

Molecular mechanisms of cellular transformation by HTLV-1 Tax

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The HTLV Tax protein is crucial for viral replication and for initiating malignant transformation leading to the development of adult T-cell leukemia. Tax has been shown to be oncogenic, since it transforms and immortalizes rodent fibroblasts and human T-lymphocytes. Through CREB, NF- κ B and SRF pathways Tax transactivates cellular promoters including those of cytokines (IL-13, IL-15), cytokine receptors (IL-2R α) and costimulatory surface receptors (OX40/OX40L) leading to upregulated protein expression and activated signaling cascades (e.g. Jak/STAT, PI3Kinase, JNK). Tax also stimulates cell growth by direct binding to cyclin-dependent kinase holoenzymes and/or inactivating tumor suppressors (e.g. p53, DLG). Moreover, Tax silences cellular checkpoints, which guard against DNA structural damage and chromosomal missegregation, thereby favoring the manifestation of a mutator phenotype in cells.

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Introduction

The nonstructural regulatory Tax proteins are unique characteristics of delta retroviruses, a subgroup of retroviruses, which confers long-term persistent infection to mammalian lymphocytes. These proteins are crucial for productive viral replication, and they stimulate the proliferation of host lymphocytic cells. Infection of T cells with the prototype of this group, human T-cell leukemia virus type 1 (HTLV-1) causes a severe and fatal lymphoproliferative disease of helper T-cell origin, the adult T-cell leukemia (ATL) and a separate neurodegenerative disease termed tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) (Poiesz *et al.*, 1980; Yoshida *et al.*, 1984; Gessain *et al.*, 1985; Osame *et al.*, 1986). A proportion of 1–3% of HTLV-1-infected individuals develop these diseases after prolonged viral persistence (i.e. usually after two decades of infection for ATL). Even in patients

who do not develop frank leukemia, virus-infected T-lymphocytes appear to be growth-stimulated, since such cells expand clonally and persist over many years (Etoh *et al.*, 1997; Gabet *et al.*, 2000). *In vivo* infected cells are frequently capable of indefinite proliferation when propagated in tissue culture in the presence of interleukin (IL)-2. Similarly, it is also possible to generate immortal CD4⁺ and CD8⁺ T-cell lines by infecting primary blood lymphocytes with HTLV-1 *ex vivo*. Since HTLV-1 spreads very inefficiently via cell-free viral particles (Manel, Battini, Taylor and Sitbon, this issue), a critical reason for this virus to encode a cell-growth stimulatory function is to amplify its cell-associated integrated proviral genome.

HTLV-1's capacity to stimulate T-cell growth suggests that the virus encodes gene function(s) involved in clonal expansion of cells. Other than its structural genes, *gag*, *pol* and *env*, HTLV-1 contains several open reading frames in a pX region at the 3' end of its genome. The pX region has the potential to encode essential regulatory proteins (Tax, Rex) and three accessory proteins, p12, p13 and p30 which are important for viral infectivity and replication by influencing cellular signaling and gene expression (Bartoe *et al.*, 2000; Nicot *et al.*, 2001; Johnson *et al.*, 2001; D'Agostino *et al.*, 2002; Ding *et al.*, 2002; Lefebvre *et al.*, 2002). The proteins p12, p13 and p30 are dispensable for the immortalization of primary lymphocytes (Derse *et al.*, 1997; Robek *et al.*, 1998). Our current understanding is that Tax is the primary HTLV-1-encoded factor used by the virus to modulate cellular proliferation.

Structure–function features of Tax for transcriptional activation

During virus replication, the Tax protein is a transcriptional activator of the viral long terminal repeat (LTR). Tax is predominantly a nuclear phosphoprotein with a small amount distributed in the cytoplasm of cells. Recent evidence suggests that Tax is post-translationally modified by ubiquitination (Chiari *et al.*, 2004; Peloponese Jr *et al.*, 2004), and this modification attenuates Tax's transcriptional activity. Several major cellular signal transduction pathways including the transcription factors NF- κ B, CREB, SRF and AP-1 are induced by Tax (Jeang, 2001; Azran *et al.*, 2004). These Tax functions

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are detailed elsewhere in this issue, and are here only briefly summarized.

The HTLV-1 LTR is a cyclic-AMP-responsive promoter (Jeang *et al.*, 1988). Tax does not directly bind DNA, but associates through its N-terminus with the CREB protein (Baranger *et al.*, 1995; Adya and Giam, 1995), which docks at the LTR's cyclic-AMP responsive (CRE)-motifs. Tax also recruits transcriptional coactivators, CBP and p300 (Giebler *et al.*, 1997; Bex *et al.*, 1998; Harrod *et al.*, 2000), and in a less-defined manner, P/CAF (Okada and Jeang, 2002). The C-terminal activation domain of Tax (Semmes and Jeang, 1995) is thought to directly contact the TATA-box-bound TBP protein (Caron *et al.*, 1993) thereby promoting transcriptional initiation and RNA polymerase elongation (Ching *et al.*, 2004).

A second major function of Tax is to activate NF- κ B. This activation likely involves cytoplasmic and nuclear interactions. Tax and NF- κ B proteins have been shown by immunomicroscopy to colocalize (Semmes and Jeang, 1996) within nuclear bodies that contain RNA polymerase II and other transcription factors (Bex *et al.*, 1997). Recently, evidence suggests that the assembly of a transcriptionally competent 'nuclear' Tax-NF- κ B-CBP complex likely first occurs in the cytoplasm (Azran *et al.*, 2005). One prevailing view is that Tax binds the IKK γ /NEMO molecule in the cytoplasm to influence the activity of the IKK α /IKK β /IKK γ complex (Chu *et al.*, 1999; Harhaj and Sun, 1999; Jin *et al.*, 1999a). Activated IKK α /IKK β /IKK γ complexes then phosphorylate IKK α /IKK β leading to a cascade of events (reviewed elsewhere in this issue by Sun and Yamaoka) which result in nuclear migration of NF- κ B (Iha *et al.*, 2003).

***In vitro* transforming potential of Tax**

Transformation and immortalization of rodent fibroblasts

Tax has many features of an oncogene including the capacity to immortalize primary rodent cells. Expression of Tax along with a selectable marker in early-passage rat embryo fibroblasts produces immortalization without morphological changes. However, when coexpressed with Ras, Tax leads to the formation of cellular foci. Such cells are producing tumors when injected into nude mice (Pozzatti *et al.*, 1990). In rodent fibroblasts such as NIH 3T3 and Rat1, Tax-alone is sufficient to stimulate cells to grow beyond contact-inhibited saturation density, induce anchorage-independent growth in soft agar, and confer tumorigenicity to cells when introduced into nude mice (Tanaka *et al.*, 1990). Compared to rat fibroblasts transformed by classical cellular nuclear oncogenes, Tax-transformed cells also have an apparently higher resistance to the induction of apoptosis (Fujita and Shiku, 1995).

Immortalization of human T-lymphocytes

In contrast to rodent cells, primary human cells are comparatively resistant to Tax-mediated transformation. Indeed, the transformed phenotype in rat fibro-

blasts can be suppressed by normal human fibroblasts when the two cell types are fused, suggesting the existence of a dominant suppressor gene activity in human cells (Inoue *et al.*, 1994). To date, evidence suggests that Tax can immortalize primary human T-cells derived from peripheral blood or cord blood. This has been demonstrated by transducing cells with Tax expressed from rhadinoviral (Grassmann *et al.*, 1989, 1992) or retroviral (Akagi and Shimotohno, 1993) vectors. The resulting immortalized lymphocytes resemble closely the phenotype of HTLV-1-transformed T-cells, including dependence on exogenous interleukin (IL-) 2 for growth (Akagi *et al.*, 1995; Rosin *et al.*, 1998; Schmitt *et al.*, 1998). Thus, in these primary cells, a mechanism different from the previously proposed IL-2 autocrine model seems to be triggered by Tax in its dysregulation of T-cell proliferation.

