Published in final edited form as:

Future Virol. 2011 October ; 6(10): 1165–1168. doi:10.2217/fvl.11.94.

Outfoxing FoxO transcription factors: HTLV-1 Tax oncoprotein inactivates FoxO4 via the ubiquitin-proteasome pathway

Noula Shembade¹ and Edward W Harhaj^{†,1}

¹Department of Microbiology & Immunology, Sylvester Comprehensive Cancer Center, The University of Miami, Miller School of Medicine, 1550 NW 10 Avenue, Miami, FL 33136, USA

Abstract

Evaluation of: Oteiza A, Mechti N. The human T-cell leukemia virus type 1 oncoprotein tax controls forkhead box O4 activity through degradation by the proteasome. *J. Virol.* 85(13), 6480-6491 (2011). This study examines downstream signaling events of PI3K/AKT in the context of human T cell leukemia virus type 1 (HTLV-1) infection. The authors have demonstrated that the HTLV-1 Tax oncoprotein triggers the ubiquitination and proteasomal degradation of the FoxO4 transcription factor. Phosphorylation by AKT is requisite for Tax-induced FoxO4 degradation since mutation of the AKT phosphorylation sites abrogates FoxO4 degradation. Furthermore, Tax enhances the interaction between FoxO4 and the E3 ubiquitin ligase MDM2 which presumably leads to FoxO4 ubiquitination. Consistently, knockdown of MDM2 with a shRNA plasmid attenuates FoxO4 ubiquitination, revealing an important role for MDM2 in Tax-induced FoxO4 ubiquitination. Finally, Tax represses FoxO4 transcriptional activity in a dose-dependent manner. Taken together, the findings by Oteiza *et al.* suggest that Tax inactivates the tumor suppressor FoxO4 downstream of PI3K/AKT, which may play a role in HTLV-1-induced oncogenesis.

Keywords

AKT; FoxO4; HTLV-1; MDM2; proteasome; Tax; ubiquitination

Virus infection triggers the activation of innate immune pathways and transcription factors including NF- κ B and interferon regulatory factors, which in turn upregulate genes that orchestrate the antiviral response. NF- κ B and interferon regulatory factors, as well as other transcription factors, are regulated by complex post-translational modifications such as phosphorylation, ubiquitination and acetylation that influence subcellular localization, protein stability, DNA binding, transcriptional activity and protein-protein interactions. However, viral-encoded regulatory proteins may exploit post-translational modifications as a mechanism to inactivate key host transcription factors in order to circumvent an antiviral response and promote viral replication and cell survival.

The oncogenic retrovirus human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia/lymphoma (ATLL) [1] and the neurodegenerative disease

© 2011 Future Medicine Ltd

[†]Author for correspondence: Tel.: +1 305 243 7893, Fax: +1 305 243 6410, eharhaj@med.miami.edu.

Financial & competing interests disclosure: The laboratory of EW Harhaj is supported by NIH grants RO1CA135362, RO1GM083143 and PO1CA128115. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

HTLV-1-associated myelopathy/tropical spastic paraparesis [2]. HTLV-1 encodes a regulatory protein Tax in the pX region of the viral genome that deregulates host signaling pathways and the cell cycle machinery in CD4⁺ T lymphocytes, ultimately leading to genomic instability, malignant transformation and the onset of ATLL after a 20–40 year latency period [1]. HTLV-1 Tax is a potent activator of the NF- κ B transcription factor family which plays an essential role in HTLV-1-mediated T-cell transformation and the survival of ATLL cells [3]. The ubiquitin–proteasome pathway is a critical regulator of NF- κ B activation due to persistent phosphorylation and degradation of the inhibitory I κ B proteins in HTLV-1 transformed cells [4]. Indeed, a proteasome inhibitor blocks NF- κ B signaling and triggers cell death of HTLV-1-transformed cell lines and primary ATLL cells [5].

FoxO transcription factors (FoxO1, FoxO3, FoxO4 and FoxO6), serve important roles in cell cycle progression, cell survival, tumor suppression and DNA repair pathways [6]. With regard to virus infection, FoxO3 plays a critical role in limiting the magnitude of T-cell expansion by antagonizing the production of inflammatory cytokines within dendritic cells [7]. The phosphorylation and ubiquitination of FoxO proteins serves as a key regulatory mechanism by controlling their intracellular localization, stability, and transcriptional specificity [8]. In T lymphocytes, engagement of the T-cell antigen receptor (TCR) results in the phosphorylation of FoxO proteins by the serine/threonine kinases AKT (also known as PKB) and I κ B kinase β (IKK β), leading to FoxO3a nuclear exclusion and inactivation [9,10]. By contrast, phosphorylation of FoxO by upstream regulators such as JNK and MST1 under stress conditions is essential for nuclear localization and activation [11]. Thus, phosphorylation of FoxO proteins has divergent effects on their subcellular localization depending on the activating kinase and specific stimulus.

