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Is there a role for ubiquitin or SUMO in human T-cell leukemia virus type 2 Tax-induced NF- κ B activation?

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Abstract

It is well established that the human T-cell leukemia virus type 1-encoded oncoprotein Tax (Tax1) undergoes polyubiquitination as part of its mechanism to persistently activate NF- κ B. However, it remains unclear whether Tax2 encoded by the closely related human T-cell leukemia virus type 2 utilizes any post-translational mechanisms to activate NF- κ B. This study examines the role of ubiquitination and SUMOylation in Tax2 activation of NF- κ B. The authors have demonstrated that, in contrast to Tax1, Tax2 is not conjugated by ubiquitin or SUMO proteins. Overexpression of the E2 ubiquitin-conjugating enzyme Ubc13 specifically enhances Tax1, but not Tax2, ubiquitination and NF- κ B activation. Furthermore, a Tax2 lysineless mutant that is unable to be ubiquitinated, SUMOylated or acetylated retains NEMO/IKK γ interactions and activation of the NF- κ B pathway. Together, these results provide evidence that Tax1 and Tax2 utilize distinct mechanisms to activate NF- κ B.

Keywords

HTLV-1; HTLV-2; NF- κ B; SUMOylation; Tax; ubiquitination

Human T-cell leukemia virus type 1 (HTLV-1) and type 2 (HTLV-2) are closely related complex retroviruses that share a common genomic organization: 5' long terminal repeat–*gag-pol-env*–pX–3' long terminal repeat [1]. The pX region encodes the transactivating protein Tax, which regulates viral gene expression and also activates cellular transcription factors including NF- κ B, ATF/CREB and serum response factor. Despite many similarities, HTLV-1 and HTLV-2 display distinct tropisms and pathogenicity. HTLV-1 predominantly transforms CD4⁺ T cells, whereas HTLV-2 transforms CD8⁺ T cells *in vitro* [2]. HTLV-1 is the etiological agent of adult T-cell leukemia/lymphoma, an aggressive malignancy of CD4⁺CD25⁺ T cells [3]. HTLV-1 infection is also associated with an inflammatory neurodegenerative disease termed HTLV-1-associated myelopathy/tropical spastic paraparesis [4]. In contrast to HTLV-1, HTLV-2 infection has not been linked to leukemia or lymphoma, despite its ability to transform human T cells *in vitro* [5].

Tax1 and Tax2 exhibit 78% amino acid sequence identity, although the C-terminal regions of the two proteins are largely divergent, suggesting that differences in the C-terminus of the two proteins may account for differences in oncogenicity. Tax1, but not Tax2, contains a

PDZ binding domain in the C-terminus that mediates interactions with the tumor suppressor Dlg1 as well as other cellular PDZ domain-containing proteins [6]. The PDZ binding domain of Tax1 plays a key role in HTLV-1-induced T-cell proliferation and genetic instability *in vivo* [7]. Tax1 also contains a small domain (amino acids 225–232), lacking in Tax2, that is required for activation of the noncanonical pathway of NF- κ B controlled processing of the NF- κ B2/p100 precursor protein [8]. Tax1 is a potent activator of the noncanonical NF- κ B pathway, and is required for IL-2-independent growth of the T-cell line CTLL-2 [9]. Other notable differences between Tax1 and Tax2 include their distinct subcellular localization profiles. Tax1 is largely localized in the nucleus within nuclear speckled structures and may also be found within the Golgi or centrosome, where it activates IKK and NF- κ B [10,11]. Conversely, Tax2 localization is predominantly cytoplasmic [12].

The NF- κ B transcription factor is a key regulator of innate and adaptive immune responses to pathogens, lymphoid tissue development and survival of lymphocytes. The canonical NF- κ B pathway generally regulates inflammation and cell survival and is activated by diverse stimuli including proinflammatory cytokines TNF or IL-1 β , antigen receptor crosslinking or genotoxic stress [13]. All of these stimuli activate a multisubunit kinase complex IKK that phosphorylates the NF- κ B inhibitor I κ B α to promote its destruction by the proteasome, thus allowing NF- κ B dimers to enter the nucleus and activate gene expression. The noncanonical NF- κ B pathway is characterized by NF- κ B-inducing kinase-dependent processing of NF- κ B2/p100 to p52 and regulates lymphoid organogenesis and cell survival. The noncanonical pathway is activated by TNF receptor superfamily members such as CD40, BAFFR and LT- β R. NF- κ B activation is tightly regulated by multiple inhibitory proteins in a negative feedback loop to ensure transient activation of signaling [14]. Dysregulation of NF- κ B leading to its uncontrolled persistent activation may result in chronic inflammation and malignancy. Oncogenic viruses such as HTLV-1 and EBV usurp NF- κ B signaling to drive the proliferation and survival of virus-infected cells [15]. Tax1 expression is sufficient to trigger persistent activation of the canonical and noncanonical NF- κ B pathways, which play obligatory roles in T-cell immortalization by HTLV-1 [16]. Both Tax1 and Tax2 activate the canonical NF- κ B pathway and interact with the IKK regulatory protein IKK γ /NEMO; however, it remains unclear if Tax1 and Tax2 use the same mechanism to activate IKK and NF- κ B.

