[Frontiers in Bioscience 9, 2347-2372, September 1, 2004]

MECHANISMS OF HTLV-1 TRANSFORMATION

Kylene Kehn [1], Reem Berro [1], Cynthia de la Fuente [1], Katharine Strouss [1], Elodie Ghedin [3], Shabnam Dadgar [1], Maria Elena Bottazzi [2], Anne Pumfery [1], and Fatah Kashanchi [1,3]

[1]Department of Biochemistry and Molecular Biology, School of Medicine and Health Sciences, The George Washington University, Washington DC, 20037, USA, [2]Department of Microbiology and Tropical Medicine, School of Medicine and Health Sciences, The George Washington University, Washington DC 20037, USA, [3]The Institute for Genomic Research (TIGR), Rockville, MD 20850, USA

TABLE OF CONTENTS

1. ABSTRACT

HTLV-1 is the etiological agent of the fatal disease adult T-cell leukemia. The virus encodes many proteins including several accessory proteins, $p12^I$, $p13^{II}$, $p27^I$, and $p30^{II}$, whose roles have recently begun to be elucidated. These accessory proteins are important in T-cell activation, transcriptional regulation, viral persistence, and virus assembly. The viral oncogene Tax is thought to be largely responsible for tumorigenesis, although the precise mechanisms underlying transformation are not completely understood. Tax has a profound impact on transcription, cell growth regulation, genomic stability and apoptosis. This review will provide possible contributions of the accessory proteins to transformation as well as highlight the alterations of the above-mentioned cellular events by Tax. Animal models of both Tax and the accessory proteins are also included based on the essential information on the transformation process *in vivo* that they provide.

2. INTRODUCTION

Human T-cell leukemia virus type-1 (HTLV-1) is the first human retrovirus to be associated with a human malignancy (1, 2). It is estimated that 10-20 million people are infected worldwide (3) and infection is endemic in southern Japan, South America, Africa, the Caribbean, the Middle East, the Pacific Melanesian Islands, and Papua New Guinea (4, 5). Transmission occurs through three main routes; breast milk (mother to child), semen (sexual intercourse) and blood (transfusion). The main target cells for HTLV-1 infection are $CD4^+$ T-cells, but the virus can also infect $CD8^+$ T-cells, B-cells, and macrophages. HTLV-1 is the etiologic agent of both adult T cell leukemia (ATL) and the neurological disorder, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). In addition, it has also been implicated in other diseases such as uveitis and dermatitis (4).

ATL is a $CD4^+$ non-Hodgkin leukemia/lymphoma. HTLV-1 infection leads to disease in about 5-10% of infected individuals after a long latency period spanning decades. This long latent period reflects the inefficiency of these viruses to transform cells and the need for multiple cooperative changes in growth control mechanisms to induce tumorigenesis. ATL patients display

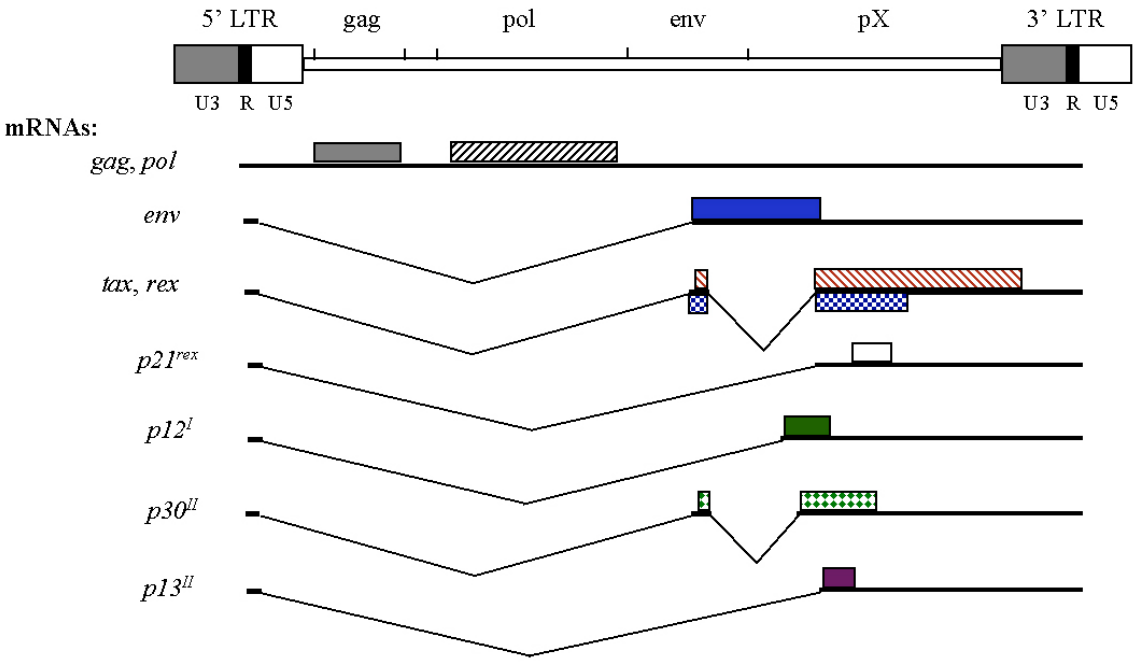

Figure 1. HTLV-1 pro-viral genome. The HTLV-1 genome contains both a 5'LTR and a 3'LTR. Each LTR region is further subdivided into a U3 region, which contains the viral CREs, an R (repeat) region, which marks the start of transcription in the 5' LTR and polyadenylation in the 3' LTR, and a U5 region. The genome encodes the structural proteins, gag, pol and env. In addition, it contains a pX region in which the viral proteins, $p12^{I}$, $p13^{II}$, $p21^{rex}$, $p27^{I}$, $p30^{II}$, Tax, and Rex are produced through alternative splicing.

abnormal lymphocytes, the so-called "flower cells", which have cleaved and convoluted nuclei. The classifications of ATL are acute, chronic, lymphoma, or smouldering ATL. The acute and lymphoma subtypes have a median survival rate of less than one year, which is predominantly due to the lack of effective treatment for ATL patients.

Compared to other animal and primate RNA viruses, HTLV-1 is a complex retrovirus. The HTLV-1 genome consists of a 5' long terminal repeat (LTR) region, group specific antigen (gag), protease (pro)/polymerase (pol), and envelope (env) genes, and a 3' LTR, which are common to most retroviruses **(**figure 1**)**. In addition, the HTLV-1 genome encodes for several regulatory and accessory proteins via alternative splicing and an internal initiation codon between env and the 3' end called the pX region. The pX region consists of four open reading frames (ORFs) (I to IV), of which pX-IV and pX-III encode the Tax and Rex proteins, respectively, as well as $p21^{rex}$, a protein of unknown function encoded by pX-ORF III. pX-I and II produce singly or doubly spliced transcripts, encoding for the four accessory proteins, $p12^{I}$, $p27^{I}$, $p13^{II}$ and $p30^{II}$. Rex is necessary for the export of unspliced viral RNA from the nucleus (6). The accessory proteins were originally thought to be unnecessary for viral replication, but studies have shown their importance in T-cell activation, transcriptional regulation, viral persistence, and virus assembly (7). Therefore, the contribution of these proteins to survival of the virus as well as possible contributions to the oncogenic process will be discussed. Next, the review will focus on the 40-kDa protein, Tax, which is the major protein involved in transformation events leading to ATL. There are four broad categories of cellular events that Tax alters which contribute to tumorigenesis, including transcription, cell growth, genomic stability, and apoptosis. Finally, animal models of Tax, which provide important insights into the *in vivo* functions of this viral protein, will also be addressed.

3. HTLV-1 ACCESSORY PROTEINS

The role of Tax as a transforming agent is very well established in HTLV-1 infected cells. Although no direct role for the accessory proteins in transformation has yet been proven, they are fundamental for viral replication and persistence of infection (11, 20), events that are a prerequisite for transformation of host cells by Tax. The accessory proteins were shown to be expressed in infected patients by detecting cytotoxic T-cells that are able to recognize pX-I and pX-II peptides as well as serum antibodies directed against recombinant proteins and peptides representing these accessory proteins (8-10). There has been several reports published in the past few years highlighting the importance of accessory proteins in viral infectivity and maintenance of a high viral load (11), host cell activation, and regulation of gene transcription (12-14).

3.1. pX-ORF-1 accessory proteins: $p12^{I}$ and $p27^{I}$

3.1.1. Structure and localization of $p12^{I}$

The $p12^{I}$ accessory protein is encoded by both singly spliced mRNA pX-ORF-I and doubly spliced mRNA pX-rex-ORF-I, which could alternatively code for

$p27^{I}$ (see the following section for details). The singly spliced $p12^{I}$ encodes a membrane-associated hydrophobic protein made up of 99 amino acid residues (15). $p12^{I}$ contains two potential transmembrane regions, TM-1 and TM-2, four SH3 binding motifs (PXXP) which are highly conserved among viral strains, a leucine zipper region that allows the formation of dimers or oligomers (16), and a di-leucine (DXXXLL) motif whose function is not yet defined. The $p12^{I}$ protein exhibits significant amino acid sequence similarity between latent membrane protein-1 (LMP-1) of Epstein-Barr virus, E5 transformation protein of bovine papillomavirus, and the transmembrane proteins of slowly transforming retroviruses (17). Similarity between $p12^{I}$ and E5 is not only restricted to the structural level but also extends to biological behavior. For example, Franchini *et al.* demonstrated that $p12^{I}$ strongly potentiated the transforming activity of E5 in mouse fibroblasts and, like E5, could bind the 16K protein, a component of the vacuolar H+ ATPase (18), although the significance of this binding is not yet completely clear.

3.1.2. $p12^{I}$: Viral infectivity and T-cell activation

Initial studies about the role of $p12^{I}$ in viral replication and transformation have revealed that mutations which are predicted to abrogate the expression of $p12^{I}$, $p13^{II}$, and $p30^{II}$, had no effect on viral replication or the immortalization of mitogen activated primary lymphocytes *in vitro* (19). However, when these mutated HTLV-1 clones were inoculated in rabbits, the results showed the essential role of HTLV-1 $p12^{I}$ in the establishment of persistent viral infection in this *in vivo* model (20). Standard *in vitro* systems, using primary lymphocytes activated by the exogenous addition of IL-2 and phytohemagglutinin (PHA), have failed to demonstrate the contribution of $p12^{I}$ to viral infectivity and ultimately cellular transformation. A study using multiple *in vitro* co-culture assays evaluated the role of $p12^{I}$ in viral infectivity in quiescent peripheral blood mononuclear cells (PBMC), co-cultured with a variety of HTLV-1-producing cells, in the absence of exogenous stimuli to more accurately reflect the virus-cell interactions *in vivo*, as observed in animal models. These kinds of assays were able to demonstrate a dramatic reduction in viral infectivity in quiescent T lymphocytes for the $p12^{I}$ mutant viral clone, ACH.p12, in comparison to the wild-type ACH clone. Moreover, the addition of mitogens to cells, such as IL-2 and phytohemagglutinin (PHA), during infection, completely rescued the ability of the mutant virus to infect primary quiescent lymphocytes. When newly infected primary lymphocytes were used to passage the virus, ACH.p12 also exhibited a reduced ability to productively infect activated lymphocytes. This study demonstrated that $p12^{I}$ is required for optimal viral infectivity in quiescent primary cells and suggests a role for $p12^{I}$ in T-lymphocyte activation (21). These findings parallel animal model data and suggest a role for $p12^{I}$ in the activation of quiescent lymphocytes as a prerequisite for effective viral replication *in vivo* (22).

Later studies have shown that $p12^{I}$ activates T-cells during early stages of infection through the elevation of intracellular calcium levels. In fact, $p12^{I}$ was found to accumulate within both the endoplasmic reticulum (ER) and cis-Golgi apparatus and directly bind to calnexin and calreticulin. Calnexin and calreticulin are calcium-binding proteins located in the ER and are important for calcium storage and release from the ER by acting as a calcium buffer (23). Nuclear factor of activated T-cells (NFAT) seems to be an important downstream readout for TCR activation (24). NFAT is a key transcription activator in T cells that is regulated by dephosphorylation by the calcium/calmodulin-dependent serine/threonine phosphatase, calcineurin, which is activated by the increase in cytoplasmic calcium. Dephosphorylated NFAT translocates from the cytoplasm to the nucleus to induce the expression of downstream genes such as IL-2 (25). Albrecht *et al.* suggested a model in which $p12^{I}$ causes the release of calcium from the ER, possibly through interaction with calnexin or calreticulin, thus leading to the activation of NFAT2, the predominantly induced NFAT isoform in activated T-cells. However, the Ras/MAPK pathway, an important pathway that leads to the activation of the AP-1 transcription factor also associated with TCR signaling, acts synergistically with $p12^{I}$-induced calcium release from the ER in order to induce NFAT-2 activation (12). Therefore, $p12^{I}$ appears to induce calcineurin-dependent NFAT activation either indirectly by affecting cytoplasmic calcium levels or directly by $p12^{I}$ binding to calcineurin. This binding occurs through a conserved calcineurin-binding motif on $p12^{I}$, highly homologous to the PXIXIT calcineurin-binding motif of NFAT (13). Although a $p12^{I}$-induced calcium release from the ER was previously observed, the exact mechanism was not addressed. Ding *et al.* demonstrated that, while $p12^{I}$ increased the intracellular calcium level, the calcium content in the ER stores was reduced. The authors suggest that this effect was partially due to IP3 receptors in the ER, since addition of an IP3 receptor inhibitor blocked calcium release as well as $p12^{I}$-mediated NFAT activation. In addition, inhibition of calcium release-activated channel (CRAC) in the plasma membrane showed that calcium influx into the cells was also important for NFAT activation by $p12^{I}$. Furthermore, this study demonstrated that although $p12^{I}$ and calreticulin were found to bind and co-localize within the cell, this binding did not correlate with NFAT activation mediated by $p12^{I}$ (26). A subsequent study has shown that $p12^{I}$ enhanced NFAT-induced IL-2 production during T-cell activation (27). $p12^{I}$ can also activate T-cells independently of the NFAT pathway. In fact, $p12^{I}$ physically binds to the cytoplasmic domain of the interleukin-2 receptor (IL-2R) beta chain that is involved in the recruitment of the Jak1 and Jak3 kinases, and enhances Stat 5 activation, conferring a proliferative advantage of PBMCs in a context of decreased requirement for IL-2 (28).

Another interesting observation suggests that $p12^{I}$ induces infection persistence by allowing the cells to evade the immune system. $p12^{I}$ physically binds to free human major histocompatibility complex class I heavy chains (MHC-1-Hc), preventing its surface presentation, and subsequently targets the heavy chains for proteasomal degradation (29).These results suggest that the interaction of $p12^{I}$ with MHC-1-Hc may interfere with antigen presentation *in vivo* and facilitate escape of HTLV-1-infected cells from immune recognition.

$p12^{I}$, which is found in the serum of ATL patients, appears to be important for viral infectivity since it modulates key pathways involved in T-cell activation. This is fundamental in HTLV-1 infection since, unlike HIV which can infect quiescent cells, successful HTLV-1 infection and subsequent transformation by Tax requires T-cell activation.

3.1.3. $p27^{I}$

The second accessory protein encoded by pX-ORF-I is the 152 amino acid protein $p27^{I}$. Unlike $p12^{I}$, produced by single splicing of ORF I, $p27^{I}$ is produced by double splicing of the same transcript. However, Koralinik *et al.* demonstrated that transfection of HA-tagged pX-rex-orf I did not lead to the production of $p27^{I}$ but to the 99 amino acid $p12^{I}$, whose translation is initiated at the internal methionine codon within the $p27^{I}$ ORF. This study proved that both singly and doubly-spliced ORF I lead to the production $p12^{I}$ (30). On the other hand, using an *in vitro* transcription and translation system, $p27^{I}$ could be produced from the doubly-spliced mRNA (31). In addition, cytotoxic T-cells (CTLs), reactive against peptides representing $p27^{I}$, were shown to be produced during the course of natural infection in HTLV-1-infected individuals along with CTLs reactive to the other three accessory proteins (10). Although analysis of $p27^{I}$ indicated that this protein was highly hydrophobic, not much information is known about its function *in vivo*.

3.2. pX-ORF II accessory proteins: $p30^{II}$ and $p13^{II}$

Both $p30^{II}$ and $p13^{II}$ are encoded by pX-ORFII by differential splicing. The smaller protein, $p13^{II}$, is initiated internally from the full-length $p30^{II}$ and represents the carboxyl-terminal 87 residues of the $p30^{II}$ protein. Inoculation of rabbits with ACH, an HTLV-1 molecular clone, containing an intact pX-ORFII or ACH clones with pX-ORFII mutations, demonstrated that loss of $p30^{II}$ and $p13^{II}$ decreases the ability of HTLV-1 to maintain a high viral load *in vivo* (11).

$p30^{II}$ localizes in the nucleolus and shares homology to the transcription factors Oct-1 and –2, Pit-1 and POU-M1, and contains a serine, threonine-rich domain (30, 31). Due to its nuclear localization as well as its homology to cellular transcription factors, it is proposed that $p30^{II}$ could act as a transcriptional activator in infected cells. A study by Zhang *et al.* provided the first evidence for the involvement of $p30^{II}$ in both HTLV-1 replication as well as cellular gene expression (32). Using a p30-Gal4 fusion protein, it was demonstrated that $p30^{II}$ transactivates a Gal4-driven luciferase reporter gene. Mutational analyses indicated that the central core region within $p30^{II}$ (amino acids 62 to 132) is essential for transcriptional activation mediated by $p30^{II}$. In addition, it was also suggested that $p30^{II}$ differentially affects both cellular and viral gene transcription by showing that high amounts of $p30^{II}$ repressed cellular CRE-driven as well as LTR-driven reporter gene activity. However, small amounts of $p30^{II}$ enhanced HTLV-1 LTR-driven reporter gene activity, independent of Tax expression.

The molecular analysis of the $p30^{II}$ transcriptional effect on cellular proteins was further investigated by the same group in a study showing that $p30^{II}$ co-localizes with p300 in cell nuclei and regulates gene expression by binding to the KIX domain of CBP/p300. $p30^{II}$ was found to disrupt CREB-Tax-CBP/p300 binding to DNA probes containing Tax response elements (TRE), suggesting that $p30^{II}$ acts as a repressor of transcription by sequestering CBP/p300 (14). The significance of this work is that $p30^{II}$ may serve to promote viral persistence by reducing viral gene expression and thus reducing immune recognition of infected cells. It would be interesting to further investigate target genes that are modulated by $p30^{II}$ and their relevance in either infectivity of the HTLV-1 virus or its transforming potential.

$p13^{II}$ is the second protein encoded by pX-ORF II. This protein represents the C-terminus of the $p30^{II}$ protein, but neither a DNA binding domain nor transcriptional activity was found to be associated with this protein (33). Little is known about $p13^{II}$ function; however, it was reported that this protein is associated with mitochondria and that it alters its morphology by disrupting the mitochondrial inner membrane potential, suggesting a potential role of this protein in apoptosis (34). A yeast two-hybrid system study showed that $p13^{II}$ interacts with two novel cellular proteins C44 and C245 (35). Similarity searches using PSI-Blast indicated that C44 shares sequence similarities with the archeal adenylate kinase. In addition, C44 was found to be expressed in Jurkat T-cells as well as in proliferating, but not resting, PBMC. C254 is a protein that shows 69% identity and 83% similarity to the C-terminus of human nonmuscle filamin (actin-binding protein; 280). The relevance of this finding is not known yet, neither is the significance of $p13^{II}$ mitochondrial localization and disruption of mitochondrial membrane potential. However, several recent findings highlighted the similarity between $p13^{II}$ and G4, an oncoprotein encoded by the bovine leukemia virus (BLV) (36, 37). Specifically, both G4 and $p13^{II}$ localize to the mitochondria (37) and interact with farnesyl pyrophosphate synthetase (FPPS) (36). FPPS is involved in the mevalonate/squalene pathway and in synthesis of farnesyl pyrophosphate, a substrate required for prenylation of Ras. Furthermore, a G4 mutant that is unable to bind to FPPS is also unable to transform primary rat embryo fibroblasts (36). The analogy between G4 and $p13^{II}$ suggests a potential role of $p13^{II}$ in oncogenesis, possibly through binding to FPPS; however, no direct evidence has been found.

