Life, Death, and Tax: Role of HTLV-I Oncoprotein in Genetic Instability and Cellular Transformation*

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Human T-cell leukemia virus type I (HTLV-I)* causes adult T-cell leukemia (ATL) (1–3). The virus is also associated with a neuropathy/myelopathy termed HTLV-associated myelopathy and tropical spastic paraparesis. ATL develops in 2–5% of HTLV-I-infected individuals after a long latent period, suggesting a multistage process of transformation occurs when both gatekeeper and caretaker functions are abrogated. Using HTLV-I as a model, we review in a Minireview the T-cell specific, granzyme B promoter (9). Leukemia has been found in mice transgenic for Tax expressed from the T-cell specific, granzyme B promoter (9).

**The Molecular Biology of HTLV-I**

HTLV-I belongs to the Deltaretrovirus genera of the Orthoretrovirinae family. In vivo, the virus has a tropism for CD4+ T-cells (11) although CD8+ T-cells may also serve as a reservoir (12). HTLV-I infection is primarily transmitted via cell-cell contact (13, 14). Recently, the human Glut1 glucose transporter has been identified as a receptor for infection by cell-free virus (15). The proviral genome of HTLV-I is roughly 9 kb, and like other retroviruses, contains two LTRs flanking structural genes encoding Gag, Pol, and Env (Fig. 1). An additional region located between env and the 3'-LTR, known as the pX region, encodes accessory proteins. The pX region has four partially overlapping reading frames (ORF, Fig. 1), of which ORF IV encodes Tax.

Tax is predominantly a nuclear phosphoprotein (16), which can shuttle into the cytoplasm using a nuclear export signal (17). The mechanism of this shuttling is unclear; however, recent findings that Tax binds tristetraprol (18) and that tristetraprol associates with nucleoprotein Nup214 (19) raise the possibility that tristetraprol may serve as a possible nucleocytoplasmic transporter for Tax. Nevertheless, the primary nuclear activity of Tax is to modulate transcription from the HTLV-I LTR (20–22) and cellular promoters including those for IL-2, IL-13, IL-15, IL-2R, c-Fos, and granulocyte macrophage colony-stimulating factor (23–30) among others. Indeed the breadth of Tax’s transcriptional reprogramming of host cell genes was verified by DNA array studies which showed that of 2000 assayed genes the expression profiles of ~300 were significantly altered (31). Tax influences so many promoters through its capacity to act in four discrete signaling pathways: CREB/ATF (reviewed in Ref. 32); NF-κB (reviewed in Ref. 33); AP-1 (34); and SRP (35). These Tax signaling cascades are discussed in greater detail elsewhere (36).

**Tax and Cell Cycle Progression**

In the course of transforming cells, viral oncoproteins such as E1A, HPV E7, and SV40 T Ag profoundly dysregulate cell cycle controls (37–39). Transition from one phase of the cell cycle to the next is normally governed by cyclin-dependent kinases (CDKs) partnered with cyclins. These CDK-cyclin complexes are in turn modulated by phosphorylation mediated through CDK-activating kinases and phosphatases, and through physical sequestration by CDK inhibitory proteins (reviewed in Refs. 40 and 41). An important cell cycle control resides at the transition from G1 to S, which is substantially governed by the retinoblastoma tumor suppressor (Rb) (42, 43). At this juncture, D- and E-cyclins with partner CDKs (reviewed in Refs. 40, 41, and 44) converge to phosphorylate Rb. Hypophosphorylated Rb sequesters and inactivates E2F factors, which are needed for the expression of genes (such as dihydrofolate reductase, DNA polymerase α, and cyclins) that are critical for S phase events (reviewed in Ref. 45). Hyperphosphorylated Rb releases E2F, activates E2F-responsive genes, and secures the passage of cells from G1 into S (45–48). Thus, regulation of Rb phosphorylation by cyclin-Cdk and CDK inhibitory proteins such as p16INK4a, p21CIP1/WAF1, and p27KIP1 is a critical mechanism for influencing gatekeeper function (37).

