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The transcription elongation factor ELL2 is specifically upregulated in HTLV-1-infected T-cells and is dependent on the viral oncoprotein tax

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ABSTRACT

The oncogene Tax of human T-cell leukemia virus type 1 (HTLV-1) is a potent transactivator of viral and cellular transcription. Here, we identified ELL2 as the sole transcription elongation factor to be specifically upregulated in HTLV-1-/Tax-transformed T-cells. Tax contributes to regulation of ELL2, since transient transfection of Tax increases *ELL2* mRNA, Tax transactivates the *ELL2* promoter, and repression of Tax results in decrease of ELL2 in transformed T-lymphocytes. However, we also measured upregulation of ELL2 in HTLV-1-transformed cells exhibiting undetectable amounts of *Tax*, suggesting that ELL2 can still be maintained independent of continuous Tax expression. We further show that Tax and ELL2 synergistically activate the HTLV-1 promoter, indicating that ELL2 cooperates with Tax in viral transactivation. This is supported by our findings that Tax and ELL2 accumulate in nuclear fractions and that they co-precipitate upon co-expression in transiently-transfected cells. Thus, upregulation of ELL2 could contribute to HTLV-1 gene regulation.

Introduction

Human T-cell leukemia virus type 1 (HTLV-1), a delta retrovirus, is the causative agent of adult T-cell leukemia/lymphoma (ATL) and of the neurodegenerative disorder HTLV-1-associated with myelopathy/tropical spastic paraparesis (HAM/TSP) (Poiesz et al., 1980; Yoshida et al., 1982; Gessain et al., 1985; Osame et al., 1986). ATL is a severe neoplasia of $CD4^+$ T lymphocytes, which develops in about 2–5% of infected carriers after lifelong persistence of the integrated provirus. Since ATL therapy remains largely unsatisfactory till now, break-out of ATL often results in death of patients (Currer et al., 2012; Matsuoka and Jeang, 2007).

The oncoprotein Tax of HTLV-1 is a potent transactivator of both cellular and viral transcription. Tax plays an important role in initiating transformation of T-cells in infected patients and promotes proliferation of infected T-cell clones. Functionally, Tax interacts with a variety of cellular proteins, thereby fostering an accumulation of genetic alterations potentially culminating in the outgrowth of ATL (Boxus et al., 2008). Tax deregulates different signaling pathways including NF-κB, cAMP response element binding (CREB), serum response factor, and PI3K/Akt (reviewed by (Currer et al., 2012; Matsuoka and Jeang, 2007; Hall and Fujii, 2005; Chan and Greene, 2012)). In addition to its interference with cellular proteins, Tax acts as a potent transactivator of viral transcription by interacting with and recruiting different transcription factors and histone-modifying enzymes like CREB, CREB binding protein (CBP), p300, and PCAF to Tax responsive elements (TRE) within the U3R region of the long terminal repeats (LTR) of the HTLV-1 provirus (reviewed in (Currer et al., 2012; Boxus et al., 2008; Nyborg et al., 2010)). The TREs are three conserved 21-bp repetitive elements called viral cAMP response elements (vCREs) which are required for Tax–pCREB complex formation (Nyborg et al., 2010). Furthermore, the positive transcription elongation factor b, P-TEFb, is recruited to the LTR by Tax (Zhou et al., 2006). P-TEFb is composed of cyclin-dependent kinase 9 (CDK9) and cyclin T1, T2 or K, and functions by phosphorylating the C-terminal domain of the largest subunit of RNA Polymerase II (RNA Pol II) and negative elongation factors DSIF and NELF. These events antagonize the actions of the negative factors, release RNA Pol II from promoter-proximal pausing, and trigger the production of full-length mRNA transcripts (Barboric and Peterlin, 2005; Peterlin and Price, 2006). P-TEFb is essential for efficient transactivation of the HTLV-1-LTR (Zhou et al., 2006; Cho et al., 2007).

