-2 to activate the non-canonical NF-κB pathway. In fact, the transforming activity of Tax-1 in CTLL-2 (cytotoxic T-lymphocyte cell lines) cells constitutively expressing the IL-2 receptor is much higher than Tax-2 and this activity has been shown to be partly mediated through the non-canonical NF-κB pathway (Tsukita et al., 2005; Kondo et al., 2006; Higuchi et al., 2007; Shoji et al., 2009). Within the same line, a constitutively active NIK, restores the transforming activity of Tax-2 to a level equivalent to Tax-1 (Hijikata et al., 2007). This inability of Tax-2 to activate the non-canonical NF-κB pathway might partially explain its inability to transform T cells and induce ATL development.
**Tax-1 AND Tax-2 POST-TRANSLATIONAL MODIFICATIONS**

Post-translational modifications of Tax-1 and Tax-2 proteins have been shown to play a critical role in their cellular localization, transactivation, and protein–protein interactions. Furthermore, Tax-1 and Tax-2 pleotropic effects and their structural organization make these proteins a target of many other potential post-translational events which still need to be discovered.

**PHOSPHORYLATION**

To date, six Tax-1 residues were identified as phosphorylation targets: Thr-48, Thr-184, Thr-215, Ser-300, Ser-301, and Ser-336 (Bex et al., 1999; Durkin et al., 2006; Figure 3). Adjacent serine residues at positions 300 and 301 in the carboxy-terminus of Tax represent the major sites for phosphorylation. Indeed, phosphorylation of at least one of these serine residues is required for Tax localization in nuclear bodies and for Tax-mediated activation of gene expression via both the ATF/CREB and NF-κB pathways (Bex et al., 1999). Furthermore, Ser-300 and Ser-301 are required for further post-translational modifications such as ubiquitylation, SUMOylation, and acetylation (Lodewick et al., 2009). On the other hand, the ser/threonine kinase CK2 phosphorylates Tax-1 at three residues: Ser-336, Ser-344, and Thr-351 within its C-terminus, which indirectly affects NF-κB activation (Higuchi et al., 2007; Bidoia et al., 2010). Some indirect evidence of the involvement of Ser-160 phosphorylation in stabilizing Tax-1 has been recently reported (Jeong et al., 2009). Although Tax-1 and Tax-2 share 85% homology in their amino acid sequences, and all the phosphorylated residues are conserved except for Ser-336, the phosphorylation status of Tax-2 is still not well determined. In vitro studies showed that CK2 does not phosphorylate Tax-2 as for Tax-1 (Bidoia et al., 2010). A detailed mutational analysis of Tax-2 residues may help in identifying Tax-2 phosphorylated residues and their impact on Tax-2 function.

**ACETYLATION**

Tax-1 has been shown to be acetylated at Lys-346 (Lodewick et al., 2009). Acetylated forms of Tax-1 were detected in both Tax-1 transfected 293 T cells and T lymphocytes (Lodewick et al., 2009). In the same study it has been suggested that phosphorylation of Ser-300/Ser-301 is essential for its nuclear translocation and hence is a prerequisite for Tax-1 acetylation through interaction with p300 (Figure 3). Tax-1 acetylation in turn participates in NF-κB activation (Lodewick et al., 2009). Although there is not much studies yet on Tax-2 acetylation, Lodewick et al. (2009) reported that Tax-2 may also be acetylated.

**UBIQUITYLATION AND SUMOylation**

Ubiquitylation and SUMOylation have been shown to play an important role in the cellular localization, function, and protein–protein interactions of both Tax-1 and Tax-2 (Chiari et al., 2004; Peloponese et al., 2004; Harhaj et al., 2007; Persani et al., 2010). Tax-1 has ten lysines (Figure 3). Five of these residues located within Tax-1 C-terminal region were found to be the major targets ubiquitylation [Lys-189 (K4), Lys-197 (K5), Lys-263 (K6), Lys-280 (K7), and Lys-284 (K8)], whereas SUMOylation takes place on Lys-280 (K7) and Lys284 (K8) (Lamsoul et al., 2005; Naar et al., 2006).