Roles for CREB, NF- κ B and SRF in cellular immortalization

Tax mutants have been used to characterize the structure-function correlate for transformation in rodent cells. Initially, only two Tax functions (NF- κ B- or CREB-activation) were considered. Surprisingly, depending on the cells, Tax mutants individually deficient in either CREB- (Smith and Greene, 1991) or NF- κ B- (Yamaoka *et al.*, 1996; Matsumoto *et al.*, 1997) activation remained competent for immortalizing rodent fibroblasts. Separately, it was reported that Tax stimulation of CARG Box function through SRF was also needed to transform primary rat fibroblasts (Matsumoto *et al.*, 1997). In the context of human lymphocytes, activation of the CREB/ATF and/or SRF pathways was required for the clonal expansion of CD4⁺ and CD8⁺ T cells (Akagi *et al.*, 1997a; Rosin *et al.*, 1998), and recently, CREB has been assigned a role as a proto-oncogene in its promotion of abnormal proliferation/survival of haematopoietic cells (Shankar *et al.*, 2005). Primary human lymphocytes transduced with a Tax mutant defective for NF- κ B activation, nevertheless, still could increase NF- κ B activity, albeit at a level reduced compared to wild-type Tax (Rosin *et al.*, 1998). This suggests that increased NF- κ B function in transformed cells emanates from cellular signaling and need not be a direct consequence of Tax. If correct, this would reconcile findings seen in some ATL cells, in which NF- κ B activity is elevated even when Tax is low to undetectable. On the other hand, it cannot be excluded that activation of the NF- κ B pathway by Tax is important for growth response to IL-2 (Akagi *et al.*, 1997a) or to the immortalization of CD4⁺ and CD8⁺ T-lymphocytes by an HTLV-1 molecular clone (Robek and Ratner, 2000).

Stimulation of cell growth through signal transduction

Comparison of gene expression profiles between HTLV-1-infected versus uninfected T-cells revealed numerous differences in expression of signaling molecules including cytokine receptors and cytokines (Ruckes *et al.*,

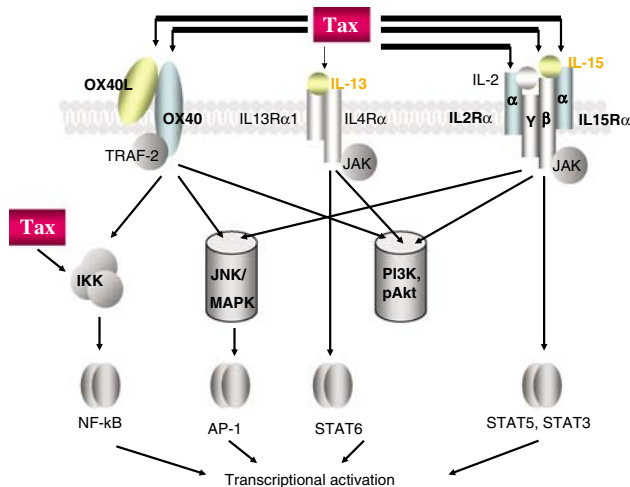


Figure 1 Cytoplasmic signaling of Tax-stimulated regulatory proteins. Tax by transactivation stimulates the gene expression of receptors OX40, IL2R α , IL15 R α 1 (blue), ligands (yellow) OX40L, IL-13 and IL-15. Receptor ligand binding results in the activation of growth and survival controlling signaling cascades and activation of transcription factors

2001; Pise-Masison *et al.*, 2002). These changes include several factors with growth promoting and/or antiapoptotic functions whose expression patterns are modulated by Tax (Ng *et al.*, 2001). Below, we summarize some salient examples (Figure 1).

IL-2 and IL-15

The α -chain of the IL-2 receptor (IL-2R α) was the first cellular gene reported to be upregulated by Tax (Ballard *et al.*, 1988; Ruben *et al.*, 1988). Together with β - and a common γ - chain, IL-2R α forms the high-affinity IL-2 receptor, which permits signaling at low IL-2 concentrations. A complementary observation that the promoter for the IL-2 gene is also activated by Tax (Hoyos *et al.*, 1989; McGuire *et al.*, 1993; Good *et al.*, 1996) led to an unifying hypothesis of T-cell proliferation through an autocrine IL-2/IL-2R loop. Actually, this hypothesis insufficiently explains ATL biology and transformed growth in culture since most Tax or HTLV-1-immortalized T-cells still require exogenous IL-2 and do not detectably express either IL-2 mRNA or protein (Akagi and Shimotohno, 1993; Schmitt *et al.*, 1998; Chung *et al.*, 2003). In parallel, IL-15, a relative of IL-2, uses the β - and γ -chains of the IL-2 receptor for signaling. IL-15 mRNA expression is increased three- to fourfold by Tax in HTLV-1-infected T-cells compared to normal lymphocytes (Azimi *et al.*, 1998). IL-15R α , the IL-15-specific binding receptor chain, is also elevated by Tax in HTLV-1-infected cells (Mariner *et al.*, 2001). Therefore, it has been suggested that an IL-15 autocrine loop may also contribute to HTLV-1 pathogenesis (Azimi *et al.*, 1999).

Interleukin-13

Stimulatory signals via the IL-4R α chain are provided by the IL-4/IL-13 receptor complex. Whereas IL-4 is

mostly absent, IL-13 is upregulated and secreted in HTLV-transformed cells, and in cultured ATL-cells derived from patients (Chung *et al.*, 2003; Wäldele *et al.*, 2004). In HTLV-cells, IL-13 expression is upregulated by Tax transactivation of the NF-AT and AP-1 elements in the promoter (Wäldele *et al.*, 2004). IL-13 is linked to leukemogenesis, since in both Hodgkin's lymphoma cells and HTLV-1-transformed cells, it appears to be operative through an autocrine mechanism.

OX40/OX40L

OX40, a member of the TNF-receptor family, is the only costimulatory T-cell molecule known to be specifically upregulated in HTLV-1-infected cells. Surface expression of OX40 is induced by Tax through OX40 promoter upregulation via two NF- κ B-like elements (Pankow *et al.*, 2000). OX40 ligand (OX40L/gp34) is a type II transmembrane molecule belonging to the tumor necrosis factor family (Baum *et al.*, 1994); it is constitutively expressed on HTLV-1-producing cells but not uninfected resting T-cells. The presence of both, ligand and receptor on the HTLV-transformed cell suggest that costimulatory signals delivered from OX40 contribute to a transformed phenotype.

Cytokine receptor signals including Jak/STAT

In response to ligand-binding, the IL-2/IL-15 receptor associated Janus tyrosine kinase (JAK3) phosphorylates signal transducer of activated T cells (Stat5a,b) transcription factors that then enter the nucleus and stimulate target gene transcription. Stat5a and Stat5b, essential for the proliferation of normal T cells, are hyper-activated in both HTLV-1-transformed human T-cell lines and lymphocytes from HTLV-1 patients (Migone *et al.*, 1995). In IL-2-independent HTLV cell lines (i.e. HuT-102 and MT-2), this pathway may be functionally redundant since its disruption with AG-490 failed to inhibit cellular proliferation (Kirken *et al.*, 2000). It is of interest that many genes upregulated in HTLV-transformed cells including cyclin D2 and several cytokines are IL-2 target genes and contain Stat5 binding sites in their promoters (Fung *et al.*, 2005).