AKT is a key regulator of cell proliferation and survival and is oncogenic in numerous cancers. AKT also activates NF- κ B via the upstream IKKs to increase the expression of antiapoptotic genes [12]. Many oncogenic viral proteins including HTLV-1 Tax utilize AKT as a mechanism to activate NF- κ B and drive cell proliferation and survival [13]. Furthermore, the PTEN and SHIP-1 phosphatases that serve as inhibitors of AKT signaling are downregulated in ATLL, which may contribute to the persistent activation of NF- κ B [14]. Therefore, both AKT and NF- κ B are constitutively activated in the context of HTLV-1 transformation, however the role of FoxO transcription factors in Tax-mediated oncogenesis has not been examined. To this end, a recent study by Oteiza and Mechti in *Journal of Virology* demonstrated that HTLV-1 Tax regulates the stability and transcriptional activity of FoxO4, which may have implications for the proliferation and survival of HTLV-1 infected T lymphocytes [15]. The authors observed proteasome-dependent degradation of FoxO4 upon overexpression of Tax in HEK293T cells. Interestingly, Tax-mediated degradation of FoxO4 was dependent on the relative ratios between transfected Tax and FoxO4 plasmids [15]. Although caution must certainly be taken when interpreting overexpression experiments, these results may be relevant since Tax expression is lost in most ATLL tumors and the expression of Tax may fluctuate *in vivo* depending on the efficiency of immune surveillance [1]. It has been previously shown that AKT phosphorylates FoxO4 on Thr32, Ser193, and Ser262 in response to TCR ligation [16]. Oteiza *et al.* have also demonstrated that these phosphorylation sites are essential for Tax-induced proteasomal degradation since mutation of these amino acids results in constitutive nuclear localization of FoxO4 and resistance to proteasomal degradation [15].

Oteiza *et al.* have also provided evidence that the E3 ubiquitin ligase MDM2 is involved in FoxO4 ubiquitination and degradation [15]. MDM2 has been previously shown to ubiquitinate FoxO3a to cause its degradation [17], as well as FoxO4 monoubiquitination and activation [18]. Indeed, Oteiza *et al.* demonstrated that Tax promotes an interaction between

FoxO4 and MDM2 leading to poly- and monoubiquitination of FoxO4 in a dose-dependent manner [15]. Consistently, knockdown of MDM2 by shRNA impaired Tax-induced FoxO4 poly- and monoubiquitination [15], however this does not exclude the involvement of other E3 ligases. Since the interaction between an E3 ubiquitin ligase and its substrates is usually transient, it will be interesting to determine if Tax physically interacts with MDM2 and FoxO4 in order to bridge these interactions. Furthermore, Oteiza *et al.* have validated the regulation of FoxO4 by Tax in a more physiological context using HTLV-1-transformed cell lines and Jurkat T cells [15].

Conclusion & future perspective

In conclusion, the study by Oteiza *et al.* has identified a transcription factor (FoxO4) that is inactivated by Tax via posttranslational modifications (Figure 1) [15]. AKT phosphorylates FoxO proteins in the nucleolus to create new binding sites for the 14-3-3 chaperone proteins, which facilitate the nuclear export of FoxO proteins in TCR-stimulated cells [9]. These findings, together with the regulation of FoxO4 by HTLV-1 Tax, raise some interesting questions that should be addressed in future studies. First, in which cellular compartment does AKT (activated by Tax) phosphorylate FoxO4? Presumably this event takes place in the nucleus, although it needs to be demonstrated experimentally. Second, is the transcriptional activity of the FoxO4 triple mutant that is refractory to AKT phosphorylation still inhibited by Tax? Third, does Tax modulate the expression and/or activity of phosphatases that counteract FoxO4 phosphorylation? Lastly, is Tax noncanonical polyubiquitination (Lys63-linked polyubiquitin chains) required for AKT activation and phosphorylation of FoxO4? It is possible that Tax polyubiquitination is required for AKT activation because Tax polyubiquitination is essential for IKK and NF- κ B activation [19]. It will also be necessary to examine the expression of FoxO4 (both mRNA and protein) in primary ATLL samples and determine the functional consequences of restoring FoxO4 protein expression in HTLV-I transformed cell lines. Because the inactivation of FoxO4 by PI3K/AKT is associated with cell proliferation and survival, this event may be a key step in the progression to ATLL.

Bibliography

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1. Matsuoka M. Human T-cell leukemia virus type I and adult T-cell leukemia. *Oncogene*. 2003; 22(33):5131–5140. [PubMed: 12910250]
2. Kiwaki T, Umehara F, Arimura Y, et al. The clinical and pathological features of peripheral neuropathy accompanied with HTLV-I associated myelopathy. *J Neurol Sci*. 2003; 206(1):17–21. [PubMed: 12480079]
3. Sun SC, Yamaoka S. Activation of NF- κ B by HTLV-I and implications for cell transformation. *Oncogene*. 2005; 24(39):5952–5964. [PubMed: 16155602]
4. Sun SC, Elwood J, Beraud C, Greene WC. Human T-cell leukemia virus type I Tax activation of NF- κ B/Rel involves phosphorylation and degradation of I κ B α and RelA (p65)-mediated induction of the *c-rel* gene. *Mol Cell Biol*. 1994; 14(11):7377–7384. [PubMed: 7935451]
5. Satou Y, Nosaka K, Koya Y, Yasunaga JI, Toyokuni S, Matsuoka M. Proteasome inhibitor, bortezomib, potentially inhibits the growth of adult T-cell leukemia cells both *in vivo* and *in vitro*. *Leukemia*. 2004; 18(8):1357–1363. [PubMed: 15190257]
6. Burgering BM. A brief introduction to FOXOlogy. *Oncogene*. 2008; 27(16):2258–2262. [PubMed: 18391968]