Tax reprograms G1 to S progression through multiple mechanistic ways (i.e., direct protein-protein binding, transcriptional induction/repression, and post-translational modification such as phosphorylation). Fig. 1B summarizes several key cell cycle factors that have been experimentally shown to be influenced by Tax. For instance, Tax can directly bind p16INK4a, Cdc2, pre-IL-16, and Cdk4 (49–56). On the other hand, p18INK4c (53, 57), CyaA (58), CyaC (53), Cdc2 (31, 51–55, 60), CyeF (51), Cdk2 (51), p21CIP1/WAF1 (53, 54, 59–63), and E2F (64–66) are regulated by Tax via transcriptional induction/repression (see Fig. 1B). Finally, Tax via an unknown mechanism influences the phosphorylation of Cdc2 (65). To properly consider this complex pattern of interactions, one should appreciate that the context of Tax’s up- or down-regulation matters. An instructive example is presented by Tax-p21CIP1/WAF1 interaction. Various studies agree that p21CIP1/WAF1 levels are significantly elevated in Tax-expressing cells (53, 54, 61–63). However, depending on whether p21CIP1/WAF1 complexes with Cdc2/Cdk2 or CyaA/Cdk2, it has been noted that the resulting ternary complex either promotes or inhibits G1/S progression (67–69). These observations, if correct, help to explain seemingly opposing effects of Tax on Cdc2 (up-regulated (31, 51–55)) and CyaA (down-regulated (58)) transcription. Indeed, enhanced transcription of Cdc2 in the face of repressed transcription of

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* This minireview will be reprinted in the 2004 Minireview Compendium, which will be available in January, 2005.

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‡ The abbreviations used are: HTLV-I, human T-cell leukemia virus type I; ATL, adult T-cell leukemia; LTR, long terminal repeat; IL, interleukin; CDK, cyclin-dependent kinase; BER, base excision repair; NER, nucleotide excision repair; HPV, human papilloma virus; Dihyd, dihydrofolate reductase, DNA polymerase α, and cyclins) that are critical for S phase events (reviewed in Ref. 45). Hyperphosphorylated Rb releases E2F, activates E2F-responsive genes, and secures the passage of cells from G1 into S (45–48). Thus, regulation of Rb phosphorylation by cyclin-Cdk and CDK inhibitory proteins such as p16INK4a, p21CIP1/WAF1, and p27KIP1 is a critical mechanism for influencing gatekeeper function (37).

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A

	

\[\text{LTR} | \text{GAG} | \text{POL} | \text{ENV} | \text{R-U5} \]

- p19
- p21
- p30
- pM
- rex
- tx

\[\text{protease} \]

\[\text{polymerase} \]

B

\[\text{Tax interaction with cell cycle factors} \]

| Gene Product | Activity | References |
|--------------|----------|------------|
| cyc A        | transcriptional repression | Köhler et al. 2001 (58) |
| cyc C        | transcriptional activation | Ng et al. 2001 (51) |
| cyc D2       | transcriptional activation and direct binding | Iwagawa et al. 2001 (51) |
|              |          | Akagi et al. 1996 (53) |
|              |          | Santiago et al. 1994 (60) |
|              |          | Huang et al. 2003 (52) |
|              |          | Ng et al. 2001 (51) |
|              |          | Haller et al. 2002 (55) |
| cyc D3       | phosphorylation | Neve et al. 1997 (65) |
| cdc 2        | transcriptional activation | Iwagawa et al. 2001 (51) |
| cdc 4        | transcriptional activation | Iwagawa et al. 2001 (51) |
| p16\[^{src}\] | inactivation by direct binding | Suzuki et al. 1998 (49) |
|              |          | Low et al. 1997 (56) |
| p18\[^{src}\] | transcriptional repression | Szankasi et al. 1999 (57) |
|              |          | Akagi et al. 1996 (55) |
| p21\[^{wall}\] | transcriptional activation | Creazzo et al. 1996 (57) |
|              |          | Akagi et al. 1996 (53) |
|              |          | dela Fuente 2000 (54) |
|              |          | Kawai et al. 2003 (61) |
|              |          | Chewburry 2003 (62) |
|              |          | Schavinsky-Khrapatsky et al. 2003 (63) |
| E2F          | transcriptional activation | Lemann et al. 1998 (64) |
|              |          | Nouvet et al. 1997 (65) |
|              |          | Obinata et al. 2000 (66) |
| pro-IL-16    | inactivation by direct binding | Wilson et al. 2003 (56) |