Tax also stimulates the expression of a growth-inhibiting protein, the transforming growth factor beta 1 (TGF- β 1). The protein is also overexpressed in ATL cells (Kim *et al.*, 1990) and may function to limit cytotoxicity. However, Tax represses TGF- β 1 signaling by reducing the DNA-binding activity of transcription factors Smad 3 and Smad 4 (Mori *et al.*, 2001; Arnulf *et al.*, 2002; Lee *et al.*, 2002).

Kinase-cascades

Phosphoinositide 3-kinase (PI3K) and its downstream target Akt are activated in response to cytokine receptors (Kelly-Welch *et al.*, 2003) and the T-cell receptor activation; this pathway provides growth stimulatory and antiapoptotic signals. PI3K-Akt was found activated in HTLV-transformed Rat-1 cells and

to be involved in cell transformation (Liu *et al.*, 2001). Currently, it remains unclear how Tax stimulates this pathway.

The ERK-JNK cascade is constitutively activated in Tax-transformed murine fibroblasts, in human lymphocytes transformed *in vitro* by HTLV-1, and in leukocytes isolated from ATL patients. However, this activation is not induced by Tax alone, and may represent an important late event when infected lymphocytes become IL-2-independent (Xu *et al.*, 1996; Jin *et al.*, 1997).

Stimulation of cell growth through cell cycle dysregulation

A major mitogenic activity of Tax is reflected in its stimulation of G1- to S-phase transition (Neuveut *et al.*, 1998; Schmitt *et al.*, 1998; Neuveut and Jeang, 2002; Liang *et al.*, 2002). In mammalian cells, G1-progression is controlled by the sequential activation of several cyclin-dependent kinases (Cdks), starting with Cdk4, Cdk6 and Cdk2. Tax activates Cdk4, Cdk6 and Cdk2 leading to phosphorylation of the retinoblastoma (Rb) tumor suppressor family proteins and freeing E2F (Schmitt *et al.*, 1998; Iwanaga *et al.*, 2001). Tax can also increase E2F (Ohtani *et al.*, 2000) in part through upregulation of the E2F-1 promoter via an ATF binding site (Lemasson *et al.*, 1998). Several mechanisms may account for Tax's G1-S phase stimulatory action including (1) transcriptional upregulation of cyclin D2, (2) activation of kinases by direct binding of the kinase holoenzyme complex, and (3) repression of Cdk inhibitors (Figure 2).

Cyclin D binding and upregulation

HTLV-1-infected T-cell lines and patient cells contain increased levels of the early G (1) cyclin, cycD2. (Akagi *et al.*, 1996; Santiago *et al.*, 1999; Iwanaga *et al.*, 2001). The cycD2 expression is also upregulated by IL-2 receptor signals and in part accounts for the T-cell proliferation stimulated by IL-2. The IL-2 receptor induces cycD2 by activating transcription factor Stat5,

which binds directly to a cognate site in the cyclin D2 promoter (Martino *et al.*, 2001; Moon *et al.*, 2004; Fung *et al.*, 2005). Tax may cooperate with IL-2-signaling either indirectly through stimulating the expression of IL2R α or directly by activating the cycD2 promoter (Huang *et al.*, 1997; Santiago *et al.*, 1999).

Direct binding to Cdk4/6

A further mechanistic explanation for the Cdk4 and Cdk6 activation is provided by the direct and specific interaction of these protein with Tax (Haller *et al.*, 2000; Haller *et al.*, 2002). It has been found that the N-terminus of Tax interacts with Cdk4 (Li *et al.*, 2003). Binding-deficient Tax mutants failed to activate Cdk4, indicating that direct association with Tax is required for enhanced kinase activity. The Tax/Cdk complexes represent active holoenzymes that capably phosphorylate the Rb protein *in vitro* and are resistant to repression by the p21^{Waf1/Cip1} inhibitor. Tax also interacts with the cycD component of the Cdk4/6 holoenzyme leading possibly to hyperphosphorylation of cycD3 (Neuveut *et al.*, 1998; Haller *et al.*, 2002; Neuveut and Jeang, 2002).

Inhibition of CKI function

The expression of p18^{INK4c} (Akagi *et al.*, 1996), p19^{INK4d} (Iwanaga *et al.*, 2001) and p27^{Kip1} (Iwanaga *et al.*, 2001) is reduced in the presence of Tax. Expression of p18^{INK4c} is transcriptionally repressed by Tax through the E-box element of the promoter (Suzuki *et al.*, 1999a). By contrast, p21 is strongly upregulated (Akagi *et al.*, 1996; Cereseto *et al.*, 1996). Tax activates the p21^{Waf1/Cip1} promoter in p53 null cells, and increases p21^{Waf1/Cip1} expression in Jurkat T cells. These findings suggest that Tax is at least partially responsible for the p53-independent expression of p21^{Waf1/Cip1} in HTLV-1-infected cells (Cereseto *et al.*, 1996; Chowdhury *et al.*, 2003). p21^{Waf1/Cip1} expression may enhance cell survival through its role in the antiapoptotic machinery (Kawata *et al.*, 2003). Separate from transcriptional activation, Tax also binds p16INK4A and p15INK4B, but not p21^{Waf1/Cip1} nor p27^{Kip1} (Suzuki *et al.*, 1996; Low *et al.*, 1997). Through direct binding, Tax is thought to interfere with CKI's inhibitory roles on Cdk4 and Cdk6 (Suzuki *et al.*, 1999a).

Effects of Tax on tumor suppressors and cellular apoptosis

To transform cells, oncoproteins must defeat the actions of cellular tumor suppressors. HTLV-1 Tax has evolved various strategies to negate at least three cellular tumor suppressors, p53, Rb and hDLG, and their abilities to dictate apoptosis in primary cells.

Tax and p53

p53 is a DNA-binding transcription factor that guards against cellular DNA damage and transformation. The

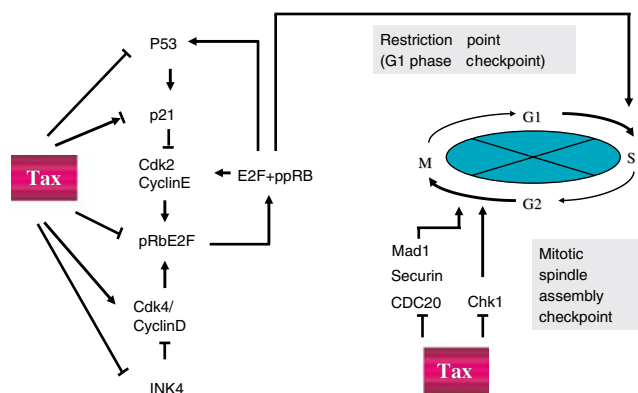


Figure 2 Effects of Tax on checkpoint factors at various points of the cell cycle are diagrammed. Tax is shown to affect p53, pRB, E2F, INK4, MAD1, Securin, CDC20, Chk1, p21^{Waf1/Cip1}, CDK4 and CDK2/cyclin E

gene for p53 is mutated in roughly 50% of all cancers. Curiously, unlike most other cancers, only a small fraction of ATL cells have p53 mutation. This paradox was elegantly resolved by independent findings that p53 function is inactivated by Tax (Reid *et al.*, 1993; Akagi *et al.*, 1997b; Mulloy *et al.*, 1998; Pise-Masison *et al.*, 1998; Ariumi *et al.*, 2000; Van *et al.*, 2001). What remains incompletely answered is how Tax inactivates p53. Some findings support that Tax abrogates p53 function by competing with it for binding the p300/CBP transcriptional coactivator (Van Orden *et al.*, 1999; Ariumi *et al.*, 2000). An alternative notion is that Tax acts through an NF- κ B/RelA(p65) pathway to perturb p53 function (Pise-Masison *et al.*, 2000a, b). Two recent papers suggest that neither the NF- κ B pathway nor the p300/CBP route fully explains Tax's inactivation of p53 (Jeong *et al.*, 2005; Miyazato *et al.*, 2005).