**UBIQUITYLATION**

Tax-1 is indeed differentially ubiquitylated by either K-48 ubiquitin chains leading to Tax degradation by the proteasome or by K-63 ubiquitin chains that mediates IκB recruitment to the centrosome and IκB activation (Kwary et al., 2008). On the other hand, Tax-1 SUMOylation is required for nuclear body formation and recruitment of RelA and IκB-γ to Tax-1-related nuclear bodies, where Tax-driven transcription is promoted (Lamsoul et al., 2005; Naar et al., 2006; Harhaj et al., 2007; Kwary et al., 2011). A RING (Really Interesting New Gene) finger domain containing protein RNF4 has recently been shown to bind putative Tax ubiquitin/SUMO modification sites K280/K284 and increase
Tax cytoplasmic enrichment and NF-κB activation (Fryrear et al., 2012). A recent report added new insights to our understanding of Tax-1 and Tax-2 ubiquitylation- and SUMOylation-dependent NF-κB activation. Bonnet et al. (2012) used Tax-1 mutants (TaxP79Q/MA) defective for nuclear body formation. Ubiquitylation levels of the mutant and the wild type protein were similar, however, the endogenous SUMOylation levels were lower in the mutant. Despite low SUMOylation levels in the mutants, NF-κB activation was not affected enforcing the possibility that low levels of SUMOylation may suffice for Tax-1-induced NF-κB activation.

The involvement of Tax-2 SUMOylation and ubiquitylation in NF-κB activation remains controversial. Journou et al. (2013) showed that in contrast to Tax-1, Tax-2 SUMOylation and ubiquitylation are not essential to activate NF-κB. In their study, Tax-2 conjugation to endogenous SUMO and ubiquitin was barely detectable, however, Tax-2 was still acetylated. This low level of conjugation to endogenous ubiquitin and SUMO did not prevent Tax-2 activation of an NF-κB-dependent promoter or its interaction with IKKγ/NEMO. Furthermore, a lysine-less Tax-2 mutant, which is defective for ubiquitylation and SUMOylation but not acetylation, is still able to transactivate an NF-κB-dependent promoter and bind and activate the IKK complex to induce RAd/p65 nuclear translocation. On the other hand, using transfection methods, Turc et al. (2012) have reported that Tax-1 and Tax-2 share a common mechanism of NF-κB activation and that both depend on their ubiquitylation and SUMOylation status. Thus, they show that patterns and levels of ubiquitylation between Tax-1 and Tax-2 are conserved, except for a reduced representation of the Tax-2 mono-ubiquitylated form compared to Tax-1.

INHIBITION OF APOPTOSIS AND INDUCTION OF DNA DAMAGE BY Tax-1 AND Tax-2

Induction of p53-dependent cell death by Tax-1 has been shown in many studies using both in vitro Tax-1 inducible cell lines (Ray and Gottlieb, 1993) and in vivo transgenic mice. Indeed, Tax-1 transgenic mice are characterized by enhanced apoptosis which is associated with elevated levels of oncoproteins such as Myc, Fos, Jun, and p53 expression (Hall et al., 1998). It is important to mention that ATL malignant transformation involves complex and multi-step mechanisms such as apoptosis.

