Transcriptional activity of HTLV-I Tax influences the expression of marker genes associated with cellular transformation

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Human T cell leukemia virus type I (HTLV-I) has been identified as the etiologic agent of adult T cell leukemia (ATL). HTLV-I encodes a transcriptional regulatory protein, Tax, which also functions as the viral transforming protein. Through interactions with a number of cellular transcription factors Tax can modulate cellular gene expression. Since the majority of Tax-responsive cellular genes are important regulators of cellular proliferation, the transactivating functions of Tax appear to be necessary for cellular transformation by HTLV-I. Gaining a complete understanding of the broad range of genes regulated by Tax, the temporal pattern of their expression, and their effects on cell function may identify early markers of disease progression mediated by this virus.

1. Introduction

Infection with human T cell leukemia virus type I (HTLV-I) is a prerequisite for the development of two fatal human diseases: adult T cell leukemia/lymphoma (ATL) and tropical spastic paraparesis or HTLV-I associated myelopathy (TSP/HAM). ATL is a lymphoproliferative disorder of mature T cells [39,80,118] and accounts for about 1% of all leukemias [79], while TSP/HAM is a degenerative disease of the central nervous system [30,75]. This review focuses on evaluating markers of HTLV-I infection that may be predictive of ATL development.

Approximately 20 million people worldwide are infected with HTLV-I [23]. The efficiency of HTLV-I transmission is relatively low and occurs both vertically and horizontally, predominately from mother to infant through ingestion of infected T cells in breast milk. Transmission can also occur through the exchange of infected bodily fluids during activities such as sexual contact, transfusion, and needle sharing among IV drug users. Upon infection of $CD4^+$ T lymphocytes, the HTLV-I provirus integrates randomly into the host chromosome. Infection is followed by a long period of clinical latency during which time it is difficult to detect viral gene expression. Patients seroconvert relatively early following infection despite the absence of clear clinical symptoms. Fewer than 5% of infected individuals develop ATL, and the disease typically presents 20 to 40 years after infection. The relationship between age and the development of ATL suggests that five independent events are required to complete the transformation process [74]. These features highlight the importance of defining early markers of viral infection to identify individuals with increased likelihood of developing disease. In combination with improved predictors of disease development, strategies for intervention in disease progression must also be developed.

2. The HTLV-I transcriptional activator and transforming protein, Tax

HTLV-I encodes a protein, Tax, which is essential for viral replication due to its ability to activate viral gene expression through specific Tax-responsive elements within the viral long terminal repeat [10,20,88, 91]. Tax is also the transforming protein of HTLV-I [33, 34,38,69,82,90,97,115]. Because the *tax* gene is not widely expressed in ATL cells, its role in tumor induction is likely to be an early event in cellular transformation. Tax modulates transcriptional activity by interacting with a subset of cellular transcription factors that tether Tax to the viral promoter [29,93,94,119] rather than by directly binding DNA. Through its interactions

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with cellular transcription factors, Tax also modulates expression of a variety of cellular genes, an activity that appears to play a major role in the function of Tax as a viral oncoprotein.

3. Effects of HTLV-I Tax on cellular gene expression

The transcriptional activity of Tax affects three major transcription factor pathways including cAMP response element binding protein (CREB) [1,2,11,24,57, 93,119], nuclear factor κB (NF-κB) [8,49,53,84,92], and serum response factor (SRF) [26,28]. Mechanisms through which Tax regulates these three pathways are different and have been discussed in other recent reviews [23,117]. Exploiting these pathways, Tax activates the transcription of more than thirty cellular genes (Table 1) including growth factors and cytokines, growth factor receptors, cell cycle and DNA repair control proteins, nuclear transcription factors and others (cell adhesion molecules, cytoplasmic signal transmitters, and cytoskeletal proteins). Although Tax regulation of most of these genes has been mapped to CREB, NF-κB or SRF binding sites, some of the cellular genes regulated by Tax utilize alternative transcription factor pathways for activation, and specific Tax responsive elements remain to be defined in a few of these promoters. Because of its broad ranging functions, Tax has been referred to as a promiscuous transactivator, implying that it may regulate certain universal transcription pathways.

In contrast to its transcriptional activation functions, Tax has been shown to repress transcription of five cellular genes, β-polymerase, *lck*, *bax*, p53, and p18$^{\mathrm{ink4C}}$ (Table 1). Although the element through which Tax represses β-polymerase expression has not been defined, Tax repression of *bax, lck,* p53 and p18$^{\mathrm{ink4C}}$ is mediated through E-box elements in their promoters. Only one cellular gene, p21, has been reported to be positively regulated by Tax through an E-box suggesting that E-boxes may primarily serve as a negative regulatory target for Tax [107]. Notably, four of the five cellular genes that are transrepressed by Tax (β-polymerase, *bax*, p53, and p18$^{\mathrm{ink4C}}$) have functions in regulation of cell cycle progression and DNA repair, implying that the repression of these functions may be a critical step in transformation.