7. Dejean AS, Beisner DR, Ch'en IL, et al. Transcription factor FoxO3 controls the magnitude of T cell immune responses by modulating the function of dendritic cells. *Nat Immunol.* 2009; 10(5):504–513. [PubMed: 19363483]
8. Calnan DR, Brunet A. The FoxO code. *Oncogene.* 2008; 27(16):2276–2288. [PubMed: 18391970]
- 9••. Brunet A, Bonni A, Zigmond MJ, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell.* 1999; 96(6):857–868. Demonstrated that AKT phosphorylates a forkhead transcription factor to inhibit apoptosis. [PubMed: 10102273]
10. Hu MC, Lee DF, Xia W, et al. IκB kinase promotes tumorigenesis through inhibition of forkhead FoxO3a. *Cell.* 2004; 117(2):225–237. [PubMed: 15084260]
11. Essers MA, Weijzen S, de Vries-Smits AM, et al. FoxO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J.* 2004; 23(24):4802–4812. [PubMed: 15538382]
12. Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-κB activation by tumour necrosis factor requires the Akt serine–threonine kinase. *Nature.* 1999; 401(6748):82–85. [PubMed: 10485710]
13. Jeong SJ, Pise-Masison CA, Radonovich MF, Park HU, Brady JN. Activated AKT regulates NF-κB activation, p53 inhibition and cell survival in HTLV-1-transformed cells. *Oncogene.* 2005; 24(44):6719–6728. [PubMed: 16007163]
14. Fukuda RI, Tsuchiya K, Suzuki K, et al. Human T-cell leukemia virus type I Tax down-regulates the expression of phosphatidylinositol 3,4,5-trisphosphate inositol phosphatases via the NF-κB pathway. *J Biol Chem.* 2009; 284(5):2680–2689. [PubMed: 19047050]
15. Oteiza A, Mechti N. The human T-cell leukemia virus type 1 oncoprotein Tax controls forkhead box O4 activity through degradation by the proteasome. *J Virol.* 2011; 85(13):6480–6491. [PubMed: 21525355]
16. Huang H, Tindall DJ. Dynamic FoxO transcription factors. *J Cell Sci.* 2007; 120:2479–2487. [PubMed: 17646672]
- 17••. Yang JY, Zong CS, Xia W, et al. ERK promotes tumorigenesis by inhibiting FoxO3a via MDM2-mediated degradation. *Nat Cell Biol.* 2008; 10(2):138–148. Demonstrated that ERK phosphorylates and inactivates FoxO3a via MDM2 and the ubiquitin–proteasome pathway. [PubMed: 18204439]
18. Brenkman AB, de Keizer PL, van den Broek NJ, Jochemsen AG, Burgering BM. MDM2 induces mono-ubiquitination of FoxO4. *PLoS One.* 2008; 3(7):E2819. [PubMed: 18665269]
- 19•. Shembade N, Harhaj NS, Yamamoto M, Akira S, Harhaj EW. The human T-cell leukemia virus type 1 Tax oncoprotein requires the ubiquitin-conjugating enzyme Ubc13 for NF-κB activation. *J Virol.* 2007; 81(24):13735–13742. Demonstrated that K63-linked polyubiquitination of human T-cell leukemia virus type 1 Tax is critical for NF-κB activation. [PubMed: 17942533]

Executive summary

Objectives of this study

- To determine if human T-cell leukemia virus type 1 Tax inactivates the FoxO4 transcription factor by proteasome-dependent degradation.

Methods

- HEK293T cell transfection assays and western blotting to examine FoxO4 stability and ubiquitination.
- Coimmunoprecipitation assays to detect FoxO4/MDM2 interactions in the absence or presence of Tax.
- Luciferase assays to examine FoxO4 transcriptional activity in the absence or presence of Tax.

Conclusion

- Human T-cell leukemia virus type 1 Tax targets FoxO4 for inactivation via AKT-dependent phosphorylation, which in turn triggers its nuclear export, polyubiquitination by the E3 ubiquitin ligase MDM2 and degradation by the proteasome.

Figure 1. Inactivation of FoxO4 by the human T-cell leukemia virus type 1 Tax oncoprotein
Human T-cell leukemia virus type 1 Tax activates AKT, which in turn phosphorylates FoxO4 at three conserved serine and threonine residues (Thr32, Ser193 and Ser262). The phosphorylation of FoxO4 stimulates binding to 14-3-3 proteins and the subsequent nuclear export of FoxO4 to the cytoplasm. FoxO4 then undergoes poly- and mono-ubiquitination by the E3 ubiquitin ligase MDM2 to trigger its proteosomal degradation.