4. TAX AND TRANSFORMATION

The transforming ability of HTLV-1 is thought to be mainly due to the viral protein, Tax. Tax has been shown to induce tumors and leukemias in transgenic mice and has the ability to immortalize T-cells (38, 39). In addition, Tax has been shown to eliminate T-cell dependence on IL-2 (40). For a review of the influence of Tax on the IL-2 signaling pathway readers are referred to Hollsberg (41), which comprehensively covers this topic. Tax acts mainly through protein-protein interactions, binding to transcription factors and co-activators to modulate transcription, cell cycle regulators to deregulate cell growth, DNA damage response proteins to decrease

genomic stability, and apoptotic proteins to alter the apoptotic response. These alterations by Tax will be discussed in detail below.

4.1. Tax transcriptional regulation

Tax has the ability to transactivate the HTLV-1 viral LTR through three 21 bp repeat sequences called Tax responsive elements (TREs) that are found in the U3 region of the LTR (42, 43). TREs have an imperfect central cyclic AMP response element (CRE) motif that is flanked by GC rich regions. Although Tax cannot interact directly with this response element, it binds to other DNA binding proteins to mediate this interaction. In addition, Tax modulates cellular transcription through binding to various transcription factors including, CREB/ATF, NF-kappaB, and serum response factor (SRF) (44).

4.1.1. CREB/ATF transcription factors and co-activators

Of particular importance for transactivation of the LTR are the cAMP response element binding protein (CREB)/activating transcription factor (ATF) family of transcription factors that bind to both Tax and the LTR (44, 45). Members of the CREB/ATF family are basic-leucine zipper (bZIP) proteins, whose C-terminal domains are required for DNA binding and protein dimerization. The CREB subgroup of the ATF/CREB family includes CREB, CREM, and ATF-1 (46). Because all three have the ability to stimulate viral transcription in the presence of Tax (47-50), for simplicity in the remainder of this review these proteins will be referred to collectively as CREB. Tax increases CREB binding to the TREs by stabilizing LTR bound complexes through direct contact with nucleotides flanking the CRE-like motifs (51-54) and by promoting dimerization of the bZIP domain of CREB (55). Tax binding to CREB is mediated through the bZIP domain of CREB and the amino terminus of Tax (56). In addition, Tax is found in a ternary complex containing CREB and CREB binding protein (CBP)/p300 (56).

CBP and p300 are distinct proteins, but have significant sequence identify as well as similar functions. They both contain histone acetyltransferase (HAT) activity and function as co-activators for multiple transcription factors. CREB binding to CBP/p300 is normally mediated through the phosphorylation of its KID domain (57, 58), but in the presence of Tax, CBP/p300 can bind to unphosphorylated CREB (59). Therefore, Tax forms a molecular bridge allowing the formation of a quaternary complex containing the viral CRE, CREB, CBP/p300 and Tax. The recruitment of CBP/p300 and formation of this quaternary complex enhances the transactivation of the LTR (44, 60). Both the KIX and C/H1 domains of CBP/p300 are involved in Tax binding and co-activation (59, 61-64). More recently, the carboxy terminal region 2 [(CR2) aa 1894-2212] of CBP/p300 has also been shown to bind to Tax and mediate transcriptional activation (65).

While CBP and p300 are important for viral gene expression, it appears that they are not sufficient for transactivation. This is based on data indicating that the Tax M47 mutant, which retains the ability to interact with both CBP/p300 and CREB, is defective in transactivation of the LTR (66, 67). In addition, immortalization of HTLV-1 infected cells is independent of Tax binding to CBP/p300 (68). These data indicate that there are other factors that are necessary for transactivation. An additional HAT, p300/CBP associated factor (P/CAF), is a strong candidate based on the following: Tax interacts with P/CAF *in vitro* and *in vivo*, P/CAF enhances Tax transactivation of the viral LTR, and the TaxM47 mutant is deficient for P/CAF binding (66, 69). P/CAF and CBP/p300 can form a multi-HAT/activator-enhancer complex (70-72). Interestingly, activation of the viral LTR by P/CAF is independent of its HAT activity (69, 73), while CBP/p300 function is HAT dependent (64). It is possible that both P/CAF and CBP/p300 are necessary for transactivation *in vivo*, but further studies are needed to determine exactly what function P/CAF plays at the promoter.

A second subgroup of the CREB/ATF family of transcription factors, termed ATF-4 (46), has also been implicated in Tax mediated transactivation (74-77). Tax binds to the C- terminus of ATF-4 and although ATF-4 is acetylated both *in vitro* and *in vivo*, transactivation of the LTR is independent of acetylation (75, 76). ATF-4, rather than binding to other members of the CREB/ATF family, preferentially heterodimerizes with members of the activator protein-1 (AP-1) family and C/EBP family of proteins (46). Interestingly, AP-1 family members also bind to the HTLV-1 LTR (78) and enhance viral transcription (79). However, Tax transactivation of the LTR was not affected in cells that are deficient in AP-1 activity (79), indicating that these transcription factors may not be important for LTR activation *in vivo*. Various AP-1 family members including c-Fos, JunD, c-Jun, and Fra-1 were also upregulated by Tax (80), and transgenic mice that express Tax over-expressed both c-Jun and c-Fos (81). Not only are AP-1 levels increased in response to Tax, but its activity was also affected. Tax has also been implicated in the induction of genes such as IL-8, which contains AP-1 binding sites (82, 83). Furthermore, the addition of Tax in EMSAs induced DNA binding of AP-1 proteins, providing a possible mechanism of Tax induction of AP-1 responsive genes (84). It should be noted that ATL patient cells that express little or no Tax also exhibited constitutive AP-1 DNA binding activity (85). Collectively, these studies suggest that, while ATF-4/AP-1 heterodimers are capable of activating viral transcription, the more relevant contribution *in vivo* appears to be activation of AP-1 responsive genes.

Tax recruits numerous additional proteins to the LTR including TFIIA (86, 87), TFIID via TATA binding protein (TBP) (88), and TAF128 (89). These proteins are co-factors for transcription and are therefore potentially important for transactivation of viral genes. In addition, poly (ADP-Ribose) polymerase (PARP) is a co-activator of viral gene expression as demonstrated by its ability to bind to the TREs and induce Tax activation of the LTR (90, 91). Another protein, SR-related protein TAXREB803/SRL300, binds to Tax and aids in transactivating the LTR (92). Interestingly, the use of TAXREB803 siRNA significantly

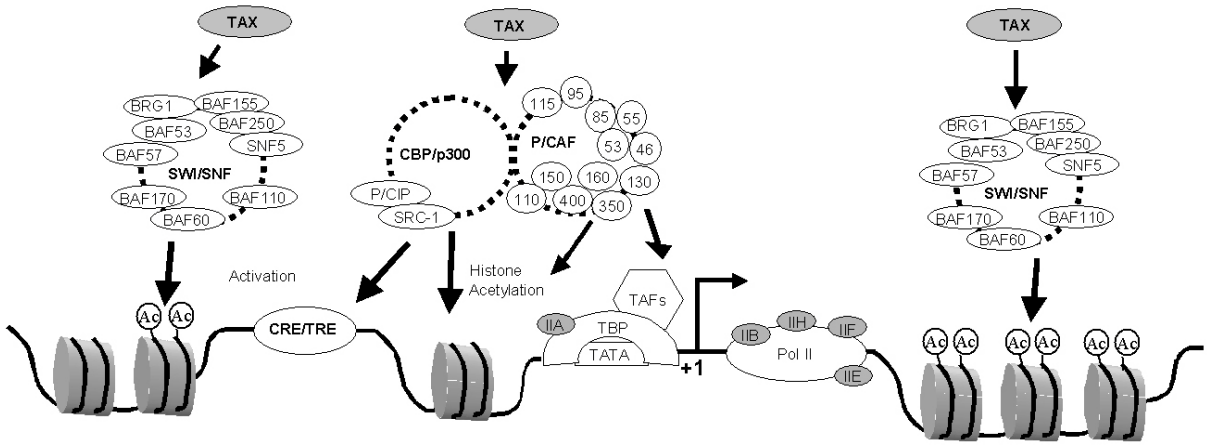

Figure 2. Proposed model of Tax and its ability to bind to chromatin remodeling (SWI/SNF) and coactivator (CBP/p300 and P/CAF) complexes. Tax binding to SWI/SNF at early G1 phase may remodel the promoter, followed by Tax/CBP/p300 interaction with DNA binding proteins (e.g. CREB), and subsequent association with the P/CAF for increased acetylation and communication with the basal transcription machinery. Tax binding to SWI/SNF may also aid in transcriptional elongation. SWI/SNF binding is through its bromodomain and binds to actelyated histone tails of H3 and H4.

decreased the transactivating ability of Tax, suggesting an important role of this Tax binding protein in LTR activation. Further studies are needed to determine which proteins, or combination of factors, are necessary for viral transcription *in vivo*.

Tax can also repress multiple cellular genes including cyclin A, Lck, c-Myb, and beta-polymerase (93-97). Although the mechanism of repression is not entirely clear, it is known that Tax competes with transcription factors, such as p53 and c-Jun, for CBP/p300 binding (98, 99). This competition involves the binding of the KIX domain of CBP/p300 and results in the sequestration of CBP/p300 by Tax. A recent study indicated that the CR2 domain (SRC-1 interaction domain) of CBP/p300 is also important in Tax binding, providing an additional family of transcription factors (SRC family) that Tax could compete with for CBP/p300 binding (65). Conversely, c-Myb repression has been shown to be independent of the interaction of Tax with CBP/p300 (96). This suggests that other factors are involved in Tax mediated repression. One possibility comes from a recent study demonstrating that the histone deacetylase, HDAC1, interacts with Tax (100). This interaction, involving the catalytic domain of HDAC1 and aa 29-97 of Tax, resulted in the repression of Tax transactivation of the viral LTR. It was suggested that HDAC1 and CBP compete for binding to Tax as increasing amounts of HDAC1 inhibited CBP/Tax binding. Furthermore, HDAC-1, 2, and 3 are found at the HTLV-1 promoter *in vivo* and inhibition of these deacetylases resulted in enhanced acetylation of histone H4 and increased viral RNA production (101). Therefore, this interaction may also have implications for the repression of cellular genes by Tax.

4.1.2. Transactivation in the context of chromatin structure

The HTLV-1 genome is integrated into the host genome, thus chromatin structure must be taken into account when studying transcriptional activation of the viral LTR. A proposed model of transcription occurring at the viral LTR in the context of chromatin is shown in figure 2, and is largely based on observations that are discussed below. Okada and Jeang made the importance of chromatin structure evident in a study where they compared the requirements for CREB, p300, and P/CAF in transiently transfected LTR plasmids versus integrated HTLV-1 virus (73). This study illustrated that plasmid DNA, without a complete chromatin structure, has different requirements for transcription factors/co-factors than the fully chromatinized integrated DNA. Specifically, neither P/CAF nor p300 had any effect on the transient LTR plasmid, whereas the integrated LTR was activated by both co-factors. In addition, through the use of *in vivo* chromatin immunoprecipitation (ChIP) assays, researchers can now examine which proteins are present at particular promoters in the presence of chromatin. Importantly, Lemasson and colleagues used *in vivo* ChIP assays to demonstrate that CREB, CREB-2 (ATF-4), ATF-1, ATF-2, c-Fos, c-Jun, CBP/p300, TAFII250, and HDAC-1, 2, and 3 were all present at the HTLV-1 promoter region (101).

Chromatin structure is greatly affected by modifications of histone tails. In particular, the acetylation of lysine residues by histone acetyltransferases (HATs) allows the relaxation of chromatin structure and transcription factors/transcriptional machinery access to promoter sequences. CBP/p300 acetylates all four histones, H2A, H2B, H3 and H4, with H3 and H4 being the preferred substrates (102, 103). Therefore, the recruitment of HATs such as CBP/p300 and P/CAF to the HTLV-1 LTR suggests that modifications to histone tails are an important step in transactivation. Lu *et al.*, using a reconstituted chromatin template, demonstrated that CBP/p300 transactivation of the viral LTR was dependent on HAT activity and that acetylation by p300 resulted in the increased recruitment of TFIID and RNA polymerase II to the LTR (104). Others assembled chromatin templates *in*

vitro with recombinant core histones to explore how chromatin structure affects Tax, CREB, and CBP/p300 activation of the viral CREs (64, 105). Interestingly, they found that CBP/p300 significantly stimulated Tax/CREB transactivation of the LTR in the presence of chromatin, but not in the presence of tailless chromatin or on naked DNA (64, 105). This study also demonstrated that a p300 mutant with minimal acetyltransferase activity was deficient for transactivation of the LTR (105). These results are intriguing in light of the fact that it is well documented that promoters around the initiation site are usually nucleosome free or contain modified nucleosomes (lack H2A and H2B), as observed in HIV-1, MMTV, and cellular promoters (106-110). Furthermore, our lab has preliminary data indicating that acetylated histone tails are necessary for activated transcription, possibly through the recruitment of factors necessary for elongation such as the chromatin remodeler, SWI/SNF. Therefore, it remains to be seen whether acetylated histone tails play an important role in elongation rather than initiation. A follow-up to this study indicated that while p300 did not provide any additional stimulation of the LTR on tailless chromatin, blocking the interaction of Tax/p300 had a detrimental effect on activation (64). Furthermore, the addition of Lys-CoA, an acetyltransferase inhibitor, resulted in inhibition of Tax transactivation on the tailless chromatin, indicating that acetylation of other substrates besides histones may play an important role in transcription. Collectively, these studies indicate that CBP/p300 are critical co-activators *in vivo* due to their ability to execute multiple tasks at the viral promoter.

In addition to HATs, ATP dependent chromatin remodeling complexes are important for transcription from chromatinized DNA *in vivo*. They function by altering nucleosome structure through movement/sliding of nucleosomes to allow cellular regulatory factors access to enable transcription (111). Mammalian SWI-SNF complexes contain either of the two highly conserved ATPase subunits: BRG1 or BRM. The SWI/SNF complex is targeted to promoters via direct interactions with transcription activators and nucleosomes, and contributes to the remodeling of chromatin structure. Our lab has recently shown the importance of BRG1/hBRM, the human homologue of the chromatin remodeling complex SWI/SNF, in HTLV-1 gene expression (112). Tax was found complexed with BRG1 and the addition of increasing amounts of BRG1 resulted in greater transactivation of the viral LTR as compared to transactivation in the presence of Tax alone. In addition, co-expression of both BRG1 and Tax resulted in acetylation of histone H4 on the HTLV-1 promoter. Furthermore, upon treatment with BRG1 siRNA, HTLV-1 viral release and transcription were decreased. These results demonstrate an important role of chromatin remodelers in viral transactivation as well as replication.

4.1.3. NF-kappaB

The NF-kappaB family of transcription factors consists of RelA (p65), RelB, c-Rel, p50/p105 (NF-kappaB1), and p52/p100 (NF-kappaB2). These proteins contain an N-terminal DNA-binding/dimerization/nuclear targeting domain called the Rel homology domain (113). NF-kappaB proteins function as either homodimers or heterodimers, with the most common combination being p50-RelA, which is referred to as NF-kappaB. NF-kappaB proteins can also be subdivided into two classes. The first class (p105 and p100) must be cleaved to form the active form of DNA binding proteins, p50 and p52, respectively. The second class (c-Rel, RelB, and RelA) contains C-terminal activation domains that are not cleaved (113). NF-kappaB proteins are normally sequestered in the cytoplasm by interactions with the IkappaB proteins, IkappaB-alpha, IkappaB-beta, and IkappaB-epsilon. Phosphorylation of IkappaB on Ser 32 and 36 by IkappaB kinases (IKK) results in the ubiquitination and subsequent proteosomal degradation of IkappaB (114). NF-kappaB is then free to enter the nucleus and stimulate transcription of NF-kappaB responsive genes.

Tax expression leads to persistent NF-kappaB activity (115). Constitutive NF-kappaB activity is also observed in ATL patients, independent of Tax (116), indicating that NF-kappaB activity is important at both early and late stages of tumorigenesis. Tax can activate NF-kappaB mediated transcription (117) and is present in distinct nuclear structures that contain both p50 and RelA (118). Furthermore, many NF-kappaB inducible genes, such as IL-2Ralpha (119), Bcl-xl (120), and IL-15 (121), are upregulated by Tax. Due to the ability of NF-kappaB to bind to CBP/p300 (122-124), it is possible that Tax activates NF-kappaB transcription in a manner similar to that observed with CREB. Although the mechanism in which Tax maintains constitutive NF-kappaB activity is not entirely clear, most research has demonstrated alterations in cytoplasmic events leading to the release of NF-kappaB.

Tax has the ability to bind to multiple members of the NF-kappaB protein family, including p50/p105 (125), p65/RelA (117), c-Rel (117), and p52/p100 (126, 127). In addition, Tax binds to IkappaB-alpha (128, 129). Therefore, it was originally thought that the interactions of these proteins resulted in the disruption of the inactive cytoplasmic complex, allowing NF-kappaB to translocate to the nucleus. Subsequently, it was demonstrated that Tax binds to the proteasome subunits HsN3 and HC9 through its N terminus, resulting in increased chymotryptic and tryptic activity (130-132). IkappaB-alpha proteins are normally targeted for proteasomal degradation following phosphorylation by IKK. Based on the interaction of Tax with both the proteasome and IkappaB-alpha, it was proposed that Tax acts as a viral chaperone or molecular bridge for IkappaB-alpha degradation (133). In addition, Rousset *et al.* observed Tax, p105 and HC9 in a ternary complex *in vivo*, which resulted in increased p105 proteolytic processing (132). Therefore, the constitutive activation of NF-kappaB appears to be due to the targeting of NF-kappaB signaling proteins to the proteasome via the direct interaction with Tax. One study indicated that Tax was unable to activate NF-kappaB in cells that have a catalytically inactive form of IKK (134), suggesting that there are additional Tax mediated events necessary for persistent NF-kappaB activity.

An additional Tax mediated event involves modulation of IKK activity, resulting in increased IkappaB

proteasomal degradation and NF-kappaB release. One of the first observations leading to this model was that Tax expression resulted in increased phosphorylation of IkappaB-alpha (135). In addition, Tax interacted with the catalytic subunits, IKKalpha and IKKbeta of the IKK complex, stimulating its catalytic activity (135-138). The activation of IKK induced through most cytokines was transient; in contrast, Tax activation of IKK was persistent (138). Furthermore, Tax interacted with IKKgamma (NEMO), a non-catalytic regulatory component of IKK (139-141). This interaction was mediated through two leucine zippers in IKKgamma and a leucine rich region in Tax (142). It has been proposed that the Tax/IKKgamma association serves as a molecular linker, which promotes the interaction of Tax with IKKalpha and IKKbeta (143). In addition, Tax physically recruited IKKalpha to another member of the NF-kappaB family, p100, resulting in the proteolytic processing of p100 to p52 (144). Importantly, Jurkat cells with mutated IKKgamma, are deficient for NF-kappaB activity (145), demonstrating the significance of IKK in NF-kappaB signaling.

Although the association of Tax with IKK complexes is a promising model to explain the constitutive NF-kappaB activity observed in HTLV-1 infected cells, studies continue to elucidate additional steps within the NF-kappaB signaling pathway that Tax effects. For example, recent studies have indicated that Tax expression resulted in phosphorylation of both IKKbeta and IKKgamma, which was necessary for the deregulated IKK activity induced by Tax (146). In addition, IKKbeta was shown to be the kinase responsible for IKKgamma phosphorylation (147, 148). Conversely, another study has implicated MEKK-1, a member of the MAPK kinase kinase family, in the phosphorylation of IKKbeta (136). This observation is based on the ability of MEKK-1 to bind to Tax as well as dominant negative mutants of both IKKbeta and MEKK-1 resulting in a deficient NF-kappaB signaling pathway (136). Therefore, while Tax can interact at multiple steps in the NF-kappaB signaling pathway, ultimately leading to constitutive NF-kappaB activity, the precise mechanism is still under investigation.