Tax and Rb

In conjunction with p53, Rb is a major tumor suppressor that regulates G1 to S progression. Rb is epigenetically and genetically inactivated in many cancers (Scrabble *et al.*, 1990). In cell cycle studies, many have found that Tax accelerate G1 to S transition (Neuveut *et al.*, 1998; Schmitt *et al.*, 1998; Kehn *et al.*, 2004), providing initial clues that HTLV-1 may inactivate Rb function (Hangaishi *et al.*, 1996; Hatta and Koeffler, 2002). There may be two explanations for how HTLV-1 inactivates Rb. First, Tax can directly bind Cdk4 (Haller *et al.*, 2002) and promote the hyperphosphorylation of Rb to inactivate its function (Neuveut *et al.*, 1998). Second, Tax may also directly bind Rb leading to its proteosomal degradation (Kehn *et al.*, 2005).

Tax and DLG

Lee *et al.* (1997) first demonstrated that the C-terminus of Tax (ETEV-COOH) contains a consensus motif (PBM; i.e. T/SXV-COOH), which binds the PDZ domains of several cellular proteins (Rousset *et al.*, 1998). One of the proteins bound by Tax is human DLG, a homologue of the *Drosophila* discs large PDZ-containing tumor suppressor. Comparing HTLV-1 and HTLV-2 Tax proteins revealed that the reduced ability of Tax2 to transform cells correlated with a loss of the PBM in its C-terminus (Semmes *et al.*, 1996; Endo *et al.*, 2002). Tax1's interaction with hDLG correlates with its ability to induce colony formation in rat fibroblasts, suggesting a relevant role of this interplay in cellular transformation (Suzuki *et al.*, 1999b; Hirata *et al.*, 2004). A more extensive discussion of the PDZ-binding properties of Tax is found in an accompanying article in this issue (Hall and Fujii, this issue).

Proliferation versus apoptosis

A long-standing cancer paradox is, that over-expression of oncoproteins provide proliferative advantages to cells but can also trigger cellular apoptosis. Oncogenes such

as Myc, E1A and E2F-1 all demonstrate such duality, and Tax may share this property. There is evidence that Tax protects cells from stress-induced cell cycle arrest or apoptosis (Copeland *et al.*, 1994; Brauweiler *et al.*, 1997; Torgeman *et al.*, 2001; Kasai and Jeang, 2004), but can also sensitize cells to stress-induced apoptosis (Chlichlia *et al.*, 1995, 1997, 2002; Kao *et al.*, 2000; Kasai and Jeang, 2004). This duality may be because transforming insults induce countervailing responses by the cell's tumor suppressors, which often manifest as cell cycle arrest and/or apoptosis. To transform a cell, an oncoprotein must disable the cell's apoptotic response, and this would explain why Tax exerts antiapoptotic activity perhaps through activation of NF- κ B (Kawakami *et al.*, 1999). The antiapoptotic effect of Tax may also be mediated by the transcriptional transactivation of cellular regulators of apoptosis such as Bcl-XL, Bfl1 (Bcl-2A1) (Tsukahara *et al.*, 1999; Nicot *et al.*, 2000; De La *et al.*, 2003) and HIAP-1 (Wäldele and Grassmann, unpublished observation). Likely, the choice between proliferation and death is influenced by cellular environment, cell background, and whether the cell's tumor suppressor functions have been defeated (see above). In ATL, these multiple intricacies may account for the long duration required by HTLV-1 to transform cells.

Tax and genetic damage in cells

Cancer cells can have more than 100 000 mutations (Perucho, 1996), some essential to initiating transformation and others for malignant progression. Genetic alterations initiated by Tax could be the trigger for malignant conversion in HTLV infected cells. Below, we summarize how Tax can harm the cell's genomic integrity by inducing structural DNA damage or alter chromosomal stability (Figure 2) (Loeb and Loeb, 2000).

Tax and DNA structural damage

Structurally damaged DNA is frequent in HTLV-1-transformed cells (Marriott *et al.*, 2002; see also Marriott and Semmes, this issue), suggesting the loss of checkpoint and repair functions which normally sense and eliminate such lesions. Indeed, Tax impairs the DNA-damage-induced checkpoint normally operative during G2/M transition (Liang *et al.*, 2002; Haoudi and Semmes, 2003). Tax also represses the expression of DNA polymerase β , an enzyme involved in base-excision repair, BER (Jeang *et al.*, 1990). Reduced BER activity is seen in HTLV-1, HTLV-2 and bovine leukemia virus transformed cells (Philpott and Buehring, 1999). Further, Tax independently suppresses a second repair process, nucleotide excision repair (NER), which is normally utilized by cells following UV irradiation (Kao *et al.*, 2001). Finally, chromosomal end-to-end fusion, a mistake common in cancers is also prevalent in HTLV-1 transformed cells. An explanation for the last finding may reside with the role played by telomeric repeats normally added to chromosomal ends

and DNA breaks to guard against end-to-end fusions and exonucleolytic degradation. Provocatively, Tax suppresses the expression of human telomerase (hTert; (Gabet *et al.*, 2003)), thus reducing the cell's capacity to add telomeric repeats to double-stranded breaks (DSB) and chromosomal ends (Wilkie *et al.*, 1990; Morin, 1991; Flint *et al.*, 1994). Loss of hTert function can destabilize the repair of DSB (Majone and Jeang, 2000). Consistent with its repression of cell endogenous telomerase, we recently observed that Tax cannot immortalize primary human cells unless exogenous hTert is coexpressed (Sheleg and Jeang, unpublished observation).

Tax and chromosomal instability

Cancers fall into two groups (Loeb and Loeb, 2000): those with structurally damaged chromosomes and those with aberrant chromosome numbers (i.e. aneuploidy and/or polyploidy). Most cancers are aneuploid (Cahill *et al.*, 1998), although it remains arguable whether aneuploidy is a cause, or a consequence, of transformation (Rasnick, 2002). Thus while normal peripheral blood lymphocytes are always diploid, ATL cells are ubiquitously aneuploid (Marriott *et al.*, 2002). The high correlation of ATL cells with aneuploidy, hints at a viral mechanism effectively subverting the cellular checkpoint(s) which guards against chromosomal instability (CIN).

The mitotic spindle assembly checkpoint (SAC; (Musacchio and Hardwick, 2002)) is a guardian of cellular euploidy. Two SAC proteins, MAD1 and MAD2, function as a MAD1–MAD2 heterodimer at kinetochores (Musacchio and Hardwick, 2002) in monitoring for proper chromosomal segregation during mitosis. In a knockout mouse model, it was found that heterozygous loss of just a single MAD2 allele (Michel *et al.*, 2001) heightened the risk for cellular transforma-

tion. Although knockout mouse data for MAD1 have yet to be described, there is clinical evidence obtained from two large acute myeloid leukemia (AML) studies that loss of a single MAD1 allele through monosomy of chromosome 7 holds elevated risk for human cancers (Jin *et al.*, 1999b) (Grimwade *et al.*, 1998; Byrd *et al.*, 2002). Although monosomy of the MAD1-encoding chromosome 7 (Jin *et al.*, 1999b) is uncommon in ATL cells, HTLV-1 Tax was found to bind directly MAD1 and repress its function (Jin *et al.*, 1998; Iwanaga *et al.*, 2002). Hence, the direct protein–protein interaction between a viral oncoprotein and a cellular checkpoint protein accomplishes a functional scenario equivalent to monosomy 7. Consistent with their aneuploid status, several ATL cell lines when tested *ex vivo* demonstrate defective SAC function (Kasai *et al.*, 2002).