Tax1 has been demonstrated to undergo numerous post-translational modifications (PTMs) such as ubiquitination, SUMOylation, phosphorylation and acetylation [17]. Ubiquitination of Tax1 is an important mechanism for activation of NF- κ B since a Tax1 mutant unable to be ubiquitinated is impaired in NF- κ B activation [18]. Ubiquitination occurs in an enzymatic cascade involving ubiquitin activating (E1), ubiquitin conjugating (E2) and ubiquitin ligase (E3) enzymes. Polyubiquitin chains may be linked via internal lysine (K) residues in ubiquitin (K6, K11, K27, K29, K33, K48 and K63), although K48- and K63-linked polyubiquitin chains are the best characterized. Whereas K48-linked polyubiquitin chains mark proteins for proteasomal degradation, K63-linked chains mostly serve nondegradative roles in signal transduction and the DNA damage response by acting as scaffolds for protein complexes. K63-linked polyubiquitination of Tax1 triggers NF- κ B activation by promoting Tax localization to the Golgi and facilitating an interaction with NEMO/IKK γ [19]. Tax1 contains ten lysine residues, and K4–8 serve as the key targets for ubiquitin conjugation [18]. Several host factors have been implicated in the regulation of Tax1 ubiquitination. The E2 ubiquitin conjugating enzyme Ubc13 has been shown to play a role in Tax1 K63-linked ubiquitination and NF- κ B activation [20]. The E3 ligase RING finger protein 4, a SUMOtargeted ubiquitin ligase, has been shown to ubiquitinate SUMOylated nuclear Tax1 to stimulate its nuclear egress upon DNA damage [21]. The deubiquitinase STAMBPL1 is similarly required for DNA damage-induced Tax nuclear export, although the target of STAMBPL1 remains unknown [11]. Another E3 ligase

PDLIM2 was shown to conjugate K48-linked polyubiquitin chains onto Tax1 to trigger its proteasomal degradation within the nuclear matrix [22]. Finally, the deubiquitinase USP20 cleaves ubiquitin chains from Tax1 as a mechanism to inhibit NF- κ B [23].

Despite the progress that has been made in our understanding of Tax1 regulation by PTMs, much less is known regarding Tax2 PTMs and their potential regulation of NF- κ B. In a recent study by Journo *et al.*, the authors first confirmed that Tax2 colocalizes with IKK γ /NEMO in the HTLV-2-infected T-cell line C19 using immunofluorescence studies [24]. Given the well-established role of ubiquitination and other PTMs in Tax1-induced NF- κ B activation, the authors conducted experiments to determine whether Tax2 was also modified by ubiquitination, SUMOylation or acetylation as a potential mechanism to activate NF- κ B. His-tagged Tax1 and Tax2 proteins were expressed in HeLa cells, purified by Ni-nitrilotriacetic acid beads and subjected to western blotting to detect conjugation of Tax with endogenous ubiquitin or SUMO molecules. As expected, Tax1 was heavily conjugated with polyubiquitin and SUMO chains; however, Tax2 was not modified with either ubiquitin or SUMO proteins at detectable levels [24]. Furthermore, overexpression of Ubc13 enhanced Tax1 ubiquitination and NF- κ B activation, but did not promote Tax2 ubiquitination or NF- κ B activation [24]. Using Tax1 and Tax2 K0 mutants in which all lysines were mutated to arginines, they further demonstrated that Tax2, but not Tax1, was still able to activate an NF- κ B luciferase reporter [24]. Consistent with these results, Tax2-K0 retained its ability to relocalize IKK γ /NEMO to perinuclear hotspots, activate IKK and promote the nuclear translocation of RelA/p65 [24]. The authors then generated a series of Tax1/2 chimeric proteins to identify the determinants of PTMs on Tax1 and Tax2. Confocal studies, ubiquitination and SUMOylation assays, and luciferase reporter assays were conducted with Tax1, Tax2, Tax1₁₋₉₀₋₂₉₁₋₁₁₃₋₁₁₄₋₃₅₃, Tax2₁₋₁₁₃₋₁₁₄₋₃₅₃ and Tax2₁₋₂₂₀₋₁₂₂₁₋₃₅₃ fusion proteins [24]. Collectively, these studies demonstrated that: the subcellular localization of Tax proteins is not responsible for the lack of Tax2 ubiquitination or SUMOylation; that the Tax1 domain containing K4–K10 is sufficient for Tax ubiquitination; and that the Tax1 domain containing K6–K10 is sufficient for Tax SUMOylation. Since K4–K8 are conserved in Tax1 and Tax2 proteins, it is likely that the sequences surrounding these lysines contain determinants for ubiquitination and SUMOylation of Tax.