In vitro, Tax-1 expression sensitizes cells to apoptotic cell death induced by DNA damaging agents (Kao et al., 2000) and by tumor necrosis factor alpha (TNF-α, Saggiorno et al., 2001). Upon UV irradiation, Tax-1 localization was increased at the cytoplasm and decreased in the nucleus and Tax-1 NES have been shown to be required for its stress-induced nucleocytoplasmic translocation (Gatta and Marriott, 2006). Caspase activity has been shown to be crucial for Tax-1-induced cell death and apoptosis whereas B cell lymphoma 2 (Bcl-2) expression has been shown to be associated with cell death prevention (Yamada et al., 1994; Chen et al., 1997; Chlichlia et al., 1997, 2002; Rivera-Walsh et al., 2001; Kasai and Jeang, 2004). Interestingly, Tax has been shown by many studies to both induce apoptosis and represses it. Many groups have shown the importance of Tax-1-mediated NF-κB activation in induction of apoptosis (Wheeler et al., 1993; Chen et al., 1997; Chlichlia et al., 1997; Los et al., 1998; Rivera-Walsh et al., 2001). Tax mutants defective in NF-κB activation have reduced apoptosis-inducing activities, and inhibition of Tax-mediated NF-κB transactivation partially inhibited apoptotic cell death (Los et al., 1998; Harrod et al., 2000; Rivera-Walsh et al., 2001). Tax also represses the transcription of the prosapoptic lce gene (Beauweiler et al., 1997). In addition, Tax inhibits the caspase cascade in an NF-κB-dependent manner through the induction of the caspase inhibitors XIAP, cIAP-1, and c-IAP-2 (Kelly et al., 1993).

Previous experiments performed on T cell lines derived from HTLV-2-infected individuals and Tax-2 expressing various cell lines have shown that Tax-2 is capable of inhibiting Fas-mediated apoptosis through the expression of bcl-x(L) messenger and protein (Zehender et al., 2001).

CONCLUDING REMARKS

To date, vast amount of knowledge has been produced regarding the HTLV-1 Tax-1 oncprotein. Many studies have provided some insights on Tax-1 transcriptional regulation, subcellular localization and post-translational modifications. However, less is known about HTLV-2 Tax-2 although many aspects of its activity and regulation is now being studied. That HTLV-2 is defective in promoting certain steps of leukemogenesis, may indeed serve as a useful comparative tool for understanding the pathogenicity of HTLV-1.

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Shirinian et al. Comparative study Tax-1 and Tax-2

Shirinian et al. Comparative study Tax-1 and Tax-2
Gallo, R. C. (1981). Growth of human \[\text{virus}\] in cell culture[J]. Nature. 289, 501–503.

Fukuda, R., Hayashi, A., Utsunomiya, A., Nakada, Y., Fukui, R., Ishi, K., et al. (2005). Role of CD8+ T cells in the elimination of HTLV-I-infected cells[J]. J. Virol. 83(21), 11056–11062.

Shimmin, et al. Comparative study Tax-1 and Tax-2
Shirinian et al. Comparative study Tax-1 and Tax-2
Peloponese, J. M. Jr., Iha, H., Yadavalli, Oteiza, A., and Mechti, N. (2011). The Shirinian et al. Comparative study Tax-1 and Tax-2 Nicot, C., Tie, F., and Giam, C. Z. (1998). Nicot, C., Opavsky, R., Mahieux, Nejmeddine, M., Barnard, A. L., Virol. 6491. doi: 10.1128/JVI.00036-11 by the proteasome. 1 oncoprotein tax controls forkhead human T-cell leukemia virus type 10.1128/JVI.78.21.11686-11695.2004 J. Virol. 85, 6480–6490. J. Virol. 85, 6480–6490.

Declining tendency of human T-cell leukemia virus type 1. Blood 381, 328–331. doi: 10.1038/sj.onc.1201567

Roucoux, D. F., and Murphy, E. (2004). The epidemiology and disease outcomes of human T-leukemic oncogene HTLV-II. J. AIDS Res. Hum. Retrovirol. 97, 2137–2144. doi: 10.4161/cc.6.21.4930