4.2. Cell growth deregulation

A key feature of the tumorigenic process is the abrogation of checkpoint controls. Tax alters cell growth through a variety of mechanisms. As discussed above (see Tax transcriptional regulation), Tax is a potent co-activator of transcription that has the ability to transactivate as well as repress a wide array of cellular genes. Among the genes that Tax induces are the immediate early serum responsive genes c-Fos, c-Jun, Egr-1, and Egr-2, suggesting that Tax can replace growth signals (80). In general, Tax tends to transactivate genes that are involved in promoting cellular growth, while repressing genes that inhibit cellular growth. In addition, Tax interacts with many proteins that are involved in the G_1/S transition and TGF-beta signaling pathway. Key deregulated processes that contribute to aberrant cellular growth are discussed below.

4.2.1. G_1/S cell cycle transition

Although proliferation is tightly regulated in T-cells, HTLV-1 infected cells have found a way to overcome this tight regulation. In particular, the G_1/S transition is highly altered in infected cells. One mechanism employed in a variety of cancers, including retinoblastomas, breast cancer, pancreatic cancer, and bladder cancer (149, 150) to bypass this restriction point, is the deregulation of the cyclin D-cdk4/p16/Rb/E2F pathway. The phosphorylation of Rb by G_1 cyclin/cdk complexes is an important regulatory event in the transition from G_1 to the S phase of the cell cycle. Rb phosphorylation allows the release of Rb bound proteins most notably, the E2F transcription factor family, which activate S phase specific genes, such as cyclin A (151, 152). Conversely, the cdk inhibitor p16/INK4A functions to slow excessive cell growth through binding and inhibiting cdk4 and cdk6. p16/INK4A has been shown to be mutated in up to 68% of acute and up to 25% of chronic ATL patients (153). In addition, p16/INK4A can be silenced through methylation of its promoter (154). While Tax has not been shown to be directly involved with these genetic and epigenetic p16/INK4A-silencing events, it does inactivate p16/INK4A through other mechanisms. Tax can directly bind to p16/INK4A, resulting in the sequestration of p16/INK4A from cyclin D/cdk4, 6 complexes (155-157). However, direct binding of Tax to p16/INK4A can not fully explain the increased cdk4 kinase activity observed in HTLV-1 infected cells, as cdk4 kinase activity was also observed in p16/INK4A null cells (158) and Tax also increased E2F dependent transcription in p16/INK4A negative T-cells (159). Rather, these results can be explained by the ability of Tax to directly bind to cyclin D3, cyclin D2, and cdk4, thereby increasing cyclin D/cdk4 associated kinase activity (158, 160, 161). While cyclin D2 is found only in complex with cdk6 in uninfected T-cells, it can also be found with cdk4 and cdk2 in HTLV-1 Tax expressing cells (162). This is an interesting observation considering that cdk2 kinase activity is normally associated with cyclin E or cyclin A complexes. It is possible that the high levels of cyclin D2, which are due to Tax transactivation of the cyclin D2 promoter (162-164), allow for the formation of these additional complexes. This new association may increase phosphorylation on those substrates targeted by cdk2. Consistent with these findings, cdk2 associated kinase activity is observed earlier in HTLV-1 infected cells (162, 165), along with an accelerated G_1 to S phase transition (165, 166). On the other hand, the cyclin A promoter, which is E2F responsive, is repressed by Tax (97). This repression of cyclin A may be useful to extend the S phase, allowing the replication of the viral genome, although this mechanism has not been experimentally verified.

In addition to the alteration of p16/INK4A, other cdk inhibitors (CDKIs) are differentially regulated by Tax. CDKIs generally act as negative regulators of the cell cycle by binding to cdks and inhibiting their phosphorylation activities. There are two families of CDKIs, the INK4 family and the CIP/KIP family. The INK4 family, consisting of p16/INK4A, p15/INK4B, p18/INK4C and p19/INK4D, specifically inhibit cdk4 and cdk6 (167-170). Conversely, the CIP/KIP family, consisting of p21/waf1, p27/Kip1, and p57/Kip2, regulate all cyclin D, E and A associated kinases (171-174). Tax transcriptionally represses the p18/INK4C promoter through an E-box

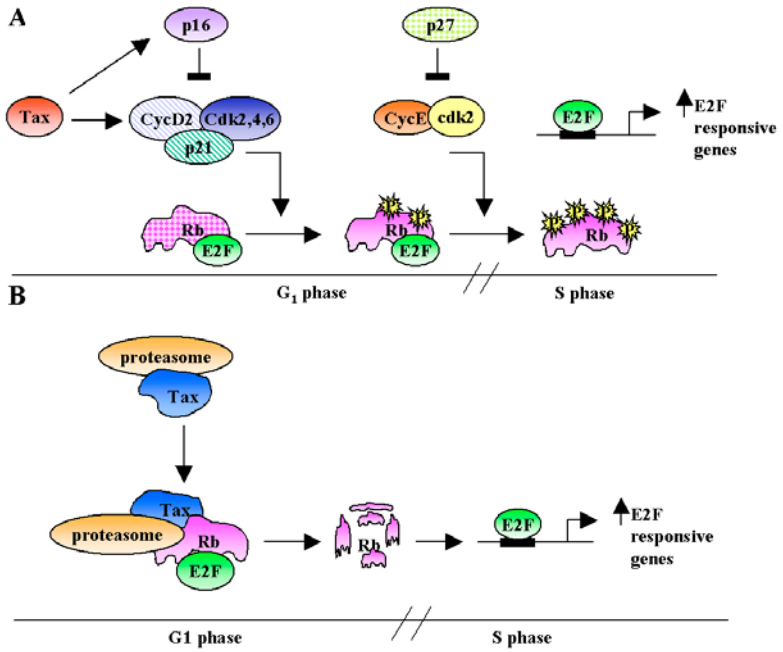

Figure 3. Alterations of the G_1/S transition. A) Tax alters the activity of cyclin/cdk complexes resulting in increased Rb phosphorylation and E2F dependent transcription. Tax can directly bind to p16/INK4A, cyclin D2 and cdk4 (see references in text). Dashed shapes indicate that these proteins (p21/waf1 and cyclin D2) are transactivated by Tax. Checkered shapes indicate that these proteins (p27 and Rb) have reduced protein expression in HTLV-1 infected cells. B) Tax acts as a molecular bridge by binding to both the proteasome and Rb, resulting in the degradation of hypophosphorylated Rb and a subsequent increase in E2F transcription.

element located at -710 and -510, resulting in reduced p18/INK4C mRNA levels in HTLV-1 infected cells (175). Conversely, Iwanaga *et al.*, through the use of IL-2 dependent Kit 225 cells, showed that p18/INK4C mRNA levels were increased after Tax expression (176). They also demonstrated a decrease of p19/INK4D mRNA and protein levels after induction of Tax expression. Although Tax expression has not been shown to affect p27/Kip1 RNA levels, protein levels were generally lower in the presence of Tax (176, 177), which corresponded to constitutive activation of cyclin E/cdk2 activity in HTLV-1 Tax expressing cells.

Another important result of Tax expression is an increase in p21/waf1 protein levels (176, 178-181). This increase in p21/waf1 expression is due to Tax transactivating the p21/waf1 promoter independently of p53 through helix-loop helix E2A sites close to the TATA box (179). The over-expression of a cdk inhibitor, such as p21/waf1, may appear to be contradictory in terms of cell growth, but it has been shown that p21/waf1 can aid the assembly of cyclin D/cdk4, 6 complexes (182-186). This is also the case in HTLV- 1 infected cells, where p21/waf1 was found in kinase active cyclin D2/cdk4 containing complexes (187). In agreement with other reports (165, 177), our lab has observed increased cdk2 kinase activity in HTLV-1 infected cells, which could be in part due to the sequestration of p21/waf1 by cyclin D2/cdk4 (162, 187).

The multiple alterations of cyclin/cdk activity by Tax are depicted in figure 3A. The expected consequences of this deregulation would be a growth advantage to infected cells due to increased hyperphosphorylation of Rb. Indeed, Tax expression ultimately results in increased Rb phosphorylation (158, 176), increased E2F transcription and protein levels (158, 176), and increased E2F mediated transcription (159). Our lab has recently observed a decrease in Rb protein levels. The diminished Rb levels are due to the direct interaction of Tax with both Rb and the proteasome, resulting in the targeting of hypophosphorylated Rb to the proteasome for degradation (unpublished results) as shown in figure 3B. Similar mechanisms have been observed for other oncogenic viruses, such as the human papilloma virus (188), and provide another significant way to overcome Rb mediated growth suppression.

Finally, it appears that in HTLV-1 infected cells cyclin E has important roles beyond its traditional role of phosphorylating Rb. It associates with both p300 and RNA Pol II and phosphorylates the carboxy terminal domain of RNA Pol II (189). Furthermore, the use of the chemical cdk inhibitor, Purvalanol A, inhibited activated, but not basal HTLV-1 transcription (189). These results indicate that cdk inhibitors can be used for the treatment of ATL. Recent data provided by cyclin E and cdk2 knockout mouse models indicated that neither cdk2 nor cyclin E was essential for cell survival as originally thought (190, 191). This evidence further supports the notion that chemical cdk inhibitors are promising options for ATL treatment based on their ability to specifically target the infected cells that rely heavily on cdks for both cell cycle progression as well as viral transcription.

4.2.2. Transforming growth factor beta

An important mediator of the G_1/S checkpoint is the cytokine transforming growth factor (TGF) beta. There are three isoforms of TGF-beta, TGF-beta-1, -2, and -3, which are encoded by separate genes on distinct chromosomes (192). TGF-beta controls cell growth, differentiation, and apoptosis, and is particularly important for cells within the hematopoietic lineages. Important downstream targets of TGF-beta are the SMAD proteins. In response to TGF-beta, SMAD2 and SMAD3 relocate to the nucleus where they can either bind directly to DNA or cooperate with other transcription factors to alter the expression of various genes. Other pathways, such as the MAPK and JNK signaling pathways, can also be altered by TGF-beta signaling. TGF-beta induces G_1 arrest through the repression of cdk transcription and activity (193, 194) and enhanced transcription of CDKIs such as p15/INK4B, p21/waf1, and p27/Kip1 (195-198). Importantly, HTLV-1 infected cells become resistant to TGF-beta1 induced growth inhibition (199). This effect appears to be mediated by Tax as Tax can inhibit downstream mediators of the TGF-beta signaling pathway. Tax competes with SMAD proteins for the co-activator CBP/p300 (200, 201). In addition, Tax binds directly to SMAD2, SMAD3, and SMAD4, preventing them from binding to their target genes (200). Tax can also induce JNK activity and c-Jun phosphorylation, resulting in SMAD3/c-Jun complexes (202). SMAD3/c-Jun complexes abrogate the ability of SMAD3 to bind to DNA providing another mechanism in which Tax can disrupt TGF-beta induced cell growth arrest. Conversely, Tax can transactivate the TGF-beta1 gene with corresponding high levels of TGF-beta1 mRNA observed in fresh leukemic cells from ATL patients (203). In addition, Tax transgenic mice display high amounts of TGF-beta1 mRNA and protein (204). It has been suggested that the TGF-beta1 produced in HTLV-1 infected cells has the ability to act on normal neighboring cells, resulting in the impairment of cytolytic T cell activity, enhanced angiogenesis, and stimulated matrix protein production (205). Therefore, the combination of promoting high levels of TGF-beta1 secretion and inhibiting TGF-beta induced cell signaling leads to a favorable environment for oncogenesis in HTLV-1 infected cells.

4.2.3. p53

The transcription factor p53 induces cell cycle arrest and/or apoptosis in response to DNA damage signals through the activation of cell cycle control proteins. The high mutation rate of this tumor suppressor protein in human cancers reflects its critical role in maintaining cellular stability as well as protecting genomic stability (discussed below). About 30% of ATL patients have mutations in the p53 gene (206). Surprisingly, in the remainder of HTLV-1 infected cells, p53 protein levels are often increased, with a corresponding increase in protein half-life, although mRNA levels are unchanged (207, 208). p53 appears to be functionally inactivated as it is unable to respond appropriately to DNA damaging agents such as UV and gamma irradiation (178, 209). While Tax does not directly bind to p53 (208, 210), nor interfere with its DNA binding ability (209, 211), it does inhibit p53 dependent transcription (209, 211-214). Tax has also been shown to inhibit the p53-related proteins, p73alpha, p73beta, and p51 (61, 215). In addition, Tax has the ability to block p53 induced G_1 arrest and p53 induced apoptosis (211).

Numerous studies have investigated the mechanism by which Tax functionally inactivates p53, but this issue still remains controversial. Inactivation appears to be through the N-terminal activation domain of p53 (amino acids 1 to 52) (209, 210) and phosphorylation of p53 has been proposed to be a contributing factor. Pise-Masison *et al.* found that phosphorylation of p53 on Ser15 was important for inactivation of p53, possibly due to a block in the interaction of p53 with the basal transcription factor, TFIID (213). In addition, it was suggested that DNA protein kinase (PK) was the kinase responsible for p53 phosphorylation (213). However, a later report by Ariumi *et al.* was not able to confirm the importance of Ser15 phosphorylation in Tax mediated inactivation of p53 (210). Furthermore, through the use of both DNA PK-/- and ATM-/- cell lines, it was demonstrated that ATM rather than DNA PK was important for Tax mediated p53 inactivation (214). This report, however, does not directly implicate ATM phosphorylation as the mechanism of p53 inhibition. Thus, there may be other Tax and ATM dependent, but phosphorylation independent, mechanisms that can account for the observed p53 inhibition.

Another point of interest is whether the inactivation of p53 is dependent on the activity of Tax through NF-kappaB or CREB/CREB binding protein (CBP) pathways. Several labs have demonstrated that Tax binding to CBP/p300 results in reduced p53 transcriptional activity (98, 210, 214, 216). p53 dependent transcription utilizes the co-activators CBP/p300 (217); therefore, Tax could indirectly inhibit p53 function by competing for the co-activators, CBP/p300. A similar competition for CBP binding has been reported in the inhibition of p73beta (61). A number of investigators demonstrated that the CREB/ATF motif of Tax was necessary for p53 inactivation (210, 211, 214), while another study indicated that the NF-kappaB pathway was important for p53 inhibition (218). Later work indicated that the observed differences were cell type specific (219). In this report, p53 phosphorylation and the NF-kappaB pathway were important in lymphoid cells such as Jurkat cells, while in non-lymphoid cells, where p300 is limiting, Tax induced p53 inactivation was CREB/ATF dependent, but independent of p53 phosphorylation. Conversely, Van *et al.*, using both lymphoid cell lines (Jurkat) as well as non-lymphoid cells (HeLa and Saos-2), demonstrated the dependence on the CREB/ATF pathway, but not the NF-kappaB pathway for inhibition of p53 (214). Furthermore, it was shown that CBP/p300 was necessary, but not sufficient, for Tax mediated inhibition of p53 (214), suggesting that there are additional mechanisms that are important in this process. Future studies are needed to uncover these additional factors that contribute to p53 inactivation. Finally, a recent report by Chowdhury *et al.* demonstrated that the Tax binding protein, TRX, is targeted by p53 for proteasomal degradation (220). Interestingly, Tax can inhibit this transcriptional independent function of p53 as well. This study suggests that Tax induced

modifications of p53 may contribute to the inhibition of many p53-associated activities.

p53 is intimately involved with many cellular pathways. As the "guardian of the genome", p53 plays a significant role in maintaining genomic integrity through the promotion of cell cycle arrest and/or apoptosis when there is DNA damage. Therefore, the inactivation of p53 in HTLV-1 infected cells not only contributes to the deregulation of cell growth, but also contributes to alteration of apoptotic pathways and genomic instability. The alterations of these cellular events by Tax are discussed below.

4.3. Genomic stability

Genetic alterations that affect critical cellular machineries are thought to contribute to the transformation of cells. Thus, the stability of the human genome is a critical factor in tumorigenesis. In viral infections, the integration of a provirus can often cause such genetic alterations (221). In the case of HTLV-1, the integration of the provirus occurs randomly (222); therefore, there appears to be no insertional mutagenic components that contribute to the development of ATL. Conversely, genomic instability is still observed in infected cells. ATL cells have a variety of chromosomal abnormalities including duplications, deletions, translocations, rearrangements, and aneuploidy (223). Deregulation of the G_1/S checkpoint and inactivation of p53 by Tax contributes to genomic instability by allowing cells to replicate in the presence of DNA damage. Indeed, it has been shown that Tax expression results in an increased mutation frequency (224). Tax can also affect genomic stability through two additional mechanisms, alteration of the DNA repair process and deregulation of mitotic checkpoint proteins.

4.3.1. DNA repair processes

One of the first indications that Tax could directly affect genomic instability was the finding that Tax represses the DNA polymerase beta promoter (225). DNA polymerase beta is an important enzyme in the DNA damage repair process of base excision repair (BER). BER involves the removal of DNA lesions resulting from exposure to UV or chemicals such as oxidizing and alkylating agents, and subsequent synthesis of DNA to fill the gaps, followed by ligation. Accordingly, Tax expressing cells have a decreased ability to repair DNA damage induced by UV light, quercetin, or hydrogen peroxide, indicating that infected cells have a defect in the BER pathway (226). Recently, Lemoine and Marriott (227) utilized a PALA assay, which measures the ability of cells to amplify the CAD gene, to determine the effect of Tax on genomic amplification. Cloned rat embryo fibroblast (CREF) cells that stably expressed Tax displayed five times more amplification of the CAD gene as compared to control cells, indicating that Tax expression increased genomic instability (227).

Tax can also affect other proteins involved in the DNA repair process. For instance, Tax transactivates the proliferation cell nuclear antigen (PCNA) promoter (228, 229). PCNA is important for the activity of DNA polymerase epsilon and delta and replication factor C (230). DNA polymerase epsilon and delta are involved in nucleotide excision repair (NER) and BER (230). Interestingly, Tax can also inhibit NER, which corresponds to increased PCNA expression (227, 228). Moreover, Tax binds to and inhibits the catalytic activity of DNA topoisomerase I (231). The inhibition of DNA relaxation could alter the ability of enzymes to repair damaged DNA sequences, further contributing to genomic instability.

A recent report by Gabet *et al.* demonstrated for the first time the ability of Tax to repress the hTERT promoter through an E-box downstream of the hTERT initiation site (232). hTERT is an essential component of the telomerase complex (233) and in the presence of Tax there was an observed decrease in telomerase activity (232). Interestingly, Tax expression also resulted in unprotected DNA ends, rather than telomere-capped ends (234), suggesting a role for Tax in inhibiting a DNA-end-stabilizing mechanism. Therefore, the ability of Tax to inhibit telomerase activity and telomere capping, alter DNA damage checkpoints, and alter DNA damage repair enzymes and processes, significantly contributes to the observed genomic alterations in HTLV-1 infected cells.

4.3.2. Mitotic checkpoints

The mitotic spindle checkpoint is important for preventing the onset of anaphase in the absence of proper chromosomal alignment at the bipolar spindles. Studies in budding yeast have demonstrated the importance of the mitotic arrest defective (MAD) proteins 1 and 2 in the spindle checkpoint (235). Importantly, Tax binds to MAD1 and interferes with the ability of MAD1 to initiate this cell cycle checkpoint (236). This interference is possibly due to the binding of Tax to the domain necessary for MAD1 homodimerization (236). In addition, Tax expression reduces the stability of MAD1. In agreement with these findings, treatment of HTLV-1 infected cells with microtubule inhibitors did not result in cell cycle arrest at mitosis, suggesting that infected cells were defective in the mitotic spindle assembly checkpoint (237). Upon closer examination, it was observed that both MAD1 and MAD2 were inappropriately localized in the cytoplasm in infected cells (237). The importance of this study lies in the implication that microtubule inhibitors should not be used alone as a standard chemotheraputic treatment for ATL.