SAC loss is a recessive change, which explains cellular tolerance of aneuploidy, but such loss in itself cannot create chromosomal mistakes. The following findings suggest that Tax may trigger chromosomal separation errors in several ways. (1) We find that Tax, like the HPV E7 oncoprotein (Duensing and Munger, 2003), can induce abnormal amplification of cellular centrosomes (Peloponese, Haller and Jeang, unpublished). Centrosomal amplification is seen in diverse tumors, and is considered a frequent first step shared by many cancers in developing CIN (Storchova and Pellman, 2004). Aberrant centrosomal amplification can directly initiate chromosomal missegregation. (2) Tax can promote unscheduled degradation of securin and cyclin B1 most likely through the premature activation of the CDC20-associated anaphase promoting complex (Liu *et al.*, 2005). This can lead to faulty chromosomal segregation and ensuing aneuploidy. (3) There is a paradigm that polyploidy is the precursor to aneuploidy (Margolis *et al.*, 2003). Tax expression frequently engenders multinucleated polyploid cells (Jin *et al.*, 1998; Liang *et al.*, 2002). Additionally, because Tax also inactivates

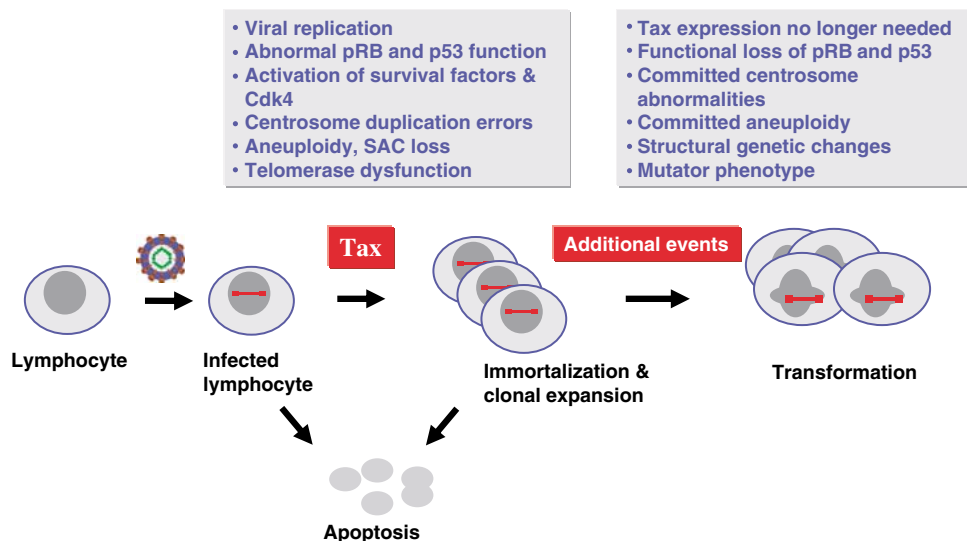


Figure 3 Progression of T-lymphocytes from immortalization and clonal expansion to transformation. Various changes in the cell during the different stages are listed in the shaded boxes. As shown, virus-infected cells can also go into apoptosis

p53 and Rb (Akagi *et al.*, 1997a; Neuveut *et al.*, 1998; Pise-Masison *et al.*, 1998; Takemoto *et al.*, 2000; Van *et al.*, 2001), two factors essential to a G1 tetraploid/polyploid checkpoint (Margolis *et al.*, 2003), it is reasonable that the polyploidy to aneuploidy mechanism can contribute to ATL transformation.

Conclusion

This article summarizes our current, albeit incomplete, understanding of how HTLV-1 transforms cells. *In vitro* cell transformation assays have clearly assigned a fundamental role to Tax in several important correlates of transformation. Conceptually, it is important to note that initiation and maintenance of transformation are separate processes, which require different biological events. Currently, based on the absence of Tax expression in many late-stage ATL cells, this viral oncoprotein is likely to be needed only for the former but not for the latter. Transformation initiation may start with mitogenic activation by Tax of lymphocytes in G1 with concomitant interference with tumor suppressor function and enhanced survival function (Figure 3). Initia-

tion of transformation then transits to the maintenance of a committed ATL-transformed phenotype, which likely arises from the cell's imprinted damaged genetic content. In this regards, we also propose that Tax plays a role in the creation of damaged DNA in cells. We note that two discrete requirements must be met for damaged DNA to manifest and persist. First, DNA damage must be created, either as a consequence of ambient errors or because Tax induces centrosomal amplification and/or premature APC-degradation of mitotic cyclins. Second, tolerance of created DNA errors must also occur. Tolerance can only happen if checkpoints that monitor and repair errors are extinguished. Thus, initiation of mitogenesis, creation of DNA errors, and inactivation of checkpoints represent a series of linked events overcome by Tax for cellular transformation. The requirement that HTLV-1 surmounts several biological barriers may explain the long latency period required for the virus to engender ATL.

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References

- Adya N and Giam CZ. (1995). *J. Virol.*, **69**, 1834–1841.
- Akagi T, Ono H, Nyunoya H and Shimotohno K. (1997a). *Oncogene*, **14**, 2071–2078.
- Akagi T, Ono H and Shimotohno K. (1995). *Blood*, **86**, 4243–4249.
- Akagi T, Ono H and Shimotohno K. (1996). *Oncogene*, **12**, 1645–1652.
- Akagi T, Ono H, Tsuchida N and Shimotohno K. (1997b). *FEBS Lett.*, **406**, 263–266.
- Akagi T and Shimotohno K. (1993). *J. Virol.*, **67**, 1211–1217.
- Ariumi Y, Kaida A, Lin JY, Hirota M, Masui O, Yamaoka S, Taya Y and Shimotohno K. (2000). *Oncogene*, **19**, 1491–1499.
- Arnulf B, Villemain A, Nicot C, Mordelet E, Charneau P, Kersual J, Zermati Y, Mauviel A, Bazarbachi A and Hermine O. (2002). *Blood*, **100**, 4129–4138.
- Azimi N, Brown K, Bamford RN, Tagaya Y, Siebenlist U and Waldmann TA. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 2452–2457.
- Azimi N, Jacobson S, Leist T and Waldmann TA. (1999). *J. Immunol.*, **163**, 4064–4072.
- Azran I, Jeang KT and Aboud M. (2005). *Oncogene*, **24**, 4521–4530.
- Azran I, Schavinsky-Khrapunsky Y and Aboud M. (2004). *Retrovirology*, **1**, 20.
- Ballard DW, Bohnlein E, Lowenthal JW, Wano Y, Franza BR and Greene WC. (1988). *Science*, **241**, 1652–1655.
- Baranger AM, Palmer CR, Hamm MK, Giebler HA, Brauweiler A, Nyborg JK and Schepartz A. (1995). *Nature*, **376**, 606–608.
- Bartoe JT, Albrecht B, Collins ND, Robek MD, Ratner L, Green PL and Lairmore MD. (2000). *J. Virol.*, **74**, 1094–1100.
- Baum PR, Gayle III RB, Ramsdell F, Srinivasan S, Sorensen RA, Watson ML, Seldin MF, Baker E, Sutherland GR and Clifford KN. (1994). *EMBO J.*, **13**, 3992–4001.
- Bex F, McDowall A, Burny A and Gaynor R. (1997). *J. Virol.*, **71**, 3484–3497.
- Bex F, Yin MJ, Burny A and Gaynor RB. (1998). *Mol. Cell. Biol.*, **18**, 2392–2405.
- Brauweiler A, Garrus JE, Reed JC and Nyborg JK. (1997). *Virology*, **231**, 135–140.
- Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA and Bloomfield CD. (2002). *Blood*, **100**, 4325–4336.
- Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW and Vogelstein B. (1998). *Nature*, **392**, 300–303.
- Caron C, Rousset R, Beraud C, Moncollin V, Egly JM and Jalinot P. (1993). *EMBO J.*, **12**, 4269–4278.
- Cereseto A, Diella F, Mulloy JC, Cara A, Michieli P, Grassmann R, Franchini G and Klotman ME. (1996). *Blood*, **88**, 1551–1560.
- Chiari E, Lamsoul I, Lodewick J, Chopin C, Bex F and Pique C. (2004). *J. Virol.*, **78**, 11823–11832.
- Ching YP, Chun AC, Chin KT, Zhang ZQ, Jeang KT and Jin DY. (2004). *Retrovirology*, **1**, 18.
- Chlichlia K, Busslinger M, Peter ME, Walczak H, Krammer PH, Schirmacher V and Khazaie K. (1997). *Oncogene*, **14**, 2265–2272.
- Chlichlia K, Los M, Schulze-Osthoff K, Gazzolo L, Schirmacher V and Khazaie K. (2002). *Antioxid. Redox Signal.*, **4**, 471–477.
- Chlichlia K, Moldenhauer G, Daniel PT, Busslinger M, Gazzolo L, Schirmacher V and Khazaie K. (1995). *Oncogene*, **10**, 269–277.
- Chowdhury IH, Farhadi A, Wang XF, Robb ML, Birx DL and Kim JH. (2003). *Int. J. Cancer*, **107**, 603–611.
- Chu ZL, Shin YA, Yang JM, DiDonato JA and Ballard DW. (1999). *J. Biol. Chem.*, **274**, 15297–15300.