Rousson, D. (2007). Integrating cell-signaling pathways with NF-kappaB and IKK function. Cell Cycle 6, 2628–2632. doi: 10.4166/c.c.21.4930 Pro-Masson, C.A., Mahannah, R., Jiang, H., Ashcroft, M., Rudnevich, M., Durral, J., et al. (2000). Inactivation of p53 by human T-cell lymphomatis virus type 1 Tax requires acquisition of the NF-kappaB pathway and is dependent on p53 phosphorylation. Mol. Cell. Biol. 20, 3377–3386. doi: 10.1128/MCB.20.15.3377-3386.2000 Poiesz, R. J., Bucossi, F. W., Gandar, A. F., Bana, P. A., Minna, J. D., and Gallis, R. C. (1986). Detection and isolation of type C retrovirus particle-induced concomitant and intercellular lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl Acad. Sci. U.S.A. 72, 7455–7459. doi: 10.1073/pnas.72.12.7455

Bianchi, L. (2001). Block of cell cycle progression by the human T-lymphocytic leukemia virus type 2 tax protein and a human type IV cell receptor genes by P19 embryonal carcinoma cells. Biochem. Biophys. Res. Commun. 271, 1427–1432. doi: 10.1016/S0006-291X(00)03026-6

Declining tendency of human T-cell leukemia virus type 1. Blood 105, 672–684. doi: 10.1182/blood-2008-03-136770

Kumar, S., Barin, S., and Chaudhuri, S. (2003). The role of calreticulin in the HTLV-I tax-expressing murine model of human T-cell leukemia virus type 2 Tax. J. Virol. 71, 812–817. Renouz, D. F., and Murphy, E. L. (2004). The epidemiology and disease outcomes of human T-leukemic oncogene HTLV-II. J. AIDS Res. Hum. Retrovirol. 6, 144–154.

Renouz, K., Dobois, C., Bantignies, F., and Janielt, F. (1996). Effects on NF-kappaB-RIP processing of the interaction between the HTLV-I transactivator Tax and the proteinase. Nature 381, 328–331. doi: 10.1038/381328a0

Rousson, R., Fabre, S., Dobois, C., Bantignies, F., and Janielt, F. (1998). The C-terminus of the HTLV-I transmembrane mutatis minus the PDG with the PDG domain of cellular proteins. Oncogene 17, 645–654. doi: 10.1038/ong.2001.567 Ryseck, R. P., Ball, P., Takemura, M., Thai, V., Ise, S., and Kudo, R. (1996). Tax oncoprotein mediates interaction with the PDG of cellular domains of proteins. Oncogene 12, 674–684. doi: 10.1038/ong.2001.567

Taggioni, D., Barp, S., and Chao- Biondani, L. (2001). Block of a mitochondrial-mitochondrial apoptotic pathway in Tax-expressing murine fibroblasts. Exp. Cell Res. 265, 245–255. doi: 10.1006/excr.2001.5310

Summit, O. J., and Kurtz, T. R. (1992). The expression of human T-cell leukemia virus type I Tax: regions necessary for function determined with 47 mutant proteins. J. Virol. 66, 7185–7192.

Summit, O. J., and Kurtz, T. R. (1996). Localization of human T-cell leukemia virus type 1 Tax: regions necessary for function determined with 47 mutant proteins. J. Virol. 66, 7185–7192.

Summit, O. J., and Kurtz, T. R. (1996). Localization of human T-cell leukemia virus type 1 Tax: regions necessary for function determined with 47 mutant proteins. J. Virol. 66, 7185–7192.

Summit, O. J., and Kurtz, T. R. (1992). The expression of human T-cell leukemia virus type I Tax: regions necessary for function determined with 47 mutant proteins. J. Virol. 66, 7185–7192.
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Turci, M., Lodewick, J., Di Gennaro, Tsuji, T., Sheehy, N., Gautier, V., Tsubata, C., Higuchi, M., Takahashi, Toro, C., Rodes, B., Bassani, S., Turci, M., Romanelli, M. G., Lorenzi, Trinh, D., Jeang, K. T., and Semmes, O. J., et al. (2012). Ubiquitination and carrier- and energy-independent virus type I (HTLV-1) tax protein is imported of the human T lymphotropic virus type 1 Tax dimer with CREB and the viral 21-base-pair repeat. J. Virol. 86, 4173–4180. doi: 10.1128/JVI.00242-08