Transcription elongation is a key step in gene regulation and frequently deregulated during the progression of cancer (Luo et al., 2012). Not only transcription initiation, but also transcription elongation is important for efficient gene regulation(Peterlin and Price 2006; Luo et al., 2012). Transcription elongation is regulated by a variety of specific classes of transcription elongation factors that influence different classes of genes (Sims et al., 2004). Among

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all known elongation factors, ELL2 is the stoichiometrically limiting component of a super elongation complex (SEC), which also contains P-TEFb, and has been identified in mixed-lineage leukemia (MLL), during HIV-transcription, and in embryonic stem cells (Luo et al., 2012; Lin et al., 2010; He et al., 2010; Sobhian et al., 2010; Lin et al., 2011). ELL2 is a member of the eleven nineteen rich-leukemia family of elongation factors (ELL family) (Shilatifard et al., 1997). Functionally, ELL2 keeps the 3'OH end of the nascent mRNA in alignment with the catalytically active center of RNA Pol II (Sims et al., 2004; He et al., 2010; Shilatifard et al., 1997). Thus, ELL2 is important for transition of RNA Pol II from its promoter-proximal paused state into its elongation state. Moreover, ELL2 had also been described to regulate pre-mRNA processing and polyadenylation of nascent mRNAs during plasma cell differentiation (Martincic et al., 2009; Milcarek et al., 2011; Benson et al., 2012).

Despite significant efforts in the molecular characterization of the viral Tax oncoprotein, the mechanisms of gene regulation driving HTLV-1-transcription remain to be fully elucidated (Currer et al., 2012). In this study, we asked whether HTLV-1 deregulates the expression of transcription elongation factors. Here, we identify ELL2 as the sole cellular transcription elongation factor to be specifically upregulated in the presence of the viral oncoprotein Tax. We demonstrate that Tax contributes to the maintenance of ELL2 in Tax-transformed cells. However, we also show that ELL2 is still maintained in HTLV-1-infected T-cells that had lost Tax expression. Finally, we provide evidence that Tax and ELL2 synergistically activate the HTLV-1-promoter and that they are part of a common complex, indicating that upregulation of ELL2 in HTLV-1-infected cells is a novel mechanism to modulate viral gene regulation.

Results

Micro array analysis identifies upregulation of the transcription elongation factor ELL2 in HTLV-1-/Tax-transformed T-cells

To clarify whether HTLV-1/Tax could perturb components of the transcriptional elongation machinery, transcriptome analyses were performed comparing HTLV-1-transformed cells (MT-2) and ATL-derived cells (StEd) with primary $CD4^+$ T-lymphocytes (Kress et al., 2010). In addition, Tax-transformed Tesi cells were compared to Tesi/Tet cells, where Tax expression was repressed (Pichler et al., 2008). To show the integrity of the micro array, expression levels of the known Tax-target gene *OX40L* (gp34, TNFSF4) are displayed (Miura et al., 1991; Ohtani et al., 1998), which are highly upregulated in Tax-expressing MT-2, Tesi and StEd cells (Table 1). In a systematic search for classical, positive transcription elongation factors based on Sims et al. (2004), we found that the expression pattern of most elongation factors was not changed significantly in the presence of HTLV-1/Tax (Table 1). However, we identified significant upregulation of a single transcription elongation factor, the ELL-family member ELL2. Depending on the probe set used in the microarray, upregulation of ELL2 was between 2-and 11-fold. Therefore, we conclude that among all currently known positive transcription elongation factors, only *ELL2* is significantly upregulated in the presence of HTLV-1/Tax.