- Chung HK, Young HA, Goon PK, Heidecker G, Princler GL, Shimozato O, Taylor GP, Bangham CR and Derse D. (2003). *Blood*, **102**, 4130–4136.
- Copeland KF, Haaksma AG, Goudsmit J, Krammer PH and Heeney JL. (1994). *AIDS Res. Hum. Retroviruses*, **10**, 1259–1268.
- D'Agostino DM, Ranzato L, Arrigoni G, Cavallari I, Belleudi F, Torrisi MR, Silic-Benussi M, Ferro T, Petronilli V, Marin O, Chieco-Bianchi L, Bernardi P and Ciminale V. (2002). *J. Biol. Chem.*, **277**, 34424–34433.
- De La FC, Wang L, Wang D, Deng L, Wu K, Li H, Stein LD, Denny T, Coffman F, Kehn K, Baylor S, Maddukuri A, Pumfery A and Kashanchi F. (2003). *Mol. Cell Biochem.*, **245**, 99–113.
- Derse D, Mikovits J and Ruscetti F. (1997). *Virology*, **237**, 123–128.
- Ding W, Albrecht B, Kelley RE, Muthusamy N, Kim SJ, Altschuld RA and Lairmore MD. (2002). *J. Virol.*, **76**, 10374–10382.
- Duensing S and Munger K. (2003). *Prog. Cell Cycle Res.*, **5**, 383–391.
- Endo K, Hirata A, Iwai K, Sakurai M, Fukushi M, Oie M, Higuchi M, Hall WW, Gejyo F and Fujii M. (2002). *J. Virol.*, **76**, 2648–2653.
- Etoh K, Tamiya S, Yamaguchi K, Okayama A, Tsubouchi H, Ideta T, Mueller N, Takatsuki K and Matsuoka M. (1997). *Cancer Res.*, **57**, 4862–4867.
- Flint J, Craddock CF, Villegas A, Bentley DP, Williams HJ, Galanello R, Cao A, Wood WG, Ayyub H and Higgs DR. (1994). *Am. J. Hum. Genet.*, **55**, 505–512.
- Fujita M and Shiku H. (1995). *Oncogene*, **11**, 15–20.
- Fung MM, Chu YL, Fink JL, Wallace A and McGuire KL. (2005). *Oncogene*, **24**, 4624–4633.
- Gabet AS, Mortreux F, Charneau P, Riou P, Duc-Dodon M, Wu Y, Jeang KT and Wattel E. (2003). *Oncogene*, **22**, 3734–3741.
- Gabet AS, Mortreux F, Talarmin A, Plumelle Y, Leclercq I, Leroy A, Gessain A, Clity E, Joubert M and Wattel E. (2000). *Oncogene*, **19**, 4954–4960.
- Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A and de The G. (1985). *Lancet*, **2**, 407–410.
- Giebler HA, Loring JE, van Orden K, Colgin MA, Garrus JE, Escudero KW, Brauweiler A and Nyborg JK. (1997). *Mol. Cell Biol.*, **17**, 5156–5164.
- Good L, Maggirwar SB and Sun SC. (1996). *EMBO J.*, **15**, 3744–3750.
- Grassmann R, Berchtold S, Radant I, Alt M, Fleckenstein B, Sodroski JG, Haseltine WA and Ramstedt U. (1992). *J. Virol.*, **66**, 4570–4575.
- Grassmann R, Dengler C, Muller-Fleckenstein I, Fleckenstein B, McGuire K, Dokhelar MC, Sodroski JG and Haseltine WA. (1989). *Proc. Natl. Acad. Sci. USA*, **86**, 3351–3355.
- Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, Rees J, Hann I, Stevens R, Burnett A and Goldstone A. (1998). *Blood*, **92**, 2322–2333.
- Haller K, Ruckes T, Schmitt I, Saul D, Derow E and Grassmann R. (2000). *AIDS Res. Hum. Retroviruses*, **16**, 1683–1688.
- Haller K, Wu Y, Derow E, Schmitt I, Jeang KT and Grassmann R. (2002). *Mol. Cell Biol.*, **22**, 3327–3338.
- Hangaishi A, Ogawa S, Imamura N, Miyawaki S, Miura Y, Uike N, Shimazaki C, Emi N, Takeyama K, Hirokawa S, Kamada N, Kobayashi Y, Takemoto Y, Kitani T, Toyama K, Ohtake S, Yazaki Y, Ueda R and Hirai H. (1996). *Blood*, **12**, 4949–4958.
- Haoudi A and Semmes OJ. (2003). *Virology*, **305**, 229–239.
- Harhaj EW and Sun SC. (1999). *J. Biol. Chem.*, **274**, 22911–22914.
- Harrod R, Kuo YL, Tang Y, Yao Y, Vassilev A, Nakatani Y and Giam CZ. (2000). *J. Biol. Chem.*, **275**, 11852–11857.
- Hatta Y and Koeffler HP. (2002). *Leukemia*, **16**, 1069–1085.
- Hirata A, Higuchi M, Niinuma A, Ohashi M, Fukushi M, Oie M, Akiyama T, Tanaka Y, Gejyo F and Fujii M. (2004). *Virology*, **318**, 327–336.
- Hoyos B, Ballard DW, Bohnlein E, Siekevitz M and Greene WC. (1989). *Science*, **244**, 457–460.
- Huang H, Hu-Li J, Chen H, Ben Sasson SZ and Paul WE. (1997). *J. Immunol.*, **159**, 3731–3738.
- Iha H, Kibler KV, Yedavalli VR, Peloponese JM, Haller K, Miyazato A, Kasai T and Jeang KT. (2003). *Oncogene*, **22**, 8912–8923.
- Inoue H, Yamaoka S, Imamura M and Hatanaka M. (1994). *Exp. Cell Res.*, **215**, 68–74.
- Iwanaga R, Ohtani K, Hayashi T and Nakamura M. (2001). *Oncogene*, **20**, 2055–2067.
- Iwanaga Y, Kasai T, Kibler K and Jeang KT. (2002). *J. Biol. Chem.*, **277**, 31005–31013.
- Jeang KT. (2001). *Cytokine Growth Factor Rev.*, **12**, 207–217.
- Jeang KT, Boros I, Brady J, Radonovich M and Khoury G. (1988). *J. Virol.*, **62**, 4499–4509.
- Jeang KT, Widen SG, Semmes OJ and Wilson SH. (1990). *Science*, **247**, 1082–1084.
- Jeong SJ, Pise-Masison CA, Radonovich MF, Park HU and Brady JN. (2005). *J. Biol. Chem.*, **280**, 10326–10332.
- Jin DY, Giordano V, Kibler KV, Nakano H and Jeang KT. (1999a). *J. Biol. Chem.*, **274**, 17402–17405.
- Jin DY, Kozak CA, Pangilinan F, Spencer F, Green ED and Jeang KT. (1999b). *Genomics*, **55**, 363–364.
- Jin DY, Spencer F and Jeang KT. (1998). *Cell*, **93**, 81–91.
- Jin DY, Teramoto H, Giam CZ, Chun RF, Gutkind JS and Jeang KT. (1997). *J. Biol. Chem.*, **272**, 25816–25823.
- Johnson JM, Nicot C, Fullen J, Ciminale V, Casareto L, Mulloy JC, Jacobson S and Franchini G. (2001). *J. Virol.*, **75**, 6086–6094.
- Kao SY, Lemoine FJ and Mariott SJ. (2000). *Oncogene*, **19**, 2240–2248.
- Kao SY, Lemoine FJ and Marriott SJ. (2001). *Virology*, **291**, 292–298.
- Kasai T, Iwanaga Y, Iha H and Jeang KT. (2002). *J. Biol. Chem.*, **277**, 5187–5193.
- Kasai T and Jeang KT. (2004). *Retrovirology*, **1**, 7.
- Kawakami A, Nakashima T, Sakai H, Urayama S, Yamasaki S, Hida A, Tsuboi M, Nakamura H, Ida H, Migita K, Kawabe Y and Eguchi K. (1999). *Blood*, **94**, 3847–3854.
- Kawata S, Ariumi Y and Shimotohno K. (2003). *J. Virol.*, **77**, 7291–7299.
- Kehn K, Deng L, De La FC, Strouss K, Wu K, Maddukuri A, Baylor S, Rufner R, Pumfery A, Bottazzi ME and Kashanchi F. (2004). *Retrovirology*, **1**, 6.
- Kehn K, Fuente CL, Strouss K, Berro R, Jiang H, Brady J, Mahieux R, Pumfery A, Bottazzi ME and Kashanchi F. (2005). *Oncogene*, **24**, 525–540.
- Kelly-Welch AE, Hanson EM, Boothby MR and Keegan AD. (2003). *Science*, **300**, 1527–1528.
- Kim SJ, Kehrl JH, Burton J, Tendler CL, Jeang KT, Danielpour D, Thevenin C, Kim KY, Sporn MB and Roberts AB. (1990). *J. Exp. Med.*, **172**, 121–129.
- Kirken RA, Erwin RA, Wang L, Wang Y, Rui H and Farrar WL. (2000). *J. Immunol.*, **165**, 5097–5104.
- Lee DK, Kim BC, Brady JN, Jeang KT and Kim SJ. (2002). *J. Biol. Chem.*, **277**, 33766–33775.