Wu, X., and Sun, S. C. (2007). Retroviral oncoprotein Tax downregulates NF-kappaB by activating Taki and modulating the physical association of Taki with IKKbeta in vitro. J. Biol. Chem. 282, 17844–17852. doi: 10.1074/jbc.M610522200

Xia, G., Crève, M. E., Fong, A., Haba, U., Wu, M. T., Waterfield, M., et al. (2001). Retroviral oncoprotein Tax induces processing of NF-kappaB/p65 in T cells evidence for the involvement of Endo-PLD. J. Biol. Chem. 276, 4805–4815. doi: 10.1074/jbc.2004.07.083

Xu-Monette, Z. Y., Medeiros, L. J., Xiao, G., Cvijic, M. E., Fong, A., Yin, M. J., Paulssen, E., Seeler, J. S., and Green, M. R. (1993). Molecular epidemiology and evidence for the involvement of HTLV-I Tax oncoprotein is essential for viral 21-base-pair repeat. J. Virol. 69, 3668–3675. doi: 10.1128/JVI.69.10.3668-3675.1995

Yamamoto, K., Ishida, T., Nakano, K., Yamagishi, M., Yamashita, T., Tanaka, Y., et al. (2011). SMRT interacts with HTLV-1 Tax and regulates subcellular localization of Tax. Cancer Sci. 102, 260–266. doi: 10.1111/j.1349-7006.2010.01752.x

Yamazaki, J., Mizumaki, T., Takizawa, K., Karamata, M., Momose, H., Masumi, A., et al. (2009). Identification of cancer stem cells in a Tax transgenic (Tan-Tg) tumor model of adult T-lymphoid leukemia. Blood 114, 2759–2769. doi: 10.1182/blood-2008-08-174425

Yin, M. J., Christoffersen, L. B., Yamamoto, Y., Kosi, Y. T., Xu, S., Mercurio, F., et al. (1998). HTLV-I Tax protein binds to MIEK1 to stimulate JNK kinase activity and NF-kappaB activation. Cell 95, 875–884. doi: 10.1016/S0092-8674(98)70347-9

Yin, M. J., and Gaynor, R. B. (1996). Complex formation between CREB and Tax enhances the binding affinity of CREB for the human T-cell leukemia virus type 1 21-base-pair repeats. Mol. Cell. Biol. 16, 5126–5136.

Yin, M. J., Paulson, E., Seeler, J., and Gaynor, R. B. (1995a). Chimeric proteins composed of Jun and CREB define domains required for interaction with the human T-cell leukemia virus type 1 Tax protein. J. Virol. 69, 6220–6238.

Yin, M. J., Paulson, E. J., Seeler, J. S., and Gaynor, R. B. (1995b). Protein localization determinant in the N-terminal region of adult T-cell leukemia/lymphoma virus type 1 Tax dimer with CREB and the viral 21-base-pair repeat. J. Virol. 86, 6968–6976. doi: 10.1128/JVI.86.12.6968-6976.1992

Yoshida, M., Miyoshi, I., and Hinuma, Y. (1982). Isolation and characterization of HTLV-I 21-base-pair repeats of adult T-cell leukemia/lymphoma virus type 1 21-base-pair repeat. J. Virol. 69, 36607-K

Yoshida, M., Satou, Y., Y asunaga, J., Wheeler, J. F., Beck, T. L., Klatte, S., et al. (2007). The PI3K/Akt pathway and p53 pathway. Cell Cycle 6, 3668–3675. doi: 10.1089/cc.2006.6805

Yoshida, M., Oie, M., Tanaka, Y., Gejyo, F., et al. (2005). Molecular epidemiology and changes in keratin-containing filaments is permitted, provided the original author(s) and source are credited and that the original publication is not used in a new work, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

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