ELL2 mRNA and protein are specifically and significantly upregulated in HTLV-1-infected T-cell lines

To test whether ELL2 expression is a common phenotype of HTLV-1 infection, quantitative PCR (qPCR; Fig. 1A B) was performed comparing *ELL2* copy numbers in HTLV-1-infected T-cell lines and uninfected controls. *ELL2* transcripts were present in all Tax-expressing HTLV-1-infected T-cell lines including *in vitro*-transformed cell lines, ATL-and HAM/TSP-derived cells, and solely Tax-transformed T-cells (Fig. 1A). The amount of *ELL2* transcripts is significantly increased ($p<0.05$) in HTLV-1/Tax-positive cells compared to uninfected controls including cell lines derived from acute lymphoblastic leukemia, unstimulated PBMC and primary $CD4^+$ T-lymphocytes (Fig. 1B). Beyond that, ELL2 protein expression was detectable in all HTLV-1/Tax-positive cell lines as reflected by detection of a *ca.* 80 kDa band of ELL2 protein species by immunoblotting (Fig. 1C). Tax expression was validated by qPCR in all HTLV-1/Tax-positive T-cell lines (Supplementary Fig. 1A), whereas Tax protein could only be detected in 9 of the 14 infected cell lines tested (Supplementary Fig. 1B). Although the amounts of *ELL2* and *Tax* transcripts moderately correlated in Tax-expressing T-cell lines (Fig. 1A, Supplementary Fig. 1A; $R^2=0.545$, $p<0.05$, $N=14$), we did not observe a correlation between the amounts of ELL2 and Tax protein (Fig. 1C, Supplementary Fig. 1B). In summary, the transcription elongation factor ELL2 is specifically upregulated in HTLV-1-infected Tax-expressing T-cells independent of their origin on transcript and protein level.

ELL2 is not upregulated upon mitogenic stimulation of PBMC

HTLV-1-Tax-transformed cell lines express several adhesion molecules, growth-promoting chemokine receptors, and costimulatory receptors on their surface (Kress et al., 2011). Therefore, the cells accumulate in syncytia and are characterized by high proliferation rates (Matsuoka and Jeang, 2007; Popovic et al., 1983). To test whether upregulation of ELL2 is a consequence of T-cell proliferation only, PBMC were cultured in the presence of mitogenic stimulation (PHA-P, IL-2) and samples for isolation of RNA and protein were taken at different time points post stimulation. Detection of transcripts of the costimulatory tumor necrosis superfamily receptor *4-1BB* served as a control for mitogenic stimulation. Transcripts of *4-1BB* were only slightly expressed in unstimulated PBMC, but increased following stimulation (Supplementary Fig. 2A). Despite efficient mitogenic stimulation, we could neither observe an increase of *ELL2* transcripts (Supplementary Fig. 2B) nor of ELL2 protein (Supplementary Fig. 2C). 293 T cells transfected with an ELL2 expression plasmid served as a positive control for ELL2 protein expression. Collectively, these data indicate that ELL2 is not upregulated by mitogen signals.

Transient expression of Tax leads to induction of ELL2 mRNA

The expression pattern of ELL2 in HTLV-1-infected cells led us to ask, whether Tax alone is sufficient for induction of ELL2 gene expression. Therefore, transient transfections of 293 T cells were performed. Measuring *Tax* and *ELL2* transcripts by qPCR revealed that overexpression of increasing amounts of Tax led to a dose-dependent increase of *ELL2* transcripts, which correlates with *Tax* transcripts (Fig. 2A; $R^2=0.8991$, $p<0.01$). Despite high copy numbers of *Tax*, only low copy numbers of induced endogenous *ELL2* could be measured upon transfection of Tax expression plasmids. Although *ELL2* copy numbers were in the range of those of HTLV-1-transformed cells (Fig. 1A), we did not observe any changes in the amount of endogenous ELL2 protein after Tax expression independent of the amount of Tax protein expressed (Fig. 2B). Taking into consideration that ELL2 is post-translationally modified by ubiquitination and has a short half-life (Liu et al., 2012), we tested whether proteasome inhibition using MG-132 results in an accumulation of ELL2 protein upon expression of Tax. We observed an overall accumulation of ubiquitinated proteins (Fig. 2C), however, an increase of endogenous ELL2 protein was not detectable in the presence of Tax, possibly due to missing cofactors required for ELL2 protein stability (Liu et al., 2012). Endogenous ELL2 was also not inducible upon transient expression of Tax in