- Lee SS, Weiss RS and Javier RT. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 6670–6675.
- Lefebvre L, Vanderplasschen A, Ciminale V, Heremans H, Dangoisse O, Jauniaux JC, Toussaint JF, Zelnik V, Burny A, Kettmann R and Willems L. (2002). *J. Virol.*, **76**, 1400–1414.
- Lemasson I, Thebault S, Sardet C, Devaux C and Mesnard JM. (1998). *J. Biol. Chem.*, **273**, 23598–23604.
- Li J, Li H and Tsai MD. (2003). *Biochemistry*, **42**, 6921–6928.
- Liang MH, Geisbert T, Yao Y, Hinrichs SH and Giam CZ. (2002). *J. Virol.*, **76**, 4022–4033.
- Liu B, Hong S, Tang Z, Yu H and Giam CZ. (2005). *Proc. Natl. Acad. Sci. USA*, **102**, 63–68.
- Liu Y, Wang Y, Yamakuchi M, Masuda S, Tokioka T, Yamaoka S, Maruyama I and Kitajima I. (2001). *Oncogene*, **20**, 2514–2526.
- Loeb KR and Loeb LA. (2000). *Carcinogenesis*, **21**, 379–385.
- Low KG, Dorner LF, Fernando DB, Grossman J, Jeang KT and Comb MJ. (1997). *J. Virol.*, **71**, 1956–1962.
- Majone F and Jeang KT. (2000). *J. Biol. Chem.*, **275**, 32906–32910.
- Margolis RL, Lohez OD and Andreassen PR. (2003). *J. Cell Biochem.*, **88**, 673–683.
- Mariner JM, Lantz V, Waldmann TA and Azimi N. (2001). *J. Immunol.*, **166**, 2602–2609.
- Marriott SJ, Lemoine FJ and Jeang KT. (2002). *J. Biomed. Sci.*, **9**, 292–298.
- Martino A, Holmes JH, Lord JD, Moon JJ and Nelson BH. (2001). *J. Immunol.*, **166**, 1723–1729.
- Matsumoto K, Shibata H, Fujisawa JI, Inoue H, Hakura A, Tsukahara T and Fujii M. (1997). *J. Virol.*, **71**, 4445–4451.
- McGuire KL, Curtiss VE, Larson EL and Haseltine WA. (1993). *J. Virol.*, **67**, 1590–1599.
- Michel LS, Liberal V, Chatterjee A, Kirchwegger R, Pasche B, Gerald W, Dobles M, Sorger PK, Murty VV and Benezra R. (2001). *Nature*, **409**, 355–359.
- Migone TS, Lin JX, Cereseto A, Mulloy JC, O’Shea JJ, Franchini G and Leonard WJ. (1995). *Science*, **269**, 79–81.
- Miyazato A, Sheleg S, Iha H, Li Y and Jeang KT. (2005). *J. Virol.*, **79**, 9346–9350.
- Moon JJ, Rubio ED, Martino A, Krumm A and Nelson BH. (2004). *J. Biol. Chem.*, **279**, 5520–5527.
- Mori N, Morishita M, Tsukazaki T, Giam CZ, Kumatori A, Tanaka Y and Yamamoto N. (2001). *Blood*, **97**, 2137–2144.
- Morin GB. (1991). *Nature*, **353**, 454–456.
- Mulloy JC, Kislyakova T, Cereseto A, Casareto L, LoMonico A, Fullen J, Lorenzi MV, Cara A, Nicot C, Giam C and Franchini G. (1998). *J. Virol.*, **72**, 8852–8860.
- Musacchio A and Hardwick KG. (2002). *Nat. Rev. Mol. Cell Biol.*, **3**, 731–741.
- Neuveut C and Jeang KT. (2002). *Front. Biosci.*, **7**, d157–d163.
- Neuveut C, Low KG, Maldarelli F, Schmitt I, Majone F, Grassmann R and Jeang KT. (1998). *Mol. Cell Biol.*, **18**, 3620–3632.
- Ng PW, Iha H, Iwanaga Y, Bittner M, Chen Y, Jiang Y, Gooden G, Trent JM, Meltzer P, Jeang KT and Zeichner SL. (2001). *Oncogene*, **20**, 4484–4496.
- Nicot C, Mahieux R, Takemoto S and Franchini G. (2000). *Blood*, **96**, 275–281.
- Nicot C, Mulloy JC, Ferrari MG, Johnson JM, Fu K, Fukumoto R, Trovato R, Fullen J, Leonard WJ and Franchini G. (2001). *Blood*, **98**, 823–829.
- Ohtani K, Iwanaga R, Arai M, Huang Y, Matsumura Y and Nakamura M. (2000). *J. Biol. Chem.*, **275**, 11154–11163.
- Okada M and Jeang KT. (2002). *J. Virol.*, **76**, 12564–12573.
- Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M and Tara M. (1986). *Lancet*, **1**, 1031–1032.
- Pankow R, Durkop H, Latza U, Krause H, Kunzendorf U, Pohl T and Bulfone-Paus S. (2000). *J. Immunol.*, **165**, 263–270.
- Peloponese Jr JM, Iha H, Yedavalli VR, Miyazato A, Li Y, Haller K, Benkirane M and Jeang KT. (2004). *J. Virol.*, **78**, 11686–11695.
- Perucho M. (1996). *Nat. Med.*, **2**, 630–631.
- Philpott SM and Buehring GC. (1999). *J. Natl. Cancer Inst.*, **91**, 933–942.
- Pise-Masison CA, Choi KS, Radonovich M, Dittmer J, Kim SJ and Brady JN. (1998). *J. Virol.*, **72**, 1165–1170.
- Pise-Masison CA, Mahieux R, Jiang H, Ashcroft M, Radonovich M, Duvall J, Guillerme C and Brady JN. (2000a). *Mol. Cell Biol.*, **20**, 3377–3386.
- Pise-Masison CA, Mahieux R, Radonovich M, Jiang H, Duvall J, Guillerme C and Brady JN. (2000b). *AIDS Res. Hum. Retroviruses*, **16**, 1669–1675.
- Pise-Masison CA, Radonovich M, Mahieux R, Chatterjee P, Whiteford C, Duvall J, Guillerme C, Gessain A and Brady JN. (2002). *Cancer Res.*, **62**, 3562–3571.
- Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD and Gallo RC. (1980). *Proc. Natl. Acad. Sci. USA*, **77**, 7415–7419.
- Pozzatti R, Vogel J and Jay G. (1990). *Mol. Cell Biol.*, **10**, 413–417.
- Rasnick D. (2002). *Cancer Genet. Cytogenet.*, **136**, 66–72.
- Reid RL, Lindholm PF, Mireskandari A, Dittmer J and Brady JN. (1993). *Oncogene*, **8**, 3029–3036.
- Robek MD and Ratner L. (2000). *J. Virol.*, **74**, 11988–11992.
- Robek MD, Wong FH and Ratner L. (1998). *J. Virol.*, **72**, 4458–4462.
- Rosin O, Koch C, Schmitt I, Semmes OJ, Jeang KT and Grassmann R. (1998). *J. Biol. Chem.*, **273**, 6698–6703.
- Rousset R, Fabre S, Desbois C, Bantignies F and Jalinot P. (1998). *Oncogene*, **16**, 643–654.
- Ruben S, Poteat H, Tan TH, Kawakami K, Roeder R, Haseltine W and Rosen CA. (1988). *Science*, **241**, 89–92.
- Ruckes T, Saul D, Van Snick J, Hermine O and Grassmann R. (2001). *Blood*, **98**, 1150–1159.
- Santiago F, Clark E, Chong S, Molina C, Mozafari F, Mahieux R, Fujii M, Azimi N and Kashanchi F. (1999). *J. Virol.*, **73**, 9917–9927.
- Schmitt I, Rosin O, Rohwer P, Gossen M and Grassmann R. (1998). *J. Virol.*, **72**, 633–640.
- Scrable HJ, Sapienza C and Cavenee WK. (1990). *Adv. Cancer Res.*, **54**, 25–62.
- Semmes OJ and Jeang KT. (1995). *J. Virol.*, **69**, 1827–1833.
- Semmes OJ and Jeang KT. (1996). *J. Virol.*, **70**, 6347–6357.
- Semmes OJ, Majone F, Cantemir C, Turchetto L, Hjelle B and Jeang KT. (1996). *Virology*, **217**, 373–379.
- Shankar DB, Cheng JC, Kinjo K, Federman N, Moore TB, Gill A, Rao NP, Landaw EM and Sakamoto KM. (2005). *Cancer Cell*, **7**, 351–362.
- Smith MR and Greene WC. (1991). *J. Clin. Invest.*, **88**, 1038–1042.
- Storchova Z and Pellman D. (2004). *Nat. Rev. Mol. Cell Biol.*, **5**, 45–54.
- Suzuki T, Kitao S, Matsushima H and Yoshida M. (1996). *EMBO J.*, **15**, 1607–1614.
- Suzuki T, Narita T, Uchida-Toita M and Yoshida M. (1999a). *Virology*, **259**, 384–391.
- Suzuki T, Ohsugi Y, Uchida-Toita M, Akiyama T and Yoshida M. (1999b). *Oncogene*, **18**, 5967–5972.
- Takemoto S, Trovato R, Cereseto A, Nicot C, Kislyakova T, Casareto L, Waldmann T, Torelli G and Franchini G. (2000). *Blood*, **95**, 3939–3944.