There is recent literature to suggest that Tax expression also alters other aspects of the G_2 and M phases. Entry into mitosis is regulated by the activation of cyclin B/cdk1 complexes, whereas exit from mitosis is regulated through the degradation of cyclin B and other important regulators, such as securin (238). Both cyclin B and cdk1 are up-regulated in HTLV-1 infected cells and Cdc25C, which is a tyrosine phosphatase that activates cdk1 through dephosphorylation, is transactivated by Tax (239). While these alterations indicate a faster progression into the G_2 phase, this has not been experimentally verified. Conversely, naïve cells transduced with a retroviral vector encoding Tax accumulated at the G_2/M phase, suggesting either a G_2/M cell cycle block or a block in the exit from mitosis (166). Nuclear abnormalities, such as

multinucleated giant cells and cells with decondensed DNA, were observed (166). Interestingly, in transformed cell lines expressing Tax, such cell cycle alterations were not observed. Furthermore, Tax can induce both cyclin B1/Clb2p and securin/Pds1p degradation through the activation of the anaphase-promoting complex (APC) (240). The APC is an E3 ubiquitin ligase that mediates the degradation of mitotic cyclins and anaphase inhibitors to allow the onset of anaphase (238). In addition, the alteration of these cell cycle proteins were observed in MAD1 null cells (240). Therefore, it appears that Tax has evolved two independent mechanisms to alter mitotic processes; through the alteration of the MAD dependent pathways and through the untimely degradation of cyclin B1 and securin.

Alteration of mitotic processes and inhibition of DNA repair mechanisms undoubtedly contributes to the observed chromosomal abnormalities in HTLV-1 infected cells. In normal uninfected cells, the occurrence of DNA damage initiates a cascade of events leading to apoptosis. Importantly, in HTLV-1 infected cells, Tax can also alter the apoptotic pathway, thus allowing mutations to persist within the genome, as discussed below.

4.4. Apoptosis

T lymphocytes may undergo apoptosis via two separate mechanisms: (i) withdrawal of growth factors and (ii) activation-induced cell death (AICD). The first mechanism is antigen-independent and can be inhibited by anti-apoptotic proteins such as Bcl-x_L and Bcl-2. Meanwhile, AICD is antigen-dependent and is mediated by CD95 (Fas) or tumor necrosis factor-alpha (TNF-alpha) signaling. This pathway links caspase-8 to death receptors expressed at the cell surface, including Fas, TNFR-1, and Death Receptor 3 (DR3). When caspase-8 is activated, it can cleave and activate an overlapping set of effector caspases resulting in protease activity within the cell (241). Furthermore, this pathway is only partially inhibited by Bcl-x_L or Bcl-2 (41, 242). The Bcl-2 family is comprised of two groups: death antagonists, which include Bcl-2, Bcl-x_L, and BAG-1; and death agonists, which include Bax, Bak, and BAD (243). Cells from ATL patients are characterized by extensive T-cell proliferation, and it is possible that disruption of normal apoptotic events in HTLV-1 infected cells could explain tumorigenesis. It is still unclear, however, as to whether Tax expression induces or suppresses apoptosis in HTLV-1 infected cells, as there has been substantial evidence to suggest both events are possible.

4.4.1. Pro-Apoptotic Effects of Tax

Several studies suggest that the viral protein Tax can induce apoptosis in HTLV-1 infected cells. For example, it has been documented that FasL-mediated apoptosis was observed in cells which had been subjected to hormone induced activation of Tax fusion proteins and the CD3 complex (244). Furthermore, Chen *et al.* demonstrated that induction of Tax was marked by up-regulation of the FasL gene and interference of the Fas/FasL pathway inhibited apoptosis (245). Importantly, it has been shown that the CD95/Fas-signaling pathway plays a substantial role in these Tax-activated apoptotic events, and that interleukin-1 beta-converting enzyme (ICE)-proteases, which are a family of Asp-directed cysteine proteases, mediate Tax-induced T-cell death (246). Conversely, Rivera-Walsh *et al.* suggested the FasL pathway was not important for Tax-mediated apoptosis induction. Instead, it was demonstrated that Tax and T-cell mitogens caused NF-kappaB signaling to induce the TNF-related apoptosis-inducing ligand (TRAIL) gene. TRAIL acts in a similar fashion as FasL (247). Consistently, Tax up-regulated TRAIL/Apo-2L in gene expression profiling studies (181).

The interaction of Tax with CREB binding protein (CBP)/p300 has also been implicated in apoptosis. Nicot *et al.* demonstrated that nuclear expression of the CBP/p300-binding domain of Tax induced caspase dependent apoptosis in HeLa cells (248). Tax CBP/p300 binding mutants exhibited reduced ability to induce apoptosis, suggesting that this complex is important for the disruption of cell survival signals. Additionally, ectopic expression of the co-activator, p300, prevented Tax induced apoptosis in a dosage dependent manner. Furthermore, under cellular stress conditions, Tax NF-kappaB binding mutants displayed a reduced ability to induce apoptosis. NF-kappaB utilizes the co-activators CBP/p300 to stimulate the transcription of various cellular factors (249). Therefore, it is possible that the persistent activation of NF-kappaB by Tax (see NF-kappaB section above) could indirectly increase the demands for CBP/p300. If the demand for CBP/p300 is not meet, the cell may be unable to efficiently transcribe critical factors and thus apoptosis would be induced.

Oxidative stress, controlled by the thioredoxin (Trx) and glutathione (GSH) systems, has been shown to evoke intracellular events such as apoptosis. Two major pathways mediate these events. One involves death receptors and is exemplified by Fas-mediated caspase-8 activation. The other pathway is via the mitochondria-mediated caspase-9 activation pathway. Both pathways result in caspase-3 activation and ultimately, nuclear degradation and cellular morphological change (250). One study indicated that expression of Tax led to rapid changes in the intracellular levels of hydrogen peroxide and glutathione, which promoted an intracellular pro-oxidant state (251). The changes in levels of hydrogen peroxide, along with concomitant activation of the NF-kappaB and CD3/TCR pathways exaggerated oxidative stress and caused DNA fragmentation in cells, a phenotype of apoptosis. Consistent with the data, anti-oxidants can block Tax-mediated damage (251, 252).

The above-described studies illustrate several possible mechanisms for Tax dependent-apoptosis. Importantly, NF-kappaB has been implicated in producing an intracellular environment that is favorable for apoptosis; however, it is important to note that NF-kappaB has also been shown to provide an anti-apoptotic environment as well.

4.4.2. Anti-Apoptotic Effects of Tax

There is a large body of evidence suggesting that Tax inhibits apoptosis in HTLV-1 infected cells.

Transactivation by Tax through NF-kappaB and CREB signaling is involved in the inhibition of Tax-mediated apoptosis (120, 253-256). NF-kappaB has been shown to produce anti-apoptotic gene products including Bcl-x_L (120, 253, 256). Importantly, both the NF-kappaB and CREB pathways are required for maximum activation of the human Bcl-x_L promoter. Consequently, this results in increased resistance to apoptosis and is necessary for infected cells to advance to leukemia *in vivo* (120). In another study, it was demonstrated that Tax transactivates Bcl-x_L via the NF-kappaB pathway. In the mouse T cell line, CTLL-2, apoptosis was induced through the deprivation of IL-2. CTLL-2/Tax transfectants were resistant to apoptosis and this resistance was associated with Bcl-x_L (253).

As it has been shown that Tax inhibits apoptosis through the NF-kappaB pathway, several studies demonstrated the contribution of NF-kappaB activity by inhibiting this pathway with an anti-inflammatory inhibitor, sodium salicylate and other NF-kappaB inhibitors (254, 255). Sodium salicylate possesses the ability to block NF-kappaB nuclear localization and bcl-xl expression (254), which ultimately rendered resistant tumor cells sensitive to apoptosis, suggesting that Tax-mediated induction of NF-kappaB activity contributes to tumorigenesis *in vivo* (254). Furthermore, in the presence of NF-kappaB inhibitors, which inhibit nuclear translocation, caspase-induced apoptosis occurred (255). These data show the inhibitory effects of Tax and suggest roles for it in the prevention of apoptosis.

p53 is an important regulator of cell cycle progression and can induce cell cycle arrest and/or apoptosis (257). According to Mulloy *et al.*, Tax indirectly interferes with the transactivating function of p53 through the CREB/ATF domain (211). Additionally, Tax inactivation of p53 transcriptional activity correlated with a loss of p53-induced G_1 arrest and apoptosis (211). Although p53 is inactive in HTLV-1 infected cells, p21/waf1 mRNA and protein levels are particularly high in HTLV-1 infected cells (178, 180), which is a result of Tax transactivating the p21 promoter (179). p21/waf1 is a mediator of p53 induced growth arrest (258). Interestingly, Kawata *et al.* demonstrated that elevated levels of p21/waf1, resulting from Tax expression, inhibited apoptosis through an unknown mechanism (259).

There have been other observations that implicate Tax in the inhibition of apoptosis. First, Tax inhibited apoptosis induced by anti-APO-1 treatment. In experiments done by Copeland *et al.*, monoclonal antibody anti-APO-1, which has been shown to induce apoptosis in human T cells, displayed a decreased ability to induce apoptosis in cells expressing Tax (260). Additionally, Tax has also been shown to repress bax expression *in vitro*. The Bax protein typically accelerates apoptosis, and ultimately, the repression of Bax leads to a decrease in apoptosis (261). On the other hand, Bcl-2 is an anti-apoptotic protein that tends to be up-regulated in HTLV-1 infections and may provide protection against virus-induced apoptosis (256, 262). Finally, although there was not a direct implication for the involvement of Tax, gene expression profiling of HTLV-1 immortalized T-cells showed genes encoding apoptosis accelerators, such caspase-8, to be down regulated while genes encoding apoptosis-inhibitors, such as apoptosis inhibitor 1 (API1), were induced (239, 263).

In conclusion, the influence of Tax on the apoptotic pathways in HTLV-1 infected cells is still quite intriguing yet unclear. Some of the discrepancy may be explained by findings from de la Fuente *et al.* whose microarray analysis confirmed the opposing and paradoxical gene expressions of a host cell upon the introduction of DNA damage stress signal in Tax expressing cells. The resulting induction of pro- and anti-apoptotic gene expressions were directly linked to whether cells were at the G_1, S, or G_2/M phases of the cell cycle. This study also provided a timeline for cell cycle stages in which treatment of the cells would be most efficient (264). Therefore, other HTLV-1 encoded proteins or proteins specific to a cell cycle phase may play a significant role in the apoptotic process.

Overall, these data do not implicate Tax as directly inducing or inhibiting apoptosis. Rather, Tax expression influences other factors such as NF-kappaB that regulate both apoptotic and anti-apoptotic genes. This allows for either a cell death or survival pathway to ensue. It is possible that the pathway chosen is dependent on the cell cycle or what cellular stress pathway is induced.

5. ANIMAL MODELS OF HTLV-1 AND TAX

Utilization of animals as model systems has proven helpful in understanding the underlying mechanisms of transformation and tumorigenicity of human cancers *in vivo*. In the case of HTLV-1, a number of investigators have utilized transgenic and severe combined immunodeficient (SCID) mice, rats, and rabbits to dissect the role of Tax and the accessory proteins, $p12^I$, $p13^{II}$, and $p30^{II}$, in replication, infectivity, and tumorigenesis.

Rabbits have proven to be an effective *in vivo* model for HTLV-1 infection (265, 266). MT-2, an HTLV-1 producing cell line, or cells co-cultivated with MT-2 inoculated into rabbits have been shown to produce two predominant pathologies resembling either chronic infection or acute ATL-like disease (265-270). Sequence comparison of the provirus variants isolated from two rabbit cell lines exhibiting these two different pathologies demonstrated about 99% sequence identity. However when compared with the prototypic Japanese HTLV-1 strain, ATK, there was not a clear correlation with lethality (271). Analysis of the proviral genome within infected peripheral blood lymphocytes revealed integration of the provirus to be primarily monoclonal (266, 272, 273). These results are consistent with a more recent report demonstrating that rabbits inoculated with infected rabbit T lymphocytes, containing a monoclonally integrated full-length HTLV-1 provirus, were shown to develop an ATL-like disease (274, 275). This pathology was characterized by thymic atrophy with high thymic proviral loads. Interestingly, this acute

phenotype was not observed in rabbits infected with different cell inoculums (generated similarly by co-cultivation with MT-2 cells) and thus resembles the phenotype of HTLV-1 infection in humans, where only a small percentage of seropositive individuals develop ATL. Other studies have demonstrated that HTLV-1-associated uveitis can develop in infected rabbits (276) and cell-free HTLV-1 virions were infectious in rabbits (277).

In order to examine the transforming ability of the tax gene, transgenic mice carrying the HTLV-1 LTR driven tax gene were developed (278, 279). Five of the eight founder mice expressed the tax transgene within the thymus and muscle tissue. However, this group exhibited extensive thymic depletion and growth retardation followed by death between 3 to 6 weeks of age. Three lines of transgenics from the remaining founder mice were found to develop neurofibromas comprised of perineural cells and fibroblasts at multiple sites between 13 to 17 weeks of age (278, 279). This was the first *in vivo* evidence to demonstrate that Tax was a viral oncoprotein. Tumors isolated from these mice and derived cell lines expressed high levels of Tax RNA and protein, suggesting that the tumors were a direct result of Tax activity within these cells. Further examination of cellular factors expressed from these tumors revealed high levels of the granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2) receptor (280). This phenotype was analogous to that observed in ATL patients and leukemic derived cell lines. Later studies were able to demonstrate other pathologies within these mice, including an exocrinopathy involving the salivary and lachrymal glands that resembles Sjogren's syndrome (280), muscular atrophy (281), inflammatory arthropathy (282, 283), inflammatory pulmonary disease involving the peribronchial areas and to a lesser extent the perivascular areas and alveolar septa within the lung (284, 285), fibrosarcomas (286), and hypercalcemia (287). Most of these pathologies have been reported as either HTLV-1 or ATL-associated conditions (288-290).

Several additional transgenic mouse models have been developed utilizing cellular promoters to target Tax expression to T lymphocytes. The CD3-epsilon promoter enhancer element was used by Hall *et al.* (291) to develop a series of Tax transgenic mice. These mouse lines exhibited a variety of pathologies, including mesenchymal tumors (also described as neurofibromas), thymic atrophy, hindleg ataxia and paresis, and mammary and salivary adenomas. Interestingly, the authors demonstrated a high level of apoptosis that strongly correlated with Tax expression in all three tumor types, lending support to the hypothesis that Tax sensitizes cells to apoptosis. Conversely, an additional mouse model was developed where Tax expression was regulated by the granzyme B promoter (39). This led to targeted expression of Tax within activated $CD4^+$ and $CD8^+$ T lymphocytes, natural killer (NK) cells, and lymphokine-activated killer cells. These mice displayed several features previously discussed such as mesenchymal tumors and thymic atrophy; however, these mice also developed peripheral large granular lymphocytic (LGL) tumors. These LGL tumors were characterized by infiltration of the lymph nodes, bone marrow, spleen, liver, and lungs, in addition to neutrophilia, reticulocytosis, and sporadic thrombocytopenia and anemia. While this data implicates Tax as a being an important contributing factor, additional evidence to support the involvement of HTLV-1 in LGL tumor development has been lacking (292). However, further studies utilizing these mice have yielded interesting insights into the role of p53 inactivation. When examining the p53 status in these mice, the authors demonstrated that p53 inactivation may be a late event in tumorigenesis (293, 294).

Another mouse model that has also been utilized is the severe combined immunodeficient (SCID) mouse. Feuer *et al.* (295) were able to propogate peripheral blood lymphocytes (PBLs) from patients diagnosed with ATL, HAM/TSP, or from asymptomatic HTLV-1-seropositive patients. These mice became persistently infected with HTLV-1 and some animals were characterized by lymphoblastic lymphomas originating from ATL PBLs. Additional studies indicated that ATL leukemic cells (containing HTLV-1 proviral DNA sequences but lacking detectable viral gene expression) in contrast to HTLV-1 immortalized cells, which express all viral gene products, were able to generate lymphomas within SCID mice (296-298). The failure of HTLV-1 immortalized cells to produce lymphomas was suggested to be due to the clearance of virus-producing transformed cells. The higher tumorigenic potential of ATL tumor cells was due to their inability to express viral proteins and as a result evade NK cell cytolysis. An observation further supported by the fact that when these mice were immunosuppressed and then inoculated with HTLV-1 transformed cells, a lymphoma phenotype was developed. Thus, repression of HTLV-1 expression and evasion of immune surveillance is thought to play a key role in tumorigenicity within ATL.

Lastly, the role of HTLV-1 infection, or more specifically Tax, in the development of a variety of pathologies has also benefited from the WKAH rat models. Expression of HTLV-1 within the spinal cords of this strain of rats was shown to result in a chronic progressive myeloneuropathy with spastic paraparesis of the hind limbs that closely resembles HAM/TSP (299-301). This pathology was characterized by high expression and production of TNF-alpha within the spinal cord and cerebrospinal fluid correlating with pX expression. Apoptosis of Schwann cells within the peripheral nerves was also observed to occur lending support to the theory that HAM/TSP results as an inflammatory response to high viral expression. Therefore, those individuals that have moderate to low viral expression may have more potential to progress from asymptomatic to ATL, since lower expression of viral products may help in bypassing immune detection.

6. SUMMARY AND PERSPECTIVE

While the accessory proteins have not been directly linked to the tumorigenic process, they still play important roles in viral persistence. The HTLV-1 accessory protein, $p12^I$, is important for both viral infectivity and T-

cell activation and, therefore may be an important precursor for Tax transforming events. In addition, transcriptional regulation via $p30^{II}$ has the potential to have a significant influence on the tumorigenesis process in HTLV-1 infected cells. More studies are needed to elucidate which genes are regulated through $p30^{II}$. Both $p30^{II}$ and $p12^{I}$ appear to play a significant role in invading the immune response and are therefore potential candidates for establishing the long latency period observed in HTLV-1 infected patients. Future studies should also be aimed towards understanding both $p13^{II}$ and $p27^{I}$, whose functions are largely unknown.

The mechanisms underlying transformation are not entirely known, but there is a large body of literature that implicates the viral oncogene Tax in tumorigenesis, including many animal models. Tax mainly acts through protein-protein interactions, being a potent transactivator of both viral and cellular transcription. Tax also induces aberrant cell growth through the deregulation of the G_1/S phase and TGF-beta signaling pathways, as well as inactivation of p53. Inactivation of p53 also provides a mechanism in which Tax effects genomic stability combined with the ability of Tax to bind and interfere with the function of multiple proteins involved in DNA repair and mitotic checkpoints. Finally, Tax appears to indirectly influence the apoptotic fate of the cell, although whether Tax induces or prevents apoptosis remains to be seen and may be dependent on other cellular events such as the cell cycle. Due to the lack of detectable Tax expression in most ATL patient samples, it is thought that Tax is important for early stages of transformation. Whether another HTLV-1 viral protein is needed to maintain the transformed phenotype or whether enough alterations at the genomic level has occurred for the cancer to persist remains to be seen.

Future studies also should be aimed at identifying effective therapy for ATL patients. Based on the multiple alterations of cyclin/cdk activity by Tax, one appealing target for drug design would be to block this hyper-activity. Indeed, some small molecule inhibitors of cdks, such as the ATP analogues Roscovitine and Flavopiridol, which are currently in clinical trials, may be used against increased cdk activity in HTLV-1 infected cells (302). Another possibility is the use of proteasome inhibitors due to the ability of Tax to manipulate the proteasome to maintain constitutive NF-kappaB activity as well as its ability to induce the degradation of Rb. This therapy is especially attractive because constitutive NF-kappaB activity is also observed in ATL patients in the absence of detectable Tax expression. Furthermore, proteasome inhibitors have been examined in clinical trials as part of anti-cancer therapies (303). More than likely, a combination of two or more drugs will provide the most effective therapy for ATL patients based on the large number of alterations observed in HTLV-1 infected cells.