**FIG. 1. HTLV-I Tax interacts with many cell cycle factors**. A, genome organization of HTLV-I with an enlarged presentation of the Tax-encoding pX region. B, a tabular summary of some of the cell cycle factors that have been found to interact with Tax.

Tax effects on cell cycle progression are complex. Tax is known to inhibit the transcriptional activity of p16\[^{src}\] and p18\[^{src}\], two cell cycle inhibitors involved in the G1/S transition. Tax also inhibits the activity of the E2F transcriptional activator, which is essential for cell cycle progression. These interactions suggest that Tax can subvert normal cell cycle control mechanisms.

**Tax and Structurally Damaged Chromosomes**

Cancer is a genetic disease. It is estimated that cancer cells can contain more than 100,000 discrete mutations (77). All cancers can be broadly divided into two groups (reviewed in Ref. 78): those arising from loss of DNA repair function (and therefore have structurally damaged chromosomes) and those with chromosomal instability (and therefore have polyploidy and/or aneuploidy). Clastogenic DNA damage is frequently found in HTLV-I-infected cells (79) and cells transfected to express Tax (80) (Fig. 2A). Clastogenic changes (point mutations, deletions, substitutions, translocations) arise and persist when defects in DNA repair mechanisms co-exist in a cell with a loss of checkpoint functions that would normally eliminate damaged DNA.

**FIG. 2. Tax causes chromosomal mis-segregation and chromosomal breakage and fusion events.** A-C, examples of monkey fibroblast cells that were transfected with Tax and stained 48 h later with propidium iodide and anti-kinetochore antisera. Arrows point to small aberrant sacules of DNA, commonly termed micronuclei. Intensely stained dots within micronuclei indicate the presence of kinetochores, which reflect inappropriate segregation of centromere-containing chromosomes. D, an example of a chromosome from Tax-expressing cells that exhibit multiple breakage and fusion events. Bright interstitial dots in the chromosome represent in situ hybridization with a telomere-specific probe. The six telomere spots indicate that this chimeric chromosome has undergone a minimum of two breakage and fusion events.

All cells acquire DNA damage at a low frequency as they transit the cell cycle. Several mechanisms, including base excision repair (BER), nucleotide excision repair (NER), recombination, and direct repair of nicks by DNA ligation act to correct genetic mistakes. In 1990, the first clue that HTLV-I subverts cellular DNA repair came from the finding that Tax repressed the expression of DNA polymerase β, an enzyme involved in BER (81). Subsequently, reduced BER activity was confirmed in HTLV-I, HTLV-II, and bovine leukemia virus-transformed cells (82). Next, Tax was found to suppress the NER normally observed following UV irradiation of cells (83). NER requires DNA polymerases δ and ε and uses proliferating cell nuclear antigen (PCNA) as a cofactor. Excessive PCNA can prompt DNA polymerase δ to synthesize inappropriately new DNA past template lesions, resulting in nucleotide misincorporation (84). Tax is believed to inhibit NER through its transcriptional up-regulation of PCNA (85); this inhibition of NER also depends, in part, on Tax's inactivation of p53 function (71-74).