- Tanaka A, Takahashi C, Yamaoka S, Nosaka T, Maki M and Hatanaka M. (1990). *Proc. Natl. Acad. Sci. USA*, **87**, 1071–1075.
- Torgeman A, Ben Aroya Z, Grunspan A, Zelin E, Butovsky E, Hallak M, Lochelt M, Flugel RM, Livneh E, Wolfson M, Kedar I and Aboud M. (2001). *Exp. Cell Res.*, **271**, 169–179.
- Tsukahara T, Kannagi M, Ohashi T, Kato H, Arai M, Nunez G, Iwanaga Y, Yamamoto N, Ohtani K, Nakamura M and Fujii M. (1999). *J. Virol.*, **73**, 7981–7987.
- Van Orden K, Yan JP, Ulloa A and Nyborg JK. (1999). *Oncogene*, **18**, 3766–3772.
- Van PL, Yim KW, Jin DY, Dapolito G, Kurimasa A and Jeang KT. (2001). *J. Virol.*, **75**, 396–407.
- Wäldele K, Schneider G, Ruckes T and Grassmann R. (2004). *J. Virol.*, **78**, 6081–6090.
- Wilkie AO, Lamb J, Harris PC, Finney RD and Higgs DR. (1990). *Nature*, **346**, 868–871.
- Xu X, Heidenreich O, Kitajima I, McGuire K, Li Q, Su B and Nerenberg M. (1996). *Oncogene*, **13**, 135–142.
- Yamaoka S, Inoue H, Sakurai M, Sugiyama T, Hazama M, Yamada T and Hatanaka M. (1996). *EMBO J.*, **15**, 873–887.
- Yoshida M, Seiki M, Yamaguchi K and Takatsuki K. (1984). *Proc. Natl. Acad. Sci. USA*, **81**, 2534–2537.