7. ACKNOWLEDGMENTS

We would like to thank the members of the Kashanchi lab for their helpful comments and suggestions and Madhur Gupta for assistance with the figures. This work was supported by grants from the NIH (AI44357, AI43894 and 13969) to FK and a research enhancement fund (REF, 111501) from The George Washington University to FK and A.Vertes. KK was supported by a pre-doctoral research fellowship from the DOD Breast Cancer Research Program DAMD17-03-1-0198.

8. REFERENCES

1. Poiesz B. J., F. W. Ruscetti, J. W. Mier, A. M. Woods, R. C. Gallo: T-cell lines established from human T-lymphocytic neoplasias by direct response to T-cell growth factor. *Proc Natl Acad Sci U S A* 77, 6815-9 (1980)

2. Poiesz B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, R. C. Gallo: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci U S A* 77, 7415-9 (1980)

3. Edlich R. F., L. G. Hill, F. M. Williams: Global epidemic of human T-cell lymphotrophic virus type-I (HTLV-I): an update. *J Long Term Eff Med Implants* 13, 127-40 (2003)

4. Manns A., M. Hisada, L. La Grenade: Human T-lymphotropic virus type I infection. *Lancet* 353, 1951-8 (1999)

5. Slattery J. P., G. Franchini, A. Gessain: Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. *Genome Res* 9, 525-40 (1999)

6. Heger P., O. Rosorius, J. Hauber, R. H. Stauber: Titration of cellular export factors, but not heteromultimerization, is the molecular mechanism of trans-dominant HTLV-1 rex mutants. *Oncogene* 18, 4080-90 (1999)

7. Albrecht B., M. D. Lairmore: Critical role of human T-lymphotropic virus type 1 accessory proteins in viral replication and pathogenesis. *Microbiol Mol Biol Rev* 66, 396-406, table of contents (2002)

8. Chen Y. M., S. H. Chen, C. Y. Fu, J. Y. Chen, M. Osame: Antibody reactivities to tumor-suppressor protein p53 and HTLV-I Tof, Rex and Tax in HTLV-I-infected people with differing clinical status. *Int J Cancer* 71, 196-202 (1997)

9. Dekaban G. A., A. A. Peters, J. C. Mulloy, J. M. Johnson, R. Trovato, E. Rivadeneira, G. Franchini: The HTLV-I orfI protein is recognized by serum antibodies from naturally infected humans and experimentally infected rabbits. *Virology* 274, 86-93 (2000)

10. Pique C., A. Ureta-Vidal, A. Gessain, B. Chancerel, O. Gout, R. Tamouza, F. Agis, M. C. Dokhelar: Evidence for the chronic *in vivo* production of human T cell leukemia virus type I Rof and Tof proteins from cytotoxic T lymphocytes directed against viral peptides. *J Exp Med* 191, 567-72 (2000)

11. Bartoe J. T., B. Albrecht, N. D. Collins, M. D. Robek, L. Ratner, P. L. Green, M. D. Lairmore: Functional role of pX open reading frame II of human T-lymphotropic virus type 1 in maintenance of viral loads *in vivo*. *J Virol* 74, 1094-100 (2000)

12. Albrecht B., C. D. D'Souza, W. Ding, S. Tridandapani, K. M. Coggeshall, M. D. Lairmore: Activation of nuclear factor of activated T cells by human T-lymphotropic virus

type 1 accessory protein p12(I). *J Virol* 76, 3493-501 (2002)
13. Kim S. J., W. Ding, B. Albrecht, P. L. Green, M. D. Lairmore: A conserved calcineurin-binding motif in human T lymphotropic virus type 1 p12I functions to modulate nuclear factor of activated T cell activation. *J Biol Chem* 278, 15550-7 (2003)
14. Zhang W., J. W. Nisbet, B. Albrecht, W. Ding, F. Kashanchi, J. T. Bartoe, M. D. Lairmore: Human T-lymphotropic virus type 1 p30(II) regulates gene transcription by binding CREB binding protein/p300. *J Virol* 75, 9885-95 (2001)
15. Franchini G.: Molecular mechanisms of human T-cell leukemia/lymphotropic virus type I infection. *Blood* 86, 3619-39 (1995)
16. Trovato R., J. C. Mulloy, J. M. Johnson, S. Takemoto, M. P. de Oliveira, G. Franchini: A lysine-to-arginine change found in natural alleles of the human T-cell lymphotropic/leukemia virus type 1 p12(I) protein greatly influences its stability. *J Virol* 73, 6460-7 (1999)
17. Garry R. F.: Sequence similarities between latent membrane protein LMP-1 of Epstein-Barr virus, integral membrane protein p12I of human T cell leukemia/lymphotropic virus type 1, E5 transformation protein of bovine papillomavirus, and the transmembrane proteins of slowly transforming retroviruses. *AIDS Res Hum Retroviruses* 11, 431-2 (1995)
18. Franchini G., J. C. Mulloy, I. J. Koralnik, A. Lo Monico, J. J. Sparkowski, T. Andresson, D. J. Goldstein, R. Schlegel: The human T-cell leukemia/lymphotropic virus type I p12I protein cooperates with the E5 oncoprotein of bovine papillomavirus in cell transformation and binds the 16-kilodalton subunit of the vacuolar H+ ATPase. *J Virol* 67, 7701-4 (1993)
19. Robek M. D., F. H. Wong, L. Ratner: Human T-cell leukemia virus type 1 pX-I and pX-II open reading frames are dispensable for the immortalization of primary lymphocytes. *J Virol* 72, 4458-62 (1998)
20. Collins N. D., G. C. Newbound, B. Albrecht, J. L. Beard, L. Ratner, M. D. Lairmore: Selective ablation of human T-cell lymphotropic virus type 1 p12I reduces viral infectivity *in vivo*. *Blood* 91, 4701-7 (1998)
21. Albrecht B., N. D. Collins, M. T. Burniston, J. W. Nisbet, L. Ratner, P. L. Green, M. D. Lairmore: Human T-lymphotropic virus type 1 open reading frame I p12(I) is required for efficient viral infectivity in primary lymphocytes. *J Virol* 74, 9828-35 (2000)
22. Lairmore M. D., B. Albrecht, C. D'Souza, J. W. Nisbet, W. Ding, J. T. Bartoe, P. L. Green, W. Zhang: *In vitro* and *in vivo* functional analysis of human T cell lymphotropic virus type 1 pX open reading frames I and II. *AIDS Res Hum Retroviruses* 16, 1757-64 (2000)
23. Ding W., B. Albrecht, R. Luo, W. Zhang, J. R. Stanley, G. C. Newbound, M. D. Lairmore: Endoplasmic reticulum and cis-Golgi localization of human T-lymphotropic virus type 1 p12(I): association with calreticulin and calnexin. *J Virol* 75, 7672-82 (2001)
24. Kane L. P., J. Lin, A. Weiss: Signal transduction by the TCR for antigen. *Curr Opin Immunol* 12, 242-9 (2000)
25. Crabtree G. R., E. N. Olson: NFAT signaling: choreographing the social lives of cells. *Cell* 109 Suppl, S67-79 (2002)
26. Ding W., B. Albrecht, R. E. Kelley, N. Muthusamy, S. J. Kim, R. A. Altschuld, M. D. Lairmore: Human T-cell lymphotropic virus type 1 p12(I) expression increases cytoplasmic calcium to enhance the activation of nuclear factor of activated T cells. *J Virol* 76, 10374-82 (2002)
27. Ding W., S. J. Kim, A. M. Nair, B. Michael, K. Boris-Lawrie, A. Tripp, G. Feuer, M. D. Lairmore: Human T-cell lymphotropic virus type 1 p12I enhances interleukin-2 production during T-cell activation. *J Virol* 77, 11027-39 (2003)
28. Nicot C., J. C. Mulloy, M. G. Ferrari, J. M. Johnson, K. Fu, R. Fukumoto, R. Trovato, J. Fullen, W. J. Leonard, G. Franchini: HTLV-1 p12(I) protein enhances STAT5 activation and decreases the interleukin-2 requirement for proliferation of primary human peripheral blood mononuclear cells. *Blood* 98, 823-9 (2001)
29. Johnson J. M., C. Nicot, J. Fullen, V. Ciminale, L. Casareto, J. C. Mulloy, S. Jacobson, G. Franchini: Free major histocompatibility complex class I heavy chain is preferentially targeted for degradation by human T-cell leukemia/lymphotropic virus type 1 p12(I) protein. *J Virol* 75, 6086-94 (2001)
30. Koralnik I. J., J. Fullen, G. Franchini: The p12I, p13II, and p30II proteins encoded by human T-cell leukemia/lymphotropic virus type I open reading frames I and II are localized in three different cellular compartments. *J Virol* 67, 2360-6 (1993)
31. Ciminale V., G. N. Pavlakis, D. Derse, C. P. Cunningham, B. K. Felber: Complex splicing in the human T-cell leukemia virus (HTLV) family of retroviruses: novel mRNAs and proteins produced by HTLV type I. *J Virol* 66, 1737-45 (1992)
32. Zhang W., J. W. Nisbet, J. T. Bartoe, W. Ding, M. D. Lairmore: Human T-lymphotropic virus type 1 p30(II) functions as a transcription factor and differentially modulates CREB-responsive promoters. *J Virol* 74, 11270-7 (2000)
33. Roithmann S., C. Pique, A. Le Cesne, L. Delamarre, D. Pham, T. Tursz, M. C. Dokhelar: The open reading frame I (ORF I)/ORF II part of the human T-cell leukemia virus type I X region is dispensable for p40tax, p27rex, or envelope expression. *J Virol* 68, 3448-51 (1994)
34. D'Agostino D. M., L. Ranzato, G. Arrigoni, I. Cavallari, F. Belleudi, M. R. Torrisi, M. Silic-Benussi, T. Ferro, V. Petronilli, O. Marin, L. Chieco-Bianchi, P. Bernardi, V. Ciminale: Mitochondrial alterations induced by the p13II protein of human T-cell leukemia virus type 1. Critical role of arginine residues. *J Biol Chem* 277, 34424-33 (2002)
35. Hou X., S. Foley, M. Cueto, M. A. Robinson: The human T-cell leukemia virus type I (HTLV-I) X region encoded protein p13(II) interacts with cellular proteins. *Virology* 277, 127-35 (2000)
36. Lefebvre L., A. Vanderplasschen, V. Ciminale, H. Heremans, O. Dangoisse, J. C. Jauniaux, J. F. Toussaint, V. Zelnik, A. Burny, R. Kettmann, L. Willems: Oncoviral bovine leukemia virus G4 and human T-cell leukemia virus type 1 p13(II) accessory proteins interact with farnesyl pyrophosphate synthetase. *J Virol* 76, 1400-14 (2002)
37. Lefebvre L., V. Ciminale, A. Vanderplasschen, D. D'Agostino, A. Burny, L. Willems, R. Kettmann: Subcellular localization of the bovine leukemia virus R3 and G4 accessory proteins. *J Virol* 76, 7843-54 (2002)

38. Grassmann R., S. Berchtold, I. Radant, M. Alt, B. Fleckenstein, J. G. Sodroski, W. A. Haseltine, U. Ramstedt: Role of human T-cell leukemia virus type 1 X region proteins in immortalization of primary human lymphocytes in culture. *J Virol* 66, 4570-5 (1992)
39. Grossman W. J., J. T. Kimata, F. H. Wong, M. Zutter, T. J. Ley, L. Ratner: Development of leukemia in mice transgenic for the tax gene of human T-cell leukemia virus type I. *Proc Natl Acad Sci U S A* 92, 1057-61 (1995)
40. Iwanaga Y., T. Tsukahara, T. Ohashi, Y. Tanaka, M. Arai, M. Nakamura, K. Ohtani, Y. Koya, M. Kannagi, N. Yamamoto, M. Fujii: Human T-cell leukemia virus type 1 tax protein abrogates interleukin-2 dependence in a mouse T-cell line. *J Virol* 73, 1271-7 (1999)
41. Hollsberg P.: Mechanisms of T-cell activation by human T-cell lymphotropic virus type I. *Microbiol Mol Biol Rev* 63, 308-33 (1999)
42. Fujisawa J., M. Seiki, M. Sato, M. Yoshida: A transcriptional enhancer sequence of HTLV-I is responsible for trans-activation mediated by p40 chi HTLV-I. *Embo J* 5, 713-8 (1986)
43. Shimotohno K., M. Takano, T. Teruuchi, M. Miwa: Requirement of multiple copies of a 21-nucleotide sequence in the U3 regions of human T-cell leukemia virus type I and type II long terminal repeats for trans-acting activation of transcription. *Proc Natl Acad Sci U S A* 83, 8112-6 (1986)
44. Yoshida M.: Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol* 19, 475-96 (2001)
45. Suzuki T., J. I. Fujisawa, M. Toita, M. Yoshida: The trans-activator tax of human T-cell leukemia virus type 1 (HTLV-1) interacts with cAMP-responsive element (CRE) binding and CRE modulator proteins that bind to the 21-base-pair enhancer of HTLV-1. *Proc Natl Acad Sci U S A* 90, 610-4 (1993)
46. Hai T., M. G. Hartman: The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. *Gene* 273, 1-11 (2001)
47. Zhao L. J., C. Z. Giam: Human T-cell lymphotropic virus type I (HTLV-I) transcriptional activator, Tax, enhances CREB binding to HTLV-I 21-base-pair repeats by protein-protein interaction. *Proc Natl Acad Sci U S A* 89, 7070-4 (1992)
48. Franklin A. A., M. F. Kubik, M. N. Uittenbogaard, A. Brauweiler, P. Utaisincharoen, M. A. Matthews, W. S. Dynan, J. P. Hoeffler, J. K. Nyborg: Transactivation by the human T-cell leukemia virus Tax protein is mediated through enhanced binding of activating transcription factor-2 (ATF-2) ATF-2 response and cAMP element-binding protein (CREB). *J Biol Chem* 268, 21225-31 (1993)
49. Yin M. J., R. B. Gaynor: HTLV-1 21 bp repeat sequences facilitate stable association between Tax and CREB to increase CREB binding affinity. *J Mol Biol* 264, 20-31 (1996)
50. Yin M. J., R. B. Gaynor: Complex formation between CREB and Tax enhances the binding affinity of CREB for the human T-cell leukemia virus type 1 21-base-pair repeats. *Mol Cell Biol* 16, 3156-68 (1996)
51. Lundblad J. R., R. P. Kwok, M. E. Laurance, M. S. Huang, J. P. Richards, R. G. Brennan, R. H. Goodman: The human T-cell leukemia virus-1 transcriptional activator Tax enhances cAMP-responsive element-binding protein (CREB) binding activity through interactions with the DNA minor groove. *J Biol Chem* 273, 19251-9 (1998)
52. Kimzey A. L., W. S. Dynan: Specific regions of contact between human T-cell leukemia virus type I Tax protein and DNA identified by photocross-linking. *J Biol Chem* 273, 13768-75 (1998)
53. Kimzey A. L., W. S. Dynan: Identification of a human T-cell leukemia virus type I tax peptide in contact with DNA. *J Biol Chem* 274, 34226-32 (1999)
54. Lenzmeier B. A., H. A. Giebler, J. K. Nyborg: Human T-cell leukemia virus type 1 Tax requires direct access to DNA for recruitment of CREB binding protein to the viral promoter. *Mol Cell Biol* 18, 721-31 (1998)
55. Wagner S., M. R. Green: HTLV-I Tax protein stimulation of DNA binding of bZIP proteins by enhancing dimerization. *Science* 262, 395-9 (1993)
56. Vendel A. C., K. J. Lumb: Molecular Recognition of the Human Coactivator CBP by the HIV-1 Transcriptional Activator Tat. *Biochemistry* 42, 910-6 (2003)
57. Parker D., K. Ferreri, T. Nakajima, V. J. LaMorte, R. Evans, S. C. Koerber, C. Hoeger, M. R. Montminy: Phosphorylation of CREB at Ser-133 induces complex formation with CREB-binding protein via a direct mechanism. *Mol Cell Biol* 16, 694-703 (1996)
58. Chrivia J. C., R. P. Kwok, N. Lamb, M. Hagiwara, M. R. Montminy, R. H. Goodman: Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature* 365, 855-9 (1993)
59. Kwok R. P., M. E. Laurance, J. R. Lundblad, P. S. Goldman, H. Shih, L. M. Connor, S. J. Marriott, R. H. Goodman: Control of cAMP-regulated enhancers by the viral transactivator Tax through CREB and the co-activator CBP. *Nature* 380, 642-6 (1996)
60. Kashanchi F., J. F. Duvall, R. P. Kwok, J. R. Lundblad, R. H. Goodman, J. N. Brady: The coactivator CBP stimulates human T-cell lymphotrophic virus type I Tax transactivation *in vitro*. *J Biol Chem* 273, 34646-52 (1998)
61. Lemasson I., J. K. Nyborg: Human T-cell leukemia virus type I tax repression of p73beta is mediated through competition for the C/H1 domain of CBP. *J Biol Chem* 276, 15720-7 (2001)
62. Giebler H. A., J. E. Loring, K. van Orden, M. A. Colgin, J. E. Garrus, K. W. Escudero, A. Brauweiler, J. K. Nyborg: Anchoring of CREB binding protein to the human T-cell leukemia virus type 1 promoter: a molecular mechanism of Tax transactivation. *Mol Cell Biol* 17, 5156-64 (1997)
63. Yan J. P., J. E. Garrus, H. A. Giebler, L. A. Stargell, J. K. Nyborg: Molecular interactions between the coactivator CBP and the human T-cell leukemia virus Tax protein. *J Mol Biol* 281, 395-400 (1998)
64. Georges S. A., H. A. Giebler, P. A. Cole, K. Luger, P. J. Laybourn, J. K. Nyborg: Tax recruitment of CBP/p300, via the KIX domain, reveals a potent requirement for acetyltransferase activity that is chromatin dependent and histone tail independent. *Mol Cell Biol* 23, 3392-404 (2003)
65. Scoggin K. E., A. Ulloa, J. K. Nyborg: The oncoprotein Tax binds the SRC-1-interacting domain of CBP/p300 to mediate transcriptional activation. *Mol Cell Biol* 21, 5520-30 (2001)