Conclusion & future perspective

In conclusion, the study by Journo *et al.* has demonstrated that Tax1 and Tax2 proteins activate NF- κ B through distinct molecular mechanisms [24]. Whereas Tax1 requires ubiquitin to engage the IKK complex and activate NF- κ B, Tax2 does not appear to require either ubiquitination or SUMOylation to activate NF- κ B (Figure 1). One possibility is that Tax2 may interact with NEMO/IKK γ and the IKK complex via an unidentified ubiquitinated protein. However, the authors have ruled out TRAF6, since Tax2 does not bind to TRAF6 [24]. A recent study by Turci *et al.* analyzed the role of Tax2 PTMs in the activation of NF- κ B and surprisingly found that Tax2 undergoes ubiquitination and SUMOylation, and that these modifications play a role in NF- κ B activation [25]. How can these contradictory results from the two studies be reconciled? The main difference in the two studies is that Journo *et al.* examined Tax modifications with endogenous ubiquitination and SUMOylation, whereas Turci *et al.* used overexpressed and epitope-tagged ubiquitin and SUMO constructs, which could lead to artifactual results. Nevertheless, additional studies are needed to confirm a lack of Tax2 PTMs in NF- κ B activation. Moving forward, it is important that future studies examining Tax PTMs are performed with endogenous proteins in more physiologically relevant cells such as primary T cells.

The study by Journo *et al.* also raises important questions about the marked differences between HTLV-1 and HTLV-2 pathogenesis. Tax1 contains additional domains lacking in

Tax2 that support activation of the noncanonical NF- κ B pathway and binding to PDZ domain proteins. Furthermore, the study by Journo *et al.* suggests that Tax2 does not undergo ubiquitination and SUMOylation as part of its mechanism to activate NF- κ B. Whether these differences in the mechanisms of NF- κ B activation translate to any qualitative or quantitative effects on the NF- κ B response and the spectrum of NF- κ B-dependent genes that are induced by the Tax proteins remains to be seen. Also, Tax1 inactivates key NF- κ B negative feedback inhibitors such as A20 by blocking phosphorylation of the TAX1BP1 adaptor [26]. It will be interesting to determine whether Tax2 possesses a similar capability to inactivate TAX1BP1 and A20. It is likely that inactivation of NF- κ B negative feedback inhibitors is a critical step in establishing a persistent NF- κ B response that enhances cell survival and immortalization. Finally, although HTLV-2 is not associated with any known neoplasms, Tax2 was recently shown to be highly efficient at transforming primary human CD4⁺ T cells by exploiting an autophagy-dependent survival pathway [27]. In stark contrast, Tax1 was shown to exert a rather weak oncogenic effect in primary human T cells and Tax1-induced immortalization of T cells was a rare event [28]. Taken together, it is likely that additional viral proteins, such as HBZ, cooperate with Tax in the oncogenic process. Future studies on Tax1 and Tax2 oncogenic activities should therefore be examined in the context of other viral proteins during HTLV infection.

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■ ■ of considerable interest

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Executive summary

Objective of the study

- To determine whether human T-cell leukemia virus Tax2 requires ubiquitination and/or SUMOylation for NF- κ B activation.

Methods

- HEK293T and HeLa cell transfection assays and Tax1/2 ubiquitination assays.
- Confocal analysis of IKK γ /NEMO localization upon expression of Tax1/2.
- Coimmunoprecipitation assays to detect binding between Tax1/2 mutants and IKK γ /NEMO.
- Luciferase assays to examine Tax1- and Tax2-mediated NF- κ B activation.

Conclusion

- Human T-cell leukemia virus Tax2 activates NF- κ B in a ubiquitin and SUMO-independent manner. Therefore, Tax1 and Tax2 appear to activate NF- κ B by distinct mechanisms.

Figure 1. Differential requirement for Tax1 and Tax2 ubiquitination and SUMOylation for NF- κ B activation

HTLV-1 Tax (Tax1) undergoes K63-linked polyubiquitination and SUMOylation to activate IKK and NF- κ B. By contrast, HTLV-2 Tax (Tax2) activates NF- κ B despite an apparent lack of conjugation with ubiquitin or SUMO.

HTLV: Human T-cell leukemia virus.