There is no evidence that Tax interferes with DNA ligation (86) or DNA recombination. However, recent data suggest that Tax represses the expression of human telomerase (hTert) (87). Repression of telomerase is significant because the telomeric repeats of chromosomes normally prevent aberrant end-to-end fusions (Fig. 2B) and protect the ends from degradation by exonucleases. Furthermore, de novo double-stranded breaks in chromosomes can also be stabilized by the transient addition of telomeric repeats (88-90). Indeed, we have documented that Tax prevents such addition of telomeric repeats to new double-stranded breaks (91) and in this way potentially interferes with a protective mechanism used to prevent inappropriate breakages-fusions (Fig. 2B). The combined effects of Tax on BER, NER, DNA end stability, telomerase, and cell cycle progression create a setting in which repair of mistakes is compromised. These combined dysregulations might explain the observed 2.8-fold increase in genomic mutation frequency (92) in HTLV-I-infected cells.

**Tax and Aneuploidy**

The majority of cancers are aneuploid (93). In transformed cells, numerical chromosomal changes that include losses or gains of entire chromosomes (aneuploidy) generally co-exist with structural chromosomal damage. Although controversial, increasingly aneuploidy is thought to be caused, rather than a consequence, of transformation (95).

During normal mitosis, human diploid cells maintain euploidy by precisely partitioning 23 pairs of chromosomes from a mother cell to two daughter cells. ATL cells, by contrast, are famously polyploid and/or aneuploid. Their nuclei are highly lobulated or

\[^{2}\]F. Mitelman, B. Johansson, and F. Mertens, personal communication.
against oncogenic stress will either prevail (that Tax binds (94) will mean either the normal cellular response or be subverted (i.e. proliferation) by HTLV-I. A clear understanding of factors in addition to Tax that guide this choice for HTLV-I-infected T-cells will be a major topic for future research.

Concluding Comments

Over 20 million individuals globally are infected with HTLV-I. It is estimated that 2–5% of these carriers will develop ATL over their lifetime. The identification and isolation of HTLV-I 25 years ago have spurred intensive mechanistic investigations into ATL transformation. Using Tax as a model system, we have learned that viral oncoprotein (38), Tax can also induce aberrant centrosomal multiplication in G1. Generating supernumerary centrosomes results in multipolar mitosis, which is another mechanism for creating aneuploidy (104).

Finally, there is a school of thought that suggests polyploidy as the precursor of aneuploidy (104). Relevant to this notion, we note that Tax expression does facilitate creation of multinucleated (i.e. polyploid) cells (76, 98). Add to this the fact that Tax can inactivate p53 and Rb (65, 71–74), two factors essential to a G1 tetraploid/polyoid checkpoint (105), and one can then further envision how this might be yet another route traveled by HTLV-I/Tax/ATL cells toward aneuploidy (Fig. 3).

Proliferation versus Apoptosis

A long standing cancer paradox is that overexpression of oncogenes does not simply provide proliferative advantages to cells but frequently also triggers cells to undergo apoptosis. Findings from oncogene-transformation factors such as Myc, E1A, and E2F-1 show this duality to be the rule rather than the exception (reviewed in Ref. 106). Indeed, it is now apparent that oncogenic insults induce countervailing responses by the cell, which are reflected in cell cycle arrest and apoptosis. We reviewed, above, how Tax defeats cellular mechanisms for braking cell cycle progression. No cell cycle and/or genetic instability manifestations of Tax can confer selective growth advantage if cells fail to tolerate such phenotypic and genotypic changes and choose instead apoptotic death. Hence, disabling the cellular apoptotic response remains a requisite for transformation.

By definition, the clinical presentation of ATL implies that in a subpopulation of CD4+ T-cells, HTLV-I infection tips the balance between proliferation and apoptosis toward the former. Nevertheless, how HTLV-I Tax oncoprotein influences this choice is not fully understood. Many have examined the contribution of Tax to stress-induced apoptosis. Overall findings have been controversial and divergent. Some found that Tax protects cells from stress-induced cell cycle arrest or apoptosis (107–109), whereas others observed that Tax sensitizes cells to stress-induced apoptosis (110–113). Likely, the decision between proliferation and death is influenced by the cellular environment, cell type genetic background, and multiple co-existing signaling events. Depending on context, which set of genes that Tax transcriptionally activates (31) and/or which cluster of gene products that Tax binds (94) will mean either the normal cellular response against oncogenic stress will either prevail (i.e. apoptosis) or be subverted.

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