4. Tax activation of genes encoding growth factors, cytokines, and growth factor receptors

The ability of Tax to activate growth factors and their receptors implies the possibility of autocrine or paracrine stimulation of cell proliferation. Tax activation of the IL-2Rα chain, a component of the high affinity IL-2 receptor (CD25), is an early event in HTLV-I infection. However, this IL-2Rα induction is not sufficient to transform cells. Tax activation of IL-2R/IL-2 gene expression probably accounts for the polyclonal proliferation of infected T cells observed during clinical latency. Monoclonal outgrowth of leukemic T cells occurs in a small portion of infected individuals. T cell lines established from HTLV-I infected individuals during clinical latency typically require the addition of exogenous IL-2 (immortalized) while those established from late stage ATL patients are IL-2 independent (transformed) even though they do not express IL-2 [6]. Tax expression is low to undetectable in transformed ATL cells, yet CD25 expression is maintained suggesting that another mechanism for IL-2R activation may exist in late stage ATL cells. Leukemic cells from ATL patients also fail to express IL-4, although Tax can transactivate this promoter [99]. Despite the absence of these growth factors in leukemic cells, their expression may play important roles in early polyclonal proliferation of HTLV-I infected cells.

OX40, a member of the tumor necrosis factor (TNF) receptor family that serves as a marker of activated T and B cells, is also expressed on the surface of HTLV-I infected cells [43]. Binding of the OX40 ligand (gp34), a member of the TNF family [31], induces T cell proliferation, modulates cytokine production, and influences T cell migration into tissues [21,31,32,42, 43]. These findings suggest that autocrine or paracrine OX40/OX40L interactions may provide necessary costimulatory signals for transformation or survival and proliferation of HTLV-I infected cells.

5. Tax activation of genes encoding cell cycle and DNA repair proteins

Cancer-causing viruses typically encode one or more proteins that disrupt cell cycle checkpoints leading to cellular transformation. HTLV-I is no exception to this rule. Tax activates genes that stimulate cell cycle progression and represses some that inhibit cell cycle progression. Cyclin D2 is a G1 cyclin that induces cell cycle progression to late G1 phase. In Tax-expressing

Table 1
Cellular Genes Regulated by HTLV-I Tax

Genes	Effect	Pathway	Reference
Growth factors/cytokines			
IL-1α	+	NF-κB	65, 66
IL-1β	+	C/EBPβ, PU.1	104
IL-2	+	NF-κB	54, 61, 89, 109
IL-3	+	?	61
IL-4	+	?	61
IL-5	+	AP-1, GATA-4*	113
IL-6	+	NF-κB	67, 116
IL-8	+	NF-κB, AP-1	63, 64
IL-15	+	NF-κB	7
NGF	+	CRE	35
proenkephalin	+	AP-1	25, 48
PTHrP	+	CRE, Sp1, Ets*	17, 18, 41, 62, 111
c-sis (PDGF)	+	Sp1, NGFI-A/Egr-1	100, 101
GM-CSF	+	NF-κB	37, 61, 71, 109
OX40 ligand (gp34)	+	NF-κB	9, 36, 60, 73
TGFβ1	+	NF-κB	50
TNF-β	+	NF-κB	77, 102
Growth factor receptors			
IL-2Rα (CD25)	+	NF-κB	8, 15, 44, 58, 84, 89, 109
OX40 (TNF receptor family)	+	NF-κB	36
egr-1 (Krox-24)	+	SRE, CRE	4
egr-2 (Krox-20)	+	SRE, CRE	4
class I MHC	+	?	87
Cell cycle/DNA repair			
PCNA	+	?	83
cyclin D2	+	CRE	3, 86
bcl-X(L)	+	NF-κB	70, 105
p21	+	E-box	3, 13, 16
bax	−	E-box	12
p18$^{\mathrm{INK4C}}$	−	E-box	3, 96
p53	−	E-box	108
DNA polymerase β	−	?	47
Transcription factors			
c-fos	+	SRE, CRE	5, 26, 27, 68
c-jun	+	?	45
c-myc	+	NF-κB	19
fra-1	+	AP-1	103
RNA polymerase III	+	CRE	78
E2F-1	+	CRE	52
Nur77	+	CRE	14
Signaling and other			
vimentin (cytoskeleton)	+	NF-κB	55, 56, 85
β-globin	+	CRE	22
ε-globin	+	CRE	22
ICAM-1 (CD-54)	+	CRE	76, 98
lyn	+	?	106, 114
lck	−	E-box	51

*Transcription factors that cooperate with Tax to activate the given promoter.

cells, expression from the cyclin D2 promoter is elevated, and cyclin D2 is found complexed with unusual cdk partners 2 and 4 [86].