66. Harrod R., Y. Tang, C. Nicot, H. S. Lu, A. Vassilev, Y. Nakatani, C. Z. Giam: An exposed KID-like domain in human T-cell lymphotropic virus type 1 Tax is responsible for the recruitment of coactivators CBP/p300. *Mol Cell Biol* 18, 5052-61 (1998)
67. Smith M. R., W. C. Greene: Identification of HTLV-I tax trans-activator mutants exhibiting novel transcriptional phenotypes. *Genes Dev* 4, 1875-85 (1990)
68. Robek M. D., L. Ratner: Immortalization of T lymphocytes by human T-cell leukemia virus type 1 is independent of the tax-CBP/p300 interaction. *J Virol* 74, 11988-92 (2000)
69. Jiang H., H. Lu, R. L. Schiltz, C. A. Pise-Masison, V. V. Ogryzko, Y. Nakatani, J. N. Brady: PCAF interacts with tax and stimulates tax transactivation in a histone acetyltransferase-independent manner. *Mol Cell Biol* 19, 8136-45 (1999)
70. Yang X. J., V. V. Ogryzko, J. Nishikawa, B. H. Howard, Y. Nakatani: A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* 382, 319-24 (1996)
71. Xu W., D. G. Edmondson, S. Y. Roth: Mammalian GCN5 and P/CAF acetyltransferases have homologous amino-terminal domains important for recognition of nucleosomal substrates. *Mol Cell Biol* 18, 5659-69 (1998)
72. Krumm A., L. Madisen, X. J. Yang, R. Goodman, Y. Nakatani, M. Groudine: Long-distance transcriptional enhancement by the histone acetyltransferase PCAF. *Proc Natl Acad Sci U S A* 95, 13501-6 (1998)
73. Okada M., K. T. Jeang: Differential requirements for activation of integrated and transiently transfected human T-cell leukemia virus type 1 long terminal repeat. *J Virol* 76, 12564-73 (2002)
74. Reddy T. R., H. Tang, X. Li, F. Wong-Staal: Functional interaction of the HTLV-1 transactivator Tax with activating transcription factor-4 (ATF4). *Oncogene* 14, 2785-92 (1997)
75. Gachon F., C. Devaux, J. M. Mesnard: Activation of HTLV-I transcription in the presence of Tax is independent of the acetylation of CREB-2 (ATF-4). *Virology* 299, 271-8 (2002)
76. Gachon F., S. Thebault, A. Peleraux, C. Devaux, J. M. Mesnard: Molecular interactions involved in the transactivation of the human T-cell leukemia virus type 1 promoter mediated by Tax and CREB-2 (ATF-4). *Mol Cell Biol* 20, 3470-81 (2000)
77. Gachon F., A. Peleraux, S. Thebault, J. Dick, I. Lemasson, C. Devaux, J. M. Mesnard: CREB-2, a cellular CRE-dependent transcription repressor, functions in association with Tax as an activator of the human T-cell leukemia virus type 1 promoter. *J Virol* 72, 8332-7 (1998)
78. Fujii M., H. Tsuchiya, X. B. Meng, M. Seiki: c-Jun, c-Fos and their family members activate the transcription mediated by three 21-bp repetitive sequences in the HTLV-I long terminal repeat. *Intervirology* 38, 221-8 (1995)
79. Jeang K. T., R. Chiu, E. Santos, S. J. Kim: Induction of the HTLV-I LTR by Jun occurs through the Tax-responsive 21-bp elements. *Virology* 181, 218-27 (1991)
80. Fujii M., T. Niki, T. Mori, T. Matsuda, M. Matsui, N. Nomura, M. Seiki: HTLV-1 Tax induces expression of various immediate early serum responsive genes. *Oncogene* 6, 1023-9 (1991)
81. Iwakura Y., M. Tosu, E. Yoshida, S. Saijo, J. Nakayama-Yamada, K. Itagaki, M. Asano, H. Siomi, M. Hatanaka, T. Takeda, *et al.*: Augmentation of c-fos and c-jun expression in transgenic mice carrying the human T-cell leukemia virus type-I tax gene. *Virus Genes* 9, 161-70 (1995)
82. Mori N., N. Mukaida, D. W. Ballard, K. Matsushima, N. Yamamoto: Human T-cell leukemia virus type I Tax transactivates human interleukin 8 gene through acting concurrently on AP-1 and nuclear factor-kappaB-like sites. *Cancer Res* 58, 3993-4000 (1998)
83. Armstrong A. P., A. A. Franklin, M. N. Uittenbogaard, H. A. Giebler, J. K. Nyborg: Pleiotropic effect of the human T-cell leukemia virus Tax protein on the DNA binding activity of eukaryotic transcription factors. *Proc Natl Acad Sci U S A* 90, 7303-7 (1993)
84. Iwai K., N. Mori, M. Oie, N. Yamamoto, M. Fujii: Human T-cell leukemia virus type 1 tax protein activates transcription through AP-1 site by inducing DNA binding activity in T cells. *Virology* 279, 38-46 (2001)
85. Mori N., M. Fujii, K. Iwai, S. Ikeda, Y. Yamasaki, T. Hata, Y. Yamada, Y. Tanaka, M. Tomonaga, N. Yamamoto: Constitutive activation of transcription factor AP-1 in primary adult T-cell leukemia cells. *Blood* 95, 3915-21 (2000)
86. Clemens K. E., G. Piras, M. F. Radonovich, K. S. Choi, J. F. Duvall, J. DeJong, R. Roeder, J. N. Brady: Interaction of the human T-cell lymphotropic virus type 1 tax transactivator with transcription factor IIA. *Mol Cell Biol* 16, 4656-64 (1996)
87. Duvall J. F., F. Kashanchi, A. Cvekl, M. F. Radonovich, G. Piras, J. N. Brady: Transactivation of the human T-cell lymphotropic virus type 1 Tax1-responsive 21-base-pair repeats requires Holo-TFIID and TFIIA. *J Virol* 69, 5077-86 (1995)
88. Caron C., R. Rousset, C. Beraud, V. Moncollin, J. M. Egly, P. Jalinot: Functional and biochemical interaction of the HTLV-I Tax1 transactivator with TBP. *Embo J* 12, 4269-78 (1993)
89. Caron C., G. Mengus, V. Dubrowskaya, A. Roisin, I. Davidson, P. Jalinot: Human TAF(II)28 interacts with the human T cell leukemia virus type I Tax transactivator and promotes its transcriptional activity. *Proc Natl Acad Sci U S A* 94, 3662-7 (1997)
90. Zhang Z., E. F. Hildebrandt, C. M. Simbulan-Rosenthal, M. G. Anderson: Sequence-specific binding of poly(ADP-ribose) polymerase-1 to the human T cell leukemia virus type-I tax responsive element. *Virology* 296, 107-16 (2002)
91. Anderson M. G., K. E. Scoggin, C. M. Simbulan-Rosenthal, J. A. Steadman: Identification of poly(ADP-ribose) polymerase as a transcriptional coactivator of the human T-cell leukemia virus type 1 Tax protein. *J Virol* 74, 2169-77 (2000)
92. Youn H. G., J. Matsumoto, Y. Tanaka, K. Shimotohno: SR-related protein TAXREB803/SRL300 is an important cellular factor for the transactivational function of human T-cell lymphotropic virus type 1 Tax. *J Virol* 77, 10015-27 (2003)
93. Lemasson I., V. Robert-Hebmann, S. Hamaia, M. Duc Dodon, L. Gazzolo, C. Devaux: Transrepression of lck gene expression by human T-cell leukemia virus type 1-encoded p40tax. *J Virol* 71, 1975-83 (1997)

94. Colgin M. A., J. K. Nyborg: The human T-cell leukemia virus type 1 oncoprotein Tax inhibits the transcriptional activity of c-Myb through competition for the CREB binding protein. *J Virol* 72, 9396-9 (1998)
95. Nicot C., R. Opavsky, R. Mahieux, J. M. Johnson, J. N. Brady, L. Wolff, G. Franchini: Tax oncoprotein trans-represses endogenous B-myb promoter activity in human T cells. *AIDS Res Hum Retroviruses* 16, 1629-32 (2000)
96. Nicot C., R. Mahieux, R. Opavsky, A. Cereseto, L. Wolff, J. N. Brady, G. Franchini: HTLV-I Tax transrepresses the human c-Myb promoter independently of its interaction with CBP or p300. *Oncogene* 19, 2155-64 (2000)
97. Kibler K. V., K. T. Jeang: CREB/ATF-dependent repression of cyclin a by human T-cell leukemia virus type 1 Tax protein. *J Virol* 75, 2161-73 (2001)
98. Van Orden K., H. A. Giebler, I. Lemasson, M. Gonzales, J. K. Nyborg: Binding of p53 to the KIX domain of CREB binding protein. A potential link to human T-cell leukemia virus, type I-associated leukemogenesis. *J Biol Chem* 274, 26321-8 (1999)
99. Van Orden K., J. P. Yan, A. Ulloa, J. K. Nyborg: Binding of the human T-cell leukemia virus Tax protein to the coactivator CBP interferes with CBP-mediated transcriptional control. *Oncogene* 18, 3766-72 (1999)
100. Ego T., Y. Ariumi, K. Shimotohno: The interaction of HTLV-1 Tax with HDAC1 negatively regulates the viral gene expression. *Oncogene* 21, 7241-6 (2002)
101. Lemasson I., N. J. Polakowski, P. J. Laybourn, J. K. Nyborg: Transcription Factor Binding and Histone Modifications on the Integrated Proviral Promoter in Human T-cell Leukemia Virus-I-infected T-cells. *J Biol Chem* 277, 49459-65 (2002)
102. Ogryzko V. V., R. L. Schiltz, V. Russanova, B. H. Howard, Y. Nakatani: The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* 87, 953-9 (1996)
103. Bannister A. J., T. Kouzarides: The CBP co-activator is a histone acetyltransferase. *Nature* 384, 641-3 (1996)
104. Lu H., C. A. Pise-Masison, T. M. Fletcher, R. L. Schiltz, A. K. Nagaich, M. Radonovich, G. Hager, P. A. Cole, J. N. Brady: Acetylation of nucleosomal histones by p300 facilitates transcription from tax-responsive human T-cell leukemia virus type 1 chromatin template. *Mol Cell Biol* 22, 4450-62 (2002)
105. Georges S. A., W. L. Kraus, K. Luger, J. K. Nyborg, P. J. Laybourn: p300-mediated tax transactivation from recombinant chromatin: histone tail deletion mimics coactivator function. *Mol Cell Biol* 22, 127-37 (2002)
106. Patel S. A., D. M. Graunke, R. O. Pieper: Aberrant silencing of the CpG island-containing human O6-methylguanine DNA methyltransferase gene is associated with the loss of nucleosome-like positioning. *Mol Cell Biol* 17, 5813-22 (1997)
107. Pumfery A., L. Deng, A. Maddukuri, C. de la Fuente, H. Li, J. D. Wade, P. Lambert, A. Kumar, F. Kashanchi: Chromatin Remodeling and Modification during HIV-1 Tat-activated Transcription. *Current HIV Research* 1, 343-362 (2003)
108. Sewack G. F., U. Hansen: Nucleosome positioning and transcription-associated chromatin alterations on the human estrogen-responsive pS2 promoter. *J Biol Chem* 272, 31118-29 (1997)
109. Truss M., J. Bartsch, C. Mows, S. Chavez, M. Beato: Chromatin structure of the MMTV promoter and its changes during hormonal induction. *Cell Mol Neurobiol* 16, 85-101 (1996)
110. Belikov S., B. Gelius, G. Almouzni, O. Wrange: Hormone activation induces nucleosome positioning *in vivo*. *Embo J* 19, 1023-33 (2000)
111. Becker P. B., W. Horz: ATP-dependent nucleosome remodeling. *Annu Rev Biochem* 71, 247-73 (2002)
112. Wu K., M. E. Bottazzi, C. De La Fuente, L. Deng, S. D. Gitlin, A. Maddukuri, S. Dadgar, H. Li, A. Vertes, A. Pumfery, F. Kashanchi: Protein profile of Tax-associated complexes. *J Biol Chem* (2003)
113. Gilmore T., M. E. Gapuzan, D. Kalaitzidis, D. Starczynowski: Rel/NF-kappa B/I kappa B signal transduction in the generation and treatment of human cancer. *Cancer Lett* 181, 1-9 (2002)
114. Baldwin A. S., Jr.: The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 14, 649-83 (1996)
115. Sun S. C., D. W. Ballard: Persistent activation of NF-kappaB by the tax transforming protein of HTLV-1: hijacking cellular IkappaB kinases. *Oncogene* 18, 6948-58 (1999)
116. Mori N., M. Fujii, S. Ikeda, Y. Yamada, M. Tomonaga, D. W. Ballard, N. Yamamoto: Constitutive activation of NF-kappaB in primary adult T-cell leukemia cells. *Blood* 93, 2360-8 (1999)
117. Suzuki T., H. Hirai, M. Yoshida: Tax protein of HTLV-1 interacts with the Rel homology domain of NF-kappa B p65 and c-Rel proteins bound to the NF-kappa B binding site and activates transcription. *Oncogene* 9, 3099-105 (1994)
118. Bex F., A. McDowall, A. Burny, R. Gaynor: The human T-cell leukemia virus type 1 transactivator protein Tax colocalizes in unique nuclear structures with NF-kappaB proteins. *J Virol* 71, 3484-97 (1997)
119. Inoue J., M. Seiki, T. Taniguchi, S. Tsuru, M. Yoshida: Induction of interleukin 2 receptor gene expression by p40x encoded by human T-cell leukemia virus type 1. *Embo J* 5, 2883-8 (1986)
120. Mori N., M. Fujii, G. Cheng, S. Ikeda, Y. Yamasaki, Y. Yamada, M. Tomonaga, N. Yamamoto: Human T-cell leukemia virus type I tax protein induces the expression of anti-apoptotic gene Bcl-xL in human T-cells through nuclear factor-kappaB and c-AMP responsive element binding protein pathways. *Virus Genes* 22, 279-87 (2001)
121. Azimi N., K. Brown, R. N. Bamford, Y. Tagaya, U. Siebenlist, T. A. Waldmann: Human T cell lymphotropic virus type I Tax protein trans-activates interleukin 15 gene transcription through an NF-kappaB site. *Proc Natl Acad Sci U S A* 95, 2452-7 (1998)
122. Gerritsen M. E., A. J. Williams, A. S. Neish, S. Moore, Y. Shi, T. Collins: CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc Natl Acad Sci U S A* 94, 2927-32 (1997)
123. Perkins N. D., L. K. Felzien, J. C. Betts, K. Leung, D. H. Beach, G. J. Nabel: Regulation of NF-kappaB by cyclin-dependent kinases associated with the p300 coactivator. *Science* 275, 523-7 (1997)
124. Zhong H., R. E. Voll, S. Ghosh: Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity

by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol Cell* 1, 661-71 (1998)
125. Hirai H., J. Fujisawa, T. Suzuki, K. Ueda, M. Muramatsu, A. Tsuboi, N. Arai, M. Yoshida: Transcriptional activator Tax of HTLV-1 binds to the NF-kappa B precursor p105. *Oncogene* 7, 1737-42 (1992)
126. Beraud C., S. C. Sun, P. Ganchi, D. W. Ballard, W. C. Greene: Human T-cell leukemia virus type I Tax associates with and is negatively regulated by the NF-kappa B2 p100 gene product: implications for viral latency. *Mol Cell Biol* 14, 1374-82 (1994)
127. Murakami T., H. Hirai, T. Suzuki, J. Fujisawa, M. Yoshida: HTLV-1 Tax enhances NF-kappa B2 expression and binds to the products p52 and p100, but does not suppress the inhibitory function of p100. *Virology* 206, 1066-74 (1995)
128. Maggirwar S. B., E. Harhaj, S. C. Sun: Activation of NF-kappa B/Rel by Tax involves degradation of I kappa B alpha and is blocked by a proteasome inhibitor. *Oncogene* 11, 993-8 (1995)
129. Suzuki T., H. Hirai, T. Murakami, M. Yoshida: Tax protein of HTLV-1 destabilizes the complexes of NF-kappa B and I kappa B-alpha and induces nuclear translocation of NF-kappa B for transcriptional activation. *Oncogene* 10, 1199-207 (1995)
130. Beraud C., W. C. Greene: Interaction of HTLV-I Tax with the human proteasome: implications for NF-kappa B induction. *J Acquir Immune Defic Syndr Hum Retrovirol* 13 Suppl 1, S76-84 (1996)
131. Hemelaar J., F. Bex, B. Booth, V. Cerundolo, A. McMichael, S. Daenke: Human T-cell leukemia virus type 1 Tax protein binds to assembled nuclear proteasomes and enhances their proteolytic activity. *J Virol* 75, 11106-15 (2001)
132. Rousset R., C. Desbois, F. Bantignies, P. Jalinot: Effects on NF-kappa B1/p105 processing of the interaction between the HTLV-1 transactivator Tax and the proteasome. *Nature* 381, 328-31 (1996)
133. Petropoulos L., J. Hiscott: Association between HTLV-1 Tax and I kappa B alpha is dependent on the I kappa B alpha phosphorylation state. *Virology* 252, 189-99 (1998)
134. Yamaoka S., G. Courtois, C. Bessia, S. T. Whiteside, R. Weil, F. Agou, H. E. Kirk, R. J. Kay, A. Israel: Complementation cloning of NEMO, a component of the IkappaB kinase complex essential for NF-kappaB activation. *Cell* 93, 1231-40 (1998)
135. Geleziunas R., S. Ferrell, X. Lin, Y. Mu, E. T. Cunningham, Jr., M. Grant, M. A. Connelly, J. E. Hambor, K. B. Marcu, W. C. Greene: Human T-cell leukemia virus type 1 Tax induction of NF-kappaB involves activation of the IkappaB kinase alpha (IKKalpha) and IKKbeta cellular kinases. *Mol Cell Biol* 18, 5157-65 (1998)
136. Yin M. J., L. B. Christerson, Y. Yamamoto, Y. T. Kwak, S. Xu, F. Mercurio, M. Barbosa, M. H. Cobb, R. B. Gaynor: HTLV-I Tax protein binds to MEKK1 to stimulate IkappaB kinase activity and NF-kappaB activation. *Cell* 93, 875-84 (1998)
137. Uhlik M., L. Good, G. Xiao, E. W. Harhaj, E. Zandi, M. Karin, S. C. Sun: NF-kappaB-inducing kinase and IkappaB kinase participate in human T-cell leukemia virus I Tax-mediated NF-kappaB activation. *J Biol Chem* 273, 21132-6 (1998)
138. Chu Z. L., J. A. DiDonato, J. Hawiger, D. W. Ballard: The tax oncoprotein of human T-cell leukemia virus type 1 associates with and persistently activates IkappaB kinases containing IKKalpha and IKKbeta. *J Biol Chem* 273, 15891-4 (1998)
139. Harhaj E. W., S. C. Sun: IKKgamma serves as a docking subunit of the IkappaB kinase (IKK) and mediates interaction of IKK with the human T-cell leukemia virus Tax protein. *J Biol Chem* 274, 22911-4 (1999)
140. Chu Z. L., Y. A. Shin, J. M. Yang, J. A. DiDonato, D. W. Ballard: IKKgamma mediates the interaction of cellular IkappaB kinases with the tax transforming protein of human T cell leukemia virus type 1. *J Biol Chem* 274, 15297-300 (1999)
141. Jin D. Y., V. Giordano, K. V. Kibler, H. Nakano, K. T. Jeang: Role of adapter function in oncoprotein-mediated activation of NF-kappaB. Human T-cell leukemia virus type I Tax interacts directly with IkappaB kinase gamma. *J Biol Chem* 274, 17402-5 (1999)
142. Xiao G., E. W. Harhaj, S. C. Sun: Domain-specific interaction with the I kappa B kinase (IKK)regulatory subunit IKK gamma is an essential step in tax-mediated activation of IKK. *J Biol Chem* 275, 34060-7 (2000)
143. Xiao G., S. C. Sun: Activation of IKKalpha and IKKbeta through their fusion with HTLV-I tax protein. *Oncogene* 19, 5198-203 (2000)
144. Xiao G., M. E. Cvijic, A. Fong, E. W. Harhaj, M. T. Uhlik, M. Waterfield, S. C. Sun: Retroviral oncoprotein Tax induces processing of NF-kappaB2/p100 in T cells: evidence for the involvement of IKKalpha. *Embo J* 20, 6805-15 (2001)
145. Harhaj E. W., L. Good, G. Xiao, M. Uhlik, M. E. Cvijic, I. Rivera-Walsh, S. C. Sun: Somatic mutagenesis studies of NF-kappa B signaling in human T cells: evidence for an essential role of IKK gamma in NF-kappa B activation by T-cell costimulatory signals and HTLV-I Tax protein. *Oncogene* 19, 1448-56 (2000)
146. Carter R. S., B. C. Geyer, M. Xie, C. A. Acevedo-Suarez, D. W. Ballard: Persistent activation of NF-kappa B by the tax transforming protein involves chronic phosphorylation of IkappaB kinase subunits IKKbeta and IKKgamma. *J Biol Chem* 276, 24445-8 (2001)
147. Prajapati S., R. B. Gaynor: Regulation of Ikappa B kinase (IKK)gamma /NEMO function by IKKbeta - mediated phosphorylation. *J Biol Chem* 277, 24331-9 (2002)
148. Carter R. S., K. N. Pennington, B. J. Ungurait, D. W. Ballard: *In vivo* identification of inducible phosphoacceptors in the IKKgamma/NEMO subunit of human IkappaB kinase. *J Biol Chem* 278, 19642-8 (2003)
149. Ortega S., M. Malumbres, M. Barbacid: Cyclin D-dependent kinases, INK4 inhibitors and cancer. *Biochim Biophys Acta* 1602, 73-87 (2002)
150. Cowgill S. M., P. Muscarella: The genetics of pancreatic cancer. *Am J Surg* 186, 279-86 (2003)
151. Nevins J. R.: The Rb/E2F pathway and cancer. *Hum Mol Genet* 10, 699-703 (2001)
152. Knudsen K. E., A. F. Fribourg, M. W. Strobeck, J. M. Blanchard, E. S. Knudsen: Cyclin A is a functional target

of retinoblastoma tumor suppressor protein-mediated cell cycle arrest. *J Biol Chem* 274, 27632-41 (1999)
153. Hatta Y., H. P. Koeffler: Role of tumor suppressor genes in the development of adult T cell leukemia/lymphoma (ATLL). *Leukemia* 16, 1069-85 (2002)
154. Trovato R., A. Cereseto, S. Takemoto, A. Gessain, T. Watanabe, T. Waldmann, G. Franchini: Deletion of the p16INK4A gene in *ex vivo* acute adult T cell lymphoma/leukemia cells and methylation of the p16INK4A promoter in HTLV type I-infected T cell lines. *AIDS Res Hum Retroviruses* 16, 709-13 (2000)
155. Suzuki T., M. Yoshida: HTLV-1 Tax protein interacts with cyclin-dependent kinase inhibitor p16Ink4a and counteracts its inhibitory activity to CDK4. *Leukemia* 11 Suppl 3, 14-6 (1997)
156. Suzuki T., S. Kitao, H. Matsushime, M. Yoshida: HTLV-1 Tax protein interacts with cyclin-dependent kinase inhibitor p16INK4A and counteracts its inhibitory activity towards CDK4. *Embo J* 15, 1607-14 (1996)
157. Low K. G., L. F. Dorner, D. B. Fernando, J. Grossman, K. T. Jeang, M. J. Comb: Human T-cell leukemia virus type 1 Tax releases cell cycle arrest induced by p16INK4a. *J Virol* 71, 1956-62 (1997)
158. Neuveut C., K. G. Low, F. Maldarelli, I. Schmitt, F. Majone, R. Grassmann, K. T. Jeang: Human T-cell leukemia virus type 1 Tax and cell cycle progression: role of cyclin D-cdk and p110Rb. *Mol Cell Biol* 18, 3620-32 (1998)
159. Lemasson I., S. Thebault, C. Sardet, C. Devaux, J. M. Mesnard: Activation of E2F-mediated transcription by human T-cell leukemia virus type I Tax protein in a p16(INK4A)-negative T-cell line. *J Biol Chem* 273, 23598-604 (1998)
160. Li J., H. Li, M. D. Tsai: Direct binding of the N-terminus of HTLV-1 tax oncoprotein to cyclin-dependent kinase 4 is a dominant path to stimulate the kinase activity. *Biochemistry* 42, 6921-8 (2003)
161. Haller K., Y. Wu, E. Derow, I. Schmitt, K. T. Jeang, R. Grassmann: Physical interaction of human T-cell leukemia virus type 1 Tax with cyclin-dependent kinase 4 stimulates the phosphorylation of retinoblastoma protein. *Mol Cell Biol* 22, 3327-38 (2002)
162. Santiago F., E. Clark, S. Chong, C. Molina, F. Mozafari, R. Mahieux, M. Fujii, N. Azimi, F. Kashanchi: Transcriptional up-regulation of the cyclin D2 gene and acquisition of new cyclin-dependent kinase partners in human T-cell leukemia virus type 1-infected cells. *J Virol* 73, 9917-27 (1999)
163. Mori N., M. Fujii, M. Hinz, K. Nakayama, Y. Yamada, S. Ikeda, Y. Yamasaki, F. Kashanchi, Y. Tanaka, M. Tomonaga, N. Yamamoto: Activation of cyclin D1 and D2 promoters by human T-cell leukemia virus type I tax protein is associated with IL-2-independent growth of T cells. *Int J Cancer* 99, 378-85 (2002)
164. Huang Y., K. Ohtani, R. Iwanaga, Y. Matsumura, M. Nakamura: Direct trans-activation of the human cyclin D2 gene by the oncogene product Tax of human T-cell leukemia virus type I. *Oncogene* 20, 1094-102 (2001)
165. Lemoine F. J., S. J. Marriott: Accelerated G(1) phase progression induced by the human T cell leukemia virus type I (HTLV-I) Tax oncoprotein. *J Biol Chem* 276, 31851-7 (2001)
166. Liang M. H., T. Geisbert, Y. Yao, S. H. Hinrichs, C. Z. Giam: Human T-lymphotropic virus type 1 oncoprotein tax promotes S-phase entry but blocks mitosis. *J Virol* 76, 4022-33 (2002)
167. Hirai H., M. F. Roussel, J. Y. Kato, R. A. Ashmun, C. J. Sherr: Novel INK4 proteins, p19 and p18, are specific inhibitors of the cyclin D-dependent kinases CDK4 and CDK6. *Mol Cell Biol* 15, 2672-81 (1995)
168. Chan F. K., J. Zhang, L. Cheng, D. N. Shapiro, A. Winoto: Identification of human and mouse p19, a novel CDK4 and CDK6 inhibitor with homology to p16ink4. *Mol Cell Biol* 15, 2682-8 (1995)
169. Guan K. L., C. W. Jenkins, Y. Li, M. A. Nichols, X. Wu, C. L. O'Keefe, A. G. Matera, Y. Xiong: Growth suppression by p18, a p16INK4/MTS1- and p14INK4B/MTS2-related CDK6 inhibitor, correlates with wild-type pRb function. *Genes Dev* 8, 2939-52 (1994)
170. Serrano M., G. J. Hannon, D. Beach: A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 366, 704-7 (1993)
171. Polyak K., M. H. Lee, H. Erdjument-Bromage, A. Koff, J. M. Roberts, P. Tempst, J. Massague: Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* 78, 59-66 (1994)
172. Polyak K., J. Y. Kato, M. J. Solomon, C. J. Sherr, J. Massague, J. M. Roberts, A. Koff: p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev* 8, 9-22 (1994)
173. Matsuoka S., M. C. Edwards, C. Bai, S. Parker, P. Zhang, A. Baldini, J. W. Harper, S. J. Elledge: p57KIP2, a structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. *Genes Dev* 9, 650-62 (1995)
174. Xiong Y., G. J. Hannon, H. Zhang, D. Casso, R. Kobayashi, D. Beach: p21 is a universal inhibitor of cyclin kinases. *Nature* 366, 701-4 (1993)
175. Suzuki T., T. Narita, M. Uchida-Toita, M. Yoshida: Down-regulation of the INK4 family of cyclin-dependent kinase inhibitors by tax protein of HTLV-1 through two distinct mechanisms. *Virology* 259, 384-91 (1999)
176. Iwanaga R., K. Ohtani, T. Hayashi, M. Nakamura: Molecular mechanism of cell cycle progression induced by the oncogene product Tax of human T-cell leukemia virus type I. *Oncogene* 20, 2055-67 (2001)
177. Cereseto A., R. Washington Parks, E. Rivadeneira, G. Franchini: Limiting amounts of p27Kip1 correlates with constitutive activation of cyclin E-CDK2 complex in HTLV-I-transformed T-cells. *Oncogene* 18, 2441-50 (1999)
178. Cereseto A., F. Diella, J. C. Mulloy, A. Cara, P. Michieli, R. Grassmann, G. Franchini, M. E. Klotman: p53 functional impairment and high p21waf1/cip1 expression in human T-cell lymphotropic/leukemia virus type I-transformed T cells. *Blood* 88, 1551-60 (1996)
179. de la Fuente C., F. Santiago, S. Y. Chong, L. Deng, T. Mayhood, P. Fu, D. Stein, T. Denny, F. Coffman, N. Azimi, R. Mahieux, F. Kashanchi: Overexpression of p21(waf1) in human T-cell lymphotropic virus type 1-

infected cells and its association with cyclin A/cdk2. *J Virol* 74, 7270-83 (2000)
180. Akagi T., H. Ono, K. Shimotohno: Expression of cell-cycle regulatory genes in HTLV-I infected T-cell lines: possible involvement of Tax1 in the altered expression of cyclin D2, p18Ink4 and p21Waf1/Cip1/Sdi1. *Oncogene* 12, 1645-52 (1996)
181. Ng P. W., H. Iha, Y. Iwanaga, M. Bittner, Y. Chen, Y. Jiang, G. Gooden, J. M. Trent, P. Meltzer, K. T. Jeang, S. L. Zeichner: Genome-wide expression changes induced by HTLV-1 Tax: evidence for MLK-3 mixed lineage kinase involvement in Tax-mediated NF-kappaB activation. *Oncogene* 20, 4484-96 (2001)
182. Cheng M., P. Olivier, J. A. Diehl, M. Fero, M. F. Roussel, J. M. Roberts, C. J. Sherr: The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *Embo J* 18, 1571-83 (1999)
183. LaBaer J., M. D. Garrett, L. F. Stevenson, J. M. Slingerland, C. Sandhu, H. S. Chou, A. Fattaey, E. Harlow: New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 11, 847-62 (1997)
184. Sherr C. J., J. M. Roberts: CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 13, 1501-12 (1999)
185. Zhang H., G. J. Hannon, D. Casso, D. Beach: p21 is a component of active cell cycle kinases. *Cold Spring Harb Symp Quant Biol* 59, 21-9 (1994)
186. Zhang H., G. J. Hannon, D. Beach: p21-containing cyclin kinases exist in both active and inactive states. *Genes Dev* 8, 1750-8 (1994)
187. Kehn K., L. Deng, C. de La Fuente, K. Strouss, K. Wu, A. Maddukuri, S. Baylor, R. Rufner, A. Pumfery, M. E. Bottazzi, F. Kashanchi: The role of cyclin D2 and p21/waf1 in human T-cell leukemia virus type 1 infected cells. *Retrovirology*. Accepted
188. Helt A. M., D. A. Galloway: Mechanisms by which DNA tumor virus oncoproteins target the Rb family of pocket proteins. *Carcinogenesis* 24, 159-69 (2003)
189. Wang L., L. Deng, K. Wu, C. de la Fuente, D. Wang, K. Kehn, A. Maddukuri, S. Baylor, F. Santiago, E. Agbottah, S. Trigon, M. Morange, R. Mahieux, F. Kashanchi: Inhibition of HTLV-1 transcription by cyclin dependent kinase inhibitors. *Mol Cell Biochem* 237, 137-53 (2002)
190. Geng Y., Q. Yu, E. Sicinska, M. Das, J. E. Schneider, S. Bhattacharya, W. M. Rideout, R. T. Bronson, H. Gardner, P. Sicinski: Cyclin E ablation in the mouse. *Cell* 114, 431-43 (2003)
191. Berthet C., E. Aleem, V. Coppola, L. Tessarollo, P. Kaldis: Cdk2 knockout mice are viable. Curr Biol 13, 1775-85 (2003)
192. Lawrence D. A.: Transforming growth factor-beta: a general review. *Eur Cytokine Netw* 7, 363-74 (1996)
193. Ewen M. E., H. K. Sluss, L. L. Whitehouse, D. M. Livingston: TGF beta inhibition of Cdk4 synthesis is linked to cell cycle arrest. *Cell* 74, 1009-20 (1993)
194. Geng Y., R. A. Weinberg: Transforming growth factor beta effects on expression of G1 cyclins and cyclin-dependent protein kinases. *Proc Natl Acad Sci U S A* 90, 10315-9 (1993)
195. Hannon G. J., D. Beach: p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* 371, 257-61 (1994)
196. Miyazaki M., R. Ohashi, T. Tsuji, K. Mihara, E. Gohda, M. Namba: Transforming growth factor-beta 1 stimulates or inhibits cell growth via down- or up-regulation of p21/Waf1. *Biochem Biophys Res Commun* 246, 873-80 (1998)
197. Ducos K., B. Panterne, N. Fortunel, A. Hatzfeld, M. N. Monier, J. Hatzfeld: p21(cip1) mRNA is controlled by endogenous transforming growth factor-beta1 in quiescent human hematopoietic stem/progenitor cells. *J Cell Physiol* 184, 80-5 (2000)
198. Pierelli L., M. Marone, G. Bonanno, S. Mozzetti, S. Rutella, R. Morosetti, C. Rumi, S. Mancuso, G. Leone, G. Scambia: Modulation of bcl-2 and p27 in human primitive proliferating hematopoietic progenitors by autocrine TGF-beta1 is a cell cycle-independent effect and influences their hematopoietic potential. *Blood* 95, 3001-9 (2000)
199. Hollsberg P., L. J. Ausubel, D. A. Hafler: Human T cell lymphotropic virus type I-induced T cell activation. Resistance to TGF-beta 1-induced suppression. *J Immunol* 153, 566-73 (1994)
200. Lee D. K., B. C. Kim, J. N. Brady, K. T. Jeang, S. J. Kim: Human T-cell lymphotropic virus type 1 tax inhibits transforming growth factor-beta signaling by blocking the association of Smad proteins with Smad-binding element. *J Biol Chem* 277, 33766-75 (2002)
201. Mori N., M. Morishita, T. Tsukazaki, C. Z. Giam, A. Kumatori, Y. Tanaka, N. Yamamoto: Human T-cell leukemia virus type I oncoprotein Tax represses Smad-dependent transforming growth factor beta signaling through interaction with CREB-binding protein/p300. *Blood* 97, 2137-44 (2001)
202. Arnulf B., A. Villemain, C. Nicot, E. Mordelet, P. Charneau, J. Kersual, Y. Zermati, A. Mauviel, A. Bazarbachi, O. Hermine: Human T-cell lymphotropic virus oncoprotein Tax represses TGF-beta 1 signaling in human T cells via c-Jun activation: a potential mechanism of HTLV-I leukemogenesis. *Blood* 100, 4129-38 (2002)
203. Kim S. J., J. H. Kehrl, J. Burton, C. L. Tendler, K. T. Jeang, D. Danielpour, C. Thevenin, K. Y. Kim, M. B. Sporn, A. B. Roberts: Transactivation of the transforming growth factor beta 1 (TGF-beta 1) gene by human T lymphotropic virus type 1 tax: a potential mechanism for the increased production of TGF-beta 1 in adult T cell leukemia. *J Exp Med* 172, 121-9 (1990)
204. Kim S. J., T. S. Winokur, H. D. Lee, D. Danielpour, K. Y. Kim, A. G. Geiser, L. S. Chen, M. B. Sporn, A. B. Roberts, G. Jay: Overexpression of transforming growth factor-beta in transgenic mice carrying the human T-cell lymphotropic virus type I tax gene. *Mol Cell Biol* 11, 5222-8 (1991)
205. Kim S. J., J. Letterio: Transforming growth factor-beta signaling in normal and malignant hematopoiesis. *Leukemia* 17, 1731-7 (2003)
206. Gatza M. L., J. C. Watt, S. J. Marriott: Cellular transformation by the HTLV-I Tax protein, a jack-of-all-trades. *Oncogene* 22, 5141-9 (2003)
207. Reid R. L., P. F. Lindholm, A. Mireskandari, J. Dittmer, J. N. Brady: Stabilization of wild-type p53 in

human T-lymphocytes transformed by HTLV-I. *Oncogene* 8, 3029-36 (1993)
208. Yamato K., T. Oka, M. Hiroi, Y. Iwahara, S. Sugito, N. Tsuchida, I. Miyoshi: Aberrant expression of the p53 tumor suppressor gene in adult T-cell leukemia and HTLV-I-infected cells. *Jpn J Cancer Res* 84, 4-8 (1993)
209. Pise-Masison C. A., K. S. Choi, M. Radonovich, J. Dittmer, S. J. Kim, J. N. Brady: Inhibition of p53 transactivation function by the human T-cell lymphotropic virus type 1 Tax protein. *J Virol* 72, 1165-70 (1998)
210. Ariumi Y., A. Kaida, J. Y. Lin, M. Hirota, O. Masui, S. Yamaoka, Y. Taya, K. Shimotohno: HTLV-1 tax oncoprotein represses the p53-mediated trans-activation function through coactivator CBP sequestration. *Oncogene* 19, 1491-9 (2000)
211. Mulloy J. C., T. Kislyakova, A. Cereseto, L. Casareto, A. LoMonico, J. Fullen, M. V. Lorenzi, A. Cara, C. Nicot, C. Giam, G. Franchini: Human T-cell lymphotropic/leukemia virus type 1 Tax abrogates p53-induced cell cycle arrest and apoptosis through its CREB/ATF functional domain. *J Virol* 72, 8852-60 (1998)
212. Akagi T., H. Ono, N. Tsuchida, K. Shimotohno: Aberrant expression and function of p53 in T-cells immortalized by HTLV-I Tax1. *FEBS Lett* 406, 263-6 (1997)
213. Pise-Masison C. A., M. Radonovich, K. Sakaguchi, E. Appella, J. N. Brady: Phosphorylation of p53: a novel pathway for p53 inactivation in human T-cell lymphotropic virus type 1-transformed cells. *J Virol* 72, 6348-55 (1998)
214. Van P. L., K. W. Yim, D. Y. Jin, G. Dapolito, A. Kurimasa, K. T. Jeang: Genetic evidence of a role for ATM in functional interaction between human T-cell leukemia virus type 1 Tax and p53. *J Virol* 75, 396-407 (2001)
215. Kaida A., Y. Ariumi, Y. Ueda, J. Y. Lin, M. Hijikata, S. Ikawa, K. Shimotohno: Functional impairment of p73 and p51, the p53-related proteins, by the human T-cell leukemia virus type 1 Tax oncoprotein. *Oncogene* 19, 827-30 (2000)
216. Suzuki T., M. Uchida-Toita, M. Yoshida: Tax protein of HTLV-1 inhibits CBP/p300-mediated transcription by interfering with recruitment of CBP/p300 onto DNA element of E-box or p53 binding site. *Oncogene* 18, 4137-43 (1999)
217. Lill N. L., S. R. Grossman, D. Ginsberg, J. DeCaprio, D. M. Livingston: Binding and modulation of p53 by p300/CBP coactivators. *Nature* 387, 823-7 (1997)
218. Pise-Masison C. A., R. Mahieux, H. Jiang, M. Ashcroft, M. Radonovich, J. Duvall, C. Guillerm, J. N. Brady: Inactivation of p53 by human T-cell lymphotropic virus type 1 Tax requires activation of the NF-kappaB pathway and is dependent on p53 phosphorylation. *Mol Cell Biol* 20, 3377-86 (2000)
219. Pise-Masison C. A., R. Mahieux, M. Radonovich, H. Jiang, J. N. Brady: Human T-lymphotropic virus type I Tax protein utilizes distinct pathways for p53 inhibition that are cell type-dependent. *J Biol Chem* 276, 200-5 (2001)
220. Chowdhury I. H., M. Radonovich, R. Mahieux, C. Pise-Masison, S. Muralidhar, J. N. Brady: P53 facilitates degradation of human T-cell leukaemia virus type I Tax-binding protein through a proteasome-dependent pathway. *J Gen Virol* 84, 897-906 (2003)
221. Klein G.: Perspectives in studies of human tumor viruses. *Front Biosci* 7, d268-74 (2002)
222. Seiki M., R. Eddy, T. B. Shows, M. Yoshida: Nonspecific integration of the HTLV provirus genome into adult T-cell leukaemia cells. *Nature* 309, 640-2 (1984)
223. Marriott S. J., F. J. Lemoine, K. T. Jeang: Damaged DNA and miscounted chromosomes: human T cell leukemia virus type I tax oncoprotein and genetic lesions in transformed cells. *J Biomed Sci* 9, 292-8 (2002)
224. Miyake H., T. Suzuki, H. Hirai, M. Yoshida: Trans-activator Tax of human T-cell leukemia virus type 1 enhances mutation frequency of the cellular genome. *Virology* 253, 155-61 (1999)
225. Jeang K. T., S. G. Widen, O. J. t. Semmes, S. H. Wilson: HTLV-I trans-activator protein, tax, is a trans-repressor of the human beta-polymerase gene. *Science* 247, 1082-4 (1990)
226. Philpott S. M., G. C. Buehring: Defective DNA repair in cells with human T-cell leukemia/bovine leukemia viruses: role of tax gene. *J Natl Cancer Inst* 91, 933-42 (1999)
227. Lemoine F. J., S. J. Marriott: Genomic instability driven by the human T-cell leukemia virus type I (HTLV-I) oncoprotein, Tax. *Oncogene* 21, 7230-4 (2002)
228. Kao S. Y., S. J. Marriott: Disruption of nucleotide excision repair by the human T-cell leukemia virus type 1 Tax protein. *J Virol* 73, 4299-304 (1999)
229. Ressler S., G. F. Morris, S. J. Marriott: Human T-cell leukemia virus type 1 Tax transactivates the human proliferating cell nuclear antigen promoter. *J Virol* 71, 1181-90 (1997)
230. Hubscher U., G. Maga, S. Spadari: Eukaryotic DNA polymerases. *Annu Rev Biochem* 71, 133-63 (2002)
231. Yoshida M., T. Suzuki: HTLV type 1 Tax oncoprotein binds to DNA topoisomerase I and inhibits its catalytic activity. *AIDS Res Hum Retroviruses* 16, 1639-45 (2000)
232. Gabet A. S., F. Mortreux, P. Charneau, P. Riou, M. Duc-Dodon, Y. Wu, K. T. Jeang, E. Wattel: Inactivation of hTERT transcription by Tax. *Oncogene* 22, 3734-41 (2003)
233. Blackburn E. H.: The end of the (DNA) line. *Nat Struct Biol* 7, 847-50 (2000)
234. Majone F., K. T. Jeang: Clastogenic effect of the human T-cell leukemia virus type I Tax oncoprotein correlates with unstabilized DNA breaks. *J Biol Chem* 275, 32906-10 (2000)
235. Li R., A. W. Murray: Feedback control of mitosis in budding yeast. *Cell* 66, 519-31 (1991)
236. Jin D. Y., F. Spencer, K. T. Jeang: Human T cell leukemia virus type 1 oncoprotein Tax targets the human mitotic checkpoint protein MAD1. *Cell* 93, 81-91 (1998)
237. Kasai T., Y. Iwanaga, H. Iha, K. T. Jeang: Prevalent loss of mitotic spindle checkpoint in adult T-cell leukemia confers resistance to microtubule inhibitors. *J Biol Chem* 277, 5187-93 (2002)
238. Smits V. A., R. H. Medema: Checking out the G(2)/M transition. *Biochim Biophys Acta* 1519, 1-12 (2001)
239. Pise-Masison C. A., M. Radonovich, R. Mahieux, P. Chatterjee, C. Whiteford, J. Duvall, C. Guillerm, A. Gessain, J. N. Brady: Transcription profile of cells infected with human T-cell leukemia virus type I compared with activated lymphocytes. *Cancer Res* 62, 3562-71 (2002)