Proliferating cell nuclear antigen (PCNA) interacts with and regulates the activity of proteins involved in DNA replication and repair, as well as proteins involved in cell cycle progression. The PCNA protein is an essential co-factor of DNA polymerase δ (pol δ), an enzyme involved in both DNA replication and repair. The interaction of PCNA with pol δ functions to increase the processivity of both leading and lagging strand DNA replication. The effect of PCNA on DNA replication and repair is thought to involve interactions with cyclins and cyclin dependent kinases (cdks). Cdk inhibitors,

such as p21, can block PCNA-dependent DNA synthesis but have no effect on PCNA-dependent DNA repair. Excess PCNA can overcome the p21 block of DNA replication, stimulate DNA synthesis past template lesions and increase nucleotide misincorporation rates. Thus, overexpression of PCNA appears to stimulate DNA synthesis even in the presence of normal negative regulatory signals.

In response to DNA damage, p53 induces p21, a cdk inhibitor that typically induces cell cycle arrest by restricting the transition from G1 to S phase. Tax represses the p53 promoter in HTLV-I infected cells, yet p53 protein levels are elevated and the protein possesses no apparent transcriptional activity. The p21 promoter is activated by Tax suggesting that Tax may function to restrict cell cycle progression; however, overexpression of p21 in uninfected T cells does not appear to block cell cycle progression [72]. As a result of these altered activities of cyclin D2, PCNA, p53 and p21, HTLV-I infected cells may be incapable of G1 arrest in the presence of DNA damage.

6. Tax activation of genes encoding transcription factors

Because some Tax-activated genes encode transcription factors, Tax can indirectly influence an even broader range of cellular genes than those it directly regulates. The products of the *c-fos* and *c-jun* immediate early growth response genes heterodimerize to activate transcription of genes that respond to the phorbol ester TPA, an activator of protein kinase C (PKC). Since deregulated *c-fos* expression can induce cellular transformation, Tax activation of this protein could contribute to the early stages of HTLV-I transformation. E2F-1, a member of the E2F transcription factor family, heterodimerizes with members of the DP family to regulate the expression of cell cycle control proteins including dihydrofolate reductase, thymidine kinase, DNA polymerase α, PCNA, histone 2A, cyclin A, cyclin E, cyclin D1, p107, pRB, *c-myc*, N-*myc*, *erb*-B, and B-*myb*. Deregulated E2F-1 expression can induce resting cells to enter S phase and stimulate cell proliferation. These proliferative effects could also play an important role at early stages of HTLV-I transformation.

Transcriptional activities of Tax are necessary, but probably not sufficient, for transformation. Despite this review's focus on the transcriptional effects of Tax, the protein has other functions including the ability to interact with and inactivate $p16^{Ink4a}$, a cyclin dependent kinase inhibitor that halts G1 phase progression [95]. This function of Tax could contribute to abnormal G1 to S phase transition. The interaction of Tax with IκB, a cytoplasmic inhibitor of NF-κB, induces release of NF-κB binding activity [40,110]. Finally, Tax has the ability to inactivate the tumor suppressor protein p53 despite the fact that Tax does not form a physical complex with p53 or induce its degradation. p53 mutations are rare in ATL cells and infected T-cell lines; however, p53 stabilization is an early event after *in vitro* HTLV-I infection of human primary peripheral blood mononuclear cells (PBMC) and thus may be a useful marker of disease progression.

7. Conclusions

The quest for markers that will predict disease susceptibility in HTLV-I infected individuals is in its infancy. In this review we have provided a comprehensive list of cellular genes regulated by the HTLV-I Tax protein and have highlighted a subset of these genes for discussion of their potential effects on cellular transformation. Since the genome is small and viral gene expression is difficult to detect in infected individuals, it is unlikely that monitoring viral gene expression patterns will be useful in this effort. HTLV-I integrates randomly into the host chromosome, and the site of viral integration does not appear to correlate with disease type or progression. Despite intense effort devoted to sequencing viral isolates from asymptomatic, as well as ATL and TSP/HAM patients, genetic subtypes clearly associated with disease have not been identified. These results suggest that disease markers are most likely to be identified from among the cellular genes whose expression patterns are altered following viral infection. Though lengthy, the list of genes regulated by Tax is probably not yet complete. Despite extensive knowledge about the molecular mechanisms used by Tax to regulate the expression of individual cellular genes, little is known about the temporal and spatial patterns of gene expression from early post-infection into the disease states. Future studies directed at detailing these events are likely to reveal important predictive markers of disease progression.

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