240. Liu B., M. H. Liang, Y. L. Kuo, W. Liao, I. Boros, T. Kleinberger, J. Blancato, C. Z. Giam: Human T-lymphotropic virus type 1 oncoprotein tax promotes unscheduled degradation of Pds1p/securin and Clb2p/cyclin B1 and causes chromosomal instability. *Mol Cell Biol* 23, 5269-81 (2003)
241. Nunez G., M. A. Benedict, Y. Hu, N. Inohara: Caspases: the proteases of the apoptotic pathway. *Oncogene* 17, 3237-45 (1998)
242. Tivol E. A., F. Borriello, A. N. Schweitzer, W. P. Lynch, J. A. Bluestone, A. H. Sharpe: Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3, 541-7 (1995)
243. Burlacu A.: Regulation of apoptosis by Bcl-2 family proteins. *J Cell Mol Med* 7, 249-57 (2003)
244. Chlichlia K., G. Moldenhauer, P. T. Daniel, M. Busslinger, L. Gazzolo, V. Schirrmacher, K. Khazaie: Immediate effects of reversible HTLV-1 tax function: T-cell activation and apoptosis. *Oncogene* 10, 269-77 (1995)
245. Chen X., V. Zachar, M. Zdravkovic, M. Guo, P. Ebbesen, X. Liu: Role of the Fas/Fas ligand pathway in apoptotic cell death induced by the human T cell lymphotropic virus type I Tax transactivator. *J Gen Virol* 78 (Pt 12), 3277-85 (1997)
246. Chlichlia K., M. Busslinger, M. E. Peter, H. Walczak, P. H. Krammer, V. Schirrmacher, K. Khazaie: ICE-proteases mediate HTLV-I Tax-induced apoptotic T-cell death. *Oncogene* 14, 2265-72 (1997)
247. Rivera-Walsh I., M. Waterfield, G. Xiao, A. Fong, S. C. Sun: NF-kappaB signaling pathway governs TRAIL gene expression and human T-cell leukemia virus-I Tax-induced T-cell death. *J Biol Chem* 276, 40385-8 (2001)
248. Nicot C., R. Harrod: Distinct p300-responsive mechanisms promote caspase-dependent apoptosis by human T-cell lymphotropic virus type 1 Tax protein. *Mol Cell Biol* 20, 8580-9 (2000)
249. Abraham E.: NF-kappaB activation. *Crit Care Med* 28, N100-4 (2000)
250. Ueda S., H. Masutani, H. Nakamura, T. Tanaka, M. Ueno, J. Yodoi: Redox control of cell death. *Antioxid Redox Signal* 4, 405-14 (2002)
251. Los M., K. Khazaie, K. Schulze-Osthoff, P. A. Baeuerle, V. Schirrmacher, K. Chlichlia: Human T cell leukemia virus-I (HTLV-I) Tax-mediated apoptosis in activated T cells requires an enhanced intracellular prooxidant state. *J Immunol* 161, 3050-5 (1998)
252. Chlichlia K., M. Los, K. Schulze-Osthoff, L. Gazzolo, V. Schirrmacher, K. Khazaie: Redox events in HTLV-1 Tax-induced apoptotic T-cell death. *Antioxid Redox Signal* 4, 471-7 (2002)
253. Tsukahara T., M. Kannagi, T. Ohashi, H. Kato, M. Arai, G. Nunez, Y. Iwanaga, N. Yamamoto, K. Ohtani, M. Nakamura, M. Fujii: Induction of Bcl-x(L) expression by human T-cell leukemia virus type 1 Tax through NF-kappaB in apoptosis-resistant T-cell transfectants with Tax. *J Virol* 73, 7981-7 (1999)
254. Portis T., J. C. Harding, L. Ratner: The contribution of NF-kappa B activity to spontaneous proliferation and resistance to apoptosis in human T-cell leukemia virus type 1 Tax-induced tumors. *Blood* 98, 1200-8 (2001)
255. Kawakami A., T. Nakashima, H. Sakai, S. Urayama, S. Yamasaki, A. Hida, M. Tsuboi, H. Nakamura, H. Ida, K. Migita, Y. Kawabe, K. Eguchi: Inhibition of caspase cascade by HTLV-I tax through induction of NF-kappaB nuclear translocation. *Blood* 94, 3847-54 (1999)
256. Nicot C., R. Mahieux, S. Takemoto, G. Franchini: Bcl-X(L) is up-regulated by HTLV-I and HTLV-II *in vitro* and in *ex vivo* ATLL samples. *Blood* 96, 275-81 (2000)
257. Levine A. J.: p53, the cellular gatekeeper for growth and division. *Cell* 88, 323-31 (1997)
258. el-Deiry W. S., T. Tokino, V. E. Velculescu, D. B. Levy, R. Parsons, J. M. Trent, D. Lin, W. E. Mercer, K. W. Kinzler, B. Vogelstein: WAF1, a potential mediator of p53 tumor suppression. *Cell* 75, 817-25 (1993)
259. Kawata S., Y. Ariumi, K. Shimotohno: p21(Waf1/Cip1/Sdi1) Prevents Apoptosis as Well as Stimulates Growth in Cells Transformed or Immortalized by Human T-Cell Leukemia Virus Type 1-Encoded Tax. *J Virol* 77, 7291-9 (2003)
260. Copeland K. F., A. G. Haaksma, J. Goudsmit, P. H. Krammer, J. L. Heeney: Inhibition of apoptosis in T cells expressing human T cell leukemia virus type I Tax. *AIDS Res Hum Retroviruses* 10, 1259-68 (1994)
261. Brauweiler A., J. E. Garrus, J. C. Reed, J. K. Nyborg: Repression of bax gene expression by the HTLV-1 Tax protein: implications for suppression of apoptosis in virally infected cells. *Virology* 231, 135-40 (1997)
262. Nicot C., T. Astier-Gin, B. Guillemain: Activation of Bcl-2 expression in human endothelial cells chronically expressing the human T-cell lymphotropic virus type I. *Virology* 236, 47-53 (1997)
263. Harhaj E. W., L. Good, G. Xiao, S. C. Sun: Gene expression profiles in HTLV-I-immortalized T cells: deregulated expression of genes involved in apoptosis regulation. *Oncogene* 18, 1341-9 (1999)
264. de la Fuente C., L. Wang, D. Wang, L. Deng, K. Wu, H. Li, L. D. Stein, T. Denny, F. Coffman, K. Kehn, S. Baylor, A. Maddukuri, A. Pumfery, F. Kashanchi: Paradoxical effects of a stress signal on pro- and anti-apoptotic machinery in HTLV-1 Tax expressing cells. *Mol Cell Biochem* 245, 99-113 (2003)
265. Miyoshi I., S. Yoshimoto, M. Fujishita, I. Kubonishi, H. Taguchi, Y. Ohtsuki: Infectious transmission of human T-cell leukemia virus to animals. *Princess Takamatsu Symp* 15, 121-7 (1984)
266. Akagi T., I. Takeda, T. Oka, Y. Ohtsuki, S. Yano, I. Miyoshi: Experimental infection of rabbits with human T-cell leukemia virus type I. *Jpn J Cancer Res* 76, 86-94 (1985)
267. Seto A., M. Kawanishi, S. Matsuda, K. Ogawa: Seronegative virus carriers in the infection of rabbits with human T lymphotropic virus type I. *J Exp Med* 168, 2409-14 (1988)
268. Seto A., M. Kawanishi, S. Matsuda, K. Ogawa, T. Eguchi, I. Miyoshi: Induction of preleukemic stage of adult T cell leukemia-like disease in rabbits. *Jpn J Cancer Res* 78, 1150-5 (1987)
269. Seto A., M. Kawanishi, S. Matsuda, K. Ogawa, I. Miyoshi: Adult T cell leukemia-like disease experimentally induced in rabbits. *Jpn J Cancer Res* 79, 335-41 (1988)
270. Cockerell G. L., M. Lairmore, B. De, J. Rovnak, T. M. Hartley, I. Miyoshi: Persistent infection of rabbits with HTLV-I: patterns of anti-viral antibody reactivity and detection of virus by gene amplification. *Int J Cancer* 45, 127-30 (1990)

271. Zhao T. M., M. A. Robinson, S. Sawasdikosol, R. M. Simpson, T. J. Kindt: Variation in HTLV-I sequences from rabbit cell lines with diverse *in vivo* effects. *Virology* 195, 271-4 (1993)
272. Sawasdikosol S., B. F. Hague, T. M. Zhao, F. S. Bowers, R. M. Simpson, M. Robinson, T. J. Kindt: Selection of rabbit CD4- CD8- T cell receptor-gamma/delta cells by *in vitro* transformation with human T lymphotropic virus-I. *J Exp Med* 178, 1337-45 (1993)
273. Leno M., R. M. Simpson, F. S. Bowers, T. J. Kindt: Human T lymphocyte virus 1 from a leukemogenic cell line mediates *in vivo* and *in vitro* lymphocyte apoptosis. *J Exp Med* 181, 1575-80 (1995)
274. Simpson R. M., M. Leno, B. S. Hubbard, T. J. Kindt: Cutaneous manifestations of human T cell leukemia virus type I infection in an experimental model. *J Infect Dis* 173, 722-6 (1996)
275. Simpson R. M., T. M. Zhao, B. S. Hubbard, S. Sawasdikosol, T. J. Kindt: Experimental acute adult T cell leukemia-lymphoma is associated with thymic atrophy in human T cell leukemia virus type I infection. *Lab Invest* 74, 696-710 (1996)
276. Taguchi H., T. Sawada, A. Fukushima, J. Iwata, Y. Ohtsuki, H. Ueno, I. Miyoshi: Bilateral uveitis in a rabbit experimentally infected with human T-lymphotropic virus type I. *Lab Invest* 69, 336-9 (1993)
277. Seto A., T. Isono, K. Ogawa: Infection of inbred rabbits with cell-free HTLV-I. *Leuk Res* 15, 105-10 (1991)
278. Hinrichs S. H., M. Nerenberg, R. K. Reynolds, G. Khoury, G. Jay: A transgenic mouse model for human neurofibromatosis. *Science* 237, 1340-3 (1987)
279. Nerenberg M., S. H. Hinrichs, R. K. Reynolds, G. Khoury, G. Jay: The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. *Science* 237, 1324-9 (1987)
280. Green J. E., C. G. Begley, D. K. Wagner, T. A. Waldmann, G. Jay: trans activation of granulocyte-macrophage colony-stimulating factor and the interleukin-2 receptor in transgenic mice carrying the human T-lymphotropic virus type 1 tax gene. *Mol Cell Biol* 9, 4731-7 (1989)
281. Nerenberg M. I., C. A. Wiley: Degeneration of oxidative muscle fibers in HTLV-1 tax transgenic mice. *Am J Pathol* 135, 1025-33 (1989)
282. Iwakura Y., M. Tosu, E. Yoshida, M. Takiguchi, K. Sato, I. Kitajima, K. Nishioka, K. Yamamoto, T. Takeda, M. Hatanaka, *et al.*: Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. *Science* 253, 1026-8 (1991)
283. Habu K., J. Nakayama-Yamada, M. Asano, S. Saijo, K. Itagaki, R. Horai, H. Yamamoto, T. Sekiguchi, T. Nosaka, M. Hatanaka, Y. Iwakura: The human T cell leukemia virus type I-tax gene is responsible for the development of both inflammatory polyarthropathy resembling rheumatoid arthritis and noninflammatory ankylotic arthropathy in transgenic mice. *J Immunol* 162, 2956-63 (1999)
284. Miyazato A., K. Kawakami, Y. Iwakura, A. Saito: Chemokine synthesis and cellular inflammatory changes in lungs of mice bearing p40tax of human T-lymphotropic virus type 1. *Clin Exp Immunol* 120, 113-24 (2000)
285. Kawakami K., A. Miyazato, Y. Iwakura, A. Saito: Induction of lymphocytic inflammatory changes in lung interstitium by human T lymphotropic virus type I. *Am J Respir Crit Care Med* 160, 995-1000 (1999)
286. Coscoy L., D. Gonzalez-Dunia, F. Tangy, S. Syan, M. Brahic, S. Ozden: Molecular mechanism of tumorigenesis in mice transgenic for the human T cell leukemia virus Tax gene. *Virology* 248, 332-41 (1998)
287. Ruddle N. H., C. B. Li, W. C. Horne, P. Santiago, N. Troiano, G. Jay, M. Horowitz, R. Baron: Mice transgenic for HTLV-I LTR-tax exhibit tax expression in bone, skeletal alterations, and high bone turnover. *Virology* 197, 196-204 (1993)
288. Fukumoto S., T. Matsumoto, K. Ikeda, T. Yamashita, T. Watanabe, K. Yamaguchi, T. Kiyokawa, K. Takatsuki, N. Shibuya, E. Ogata: Clinical evaluation of calcium metabolism in adult T-cell leukemia/lymphoma. *Arch Intern Med* 148, 921-5 (1988)
289. Nishioka K.: HTLV-I arthropathy and Sjogren syndrome. *J Acquir Immune Defic Syndr Hum Retrovirol* 13 Suppl 1, S57-62 (1996)
290. Ozden S., L. Coscoy, D. Gonzalez-Dunia: HTLV-I transgenic models: an overview. *J Acquir Immune Defic Syndr Hum Retrovirol* 13 Suppl 1, S154-61 (1996)
291. Hall A. P., J. Irvine, K. Blyth, E. R. Cameron, D. E. Onions, M. E. Campbell: Tumours derived from HTLV-I tax transgenic mice are characterized by enhanced levels of apoptosis and oncogene expression. *J Pathol* 186, 209-14 (1998)
292. Pawson R., T. F. Schulz, E. Matutes, D. Catovsky: The human T-cell lymphotropic viruses types I/II are not involved in T prolymphocytic leukemia and large granular lymphocytic leukemia. *Leukemia* 11, 1305-11 (1997)
293. Ratner L., T. Portis, M. Robek, J. Harding, W. Grossman: Studies of the immortalizing activity of HTLV type 1 Tax, using an infectious molecular clone and transgenic mice. *AIDS Res Hum Retroviruses* 16, 1647-51 (2000)
294. Portis T., W. J. Grossman, J. C. Harding, J. L. Hess, L. Ratner: Analysis of p53 inactivation in a human T-cell leukemia virus type 1 Tax transgenic mouse model. *J Virol* 75, 2185-93 (2001)
295. Feuer G., J. A. Zack, W. J. Harrington, Jr., R. Valderama, J. D. Rosenblatt, W. Wachsman, S. M. Baird, I. S. Chen: Establishment of human T-cell leukemia virus type I T-cell lymphomas in severe combined immunodeficient mice. *Blood* 82, 722-31 (1993)
296. Feuer G., S. A. Stewart, S. M. Baird, F. Lee, R. Feuer, I. S. Chen: Potential role of natural killer cells in controlling tumorigenesis by human T-cell leukemia viruses. *J Virol* 69, 1328-33 (1995)
297. Stewart S. A., G. Feuer, A. Jewett, F. V. Lee, B. Bonavida, I. S. Chen: HTLV-1 gene expression in adult T-cell leukemia cells elicits an NK cell response *in vitro* and correlates with cell rejection in SCID mice. *Virology* 226, 167-75 (1996)
298. Liu Y., K. Dole, J. R. Stanley, V. Richard, T. J. Rosol, L. Ratner, M. Lairmore, G. Feuer: Engraftment and tumorigenesis of HTLV-1 transformed T cell lines in SCID/bg and NOD/SCID mice. *Leuk Res* 26, 561-7 (2002)
299. Tomaru U., H. Ikeda, O. Ohya, M. Abe, T. Kasai, I. Yamasita, K. Morita, A. Wakisaka, T. Yoshiki: Human T lymphocyte virus type I-induced myeloneuropathy in rats: implication of local activation of the pX and tumor necrosis

factor-alpha genes in pathogenesis. *J Infect Dis* 174, 318-23 (1996)
300. Yoshiki T., H. Ikeda, U. Tomaru, O. Ohya, T. Kasai, I. Yamashita, K. Morita, H. Yamazaki, A. Ishizu, Y. Nakamaru, K. Kikuchi, S. Tanaka, A. Wakisaka: Models of HTLV-I-induced diseases. Infectious transmission of HTLV-I in inbred rats and HTVL-I env-pX transgenic rats. *Leukemia* 11 Suppl 3, 245-6 (1997)
301. Ohya O., U. Tomaru, I. Yamashita, T. Kasai, K. Morita, H. Ikeda, A. Wakisaka, T. Yoshiki: HTLV-I induced myeloneuropathy in WKAH rats: apoptosis and local activation of the HTLV-I pX and TNF-alpha genes implicated in the pathogenesis. *Leukemia* 11 Suppl 3, 255-7 (1997)
302. Senderowicz A. M.: Small-molecule cyclin-dependent kinase modulators. *Oncogene* 22, 6609-20 (2003)
303. Lenz H. J.: Clinical update: proteasome inhibitors in solid tumors. *Cancer Treat Rev* 29 Suppl 1, 41-8 (2003)

Key Words: HTLV-1, Oncogenesis, Tax, ATL, Cell Cycle, Transcription, Accessory Proteins, Apoptosis, Review

Send correspondence to: Fatah Kashanchi, Ph.D., The George Washington University, 2300 Eye St., NW, Ross Hall, Room 551, Washington DC, 20037, USA, Tel: 202-994-1781, Fax: 202-994-1780, E-mail: bcmfxk@gwumc.edu