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CELL CYCLE DYSREGULATION BY HTLV-I: ROLE OF THE TAX ONCOPROTEIN

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1. ABSTRACT

HTLV-I is a human retrovirus which is the etiological agent for adult T-cell leukemia. The virus encodes a 40 kDa oncoprotein, Tax, which has no cellular counterpart. Findings from several laboratories over the past decade have shown that over-expression of the Tax oncoprotein is wholly sufficient to transform animal cells. Emerging evidence supports that Tax transforms cells through dysregulation of several cell cycle checkpoints. Here, we review extant data on how Tax targets cyclins, inhibitors of cyclin dependant kinase, as well as cellular sentries for DNA-damage.

2. INTRODUCTION

Viruses and host cells exist in symbioses. In this balanced interaction, the former frequently subverts the cellular machineries for signal transduction, transcription and proliferation to its own advantage. In co-opting the functions of the host cell, viruses can effect an accumulation of dysfunctions and alterations which could alter the metabolism of the cell in ways leading to the promotion of cellular transformation. Accordingly, studying the biology of tumor viruses has contributed much to our understanding of mechanisms of altered cell growth in human cancers.

Human T-cell leukemia virus (HTLV-1), like human papilloma virus and human herpesvirus, is a viral etiological agent for a human cancer. Infection with HTLV-I leads to the development of adult T cell leukemia (ATL) in approximately 2 to 5% of infected individuals after a long latency period of 20 or more years. HTLV-I is also linked to a variety of neurological maladies collectively termed HAM/TSP (1). The latency period prior to the emergence of ATL suggests that infection of the cell by HTLV-I is an initiating event which leads to subsequent multistepped changes resulting in immortalization and transformation of cells.

The viral phosphoprotein Tax is believed to play a fundamental role in T-cell transfomation by HTLV-I . Indeed, Tax, a 40 kilodalton trans-activator protein that activates transcription through three 21-bp cyclic-AMP responsive elements (CREs) found in the viral long terminal repeat (LTR) (2, 3), is essential for virus replication and has been implicated as the critical viral protein for transformation of T-cells (4). Tax induces tumors and leukemia in transgenic mice and immortalizes cultured T-cells (5-8). While the exact mechanism through which Tax exerts its oncogenic potential is not completely understood, it is known that Tax can modulate the expression of several cellular genes that are involved in cellular proliferation. For example, Tax upregulates the expression of IL2, IL2-receptor, c-fos, c-Jun, erg-1 and GM-CSF (9-16). Tax can also repress the expression of β-polymerase, and some functions of c-myc and Bax (17-19). Additionally, Tax can cooperate with oncoproteins such as Ras in cellular transformation (20) and can induce morphological changes in cells via its association with intermediate filaments (21). Tax also associates with

several other cellular proteins (reviewed 22) among them, proteins involved in cell cycle promotion such as p16 INK4a, cyclin D, MAD1 (reviewed in 23) and factors involved in signal transduction (24). Finally Tax has been shown to affect the functions of proteins such as p53 (25-30), IKKγ (31-33), c-myc (18) and Bax (19).

Dysregulated cell cycles are commonly seen in transformed cells (reviewed in 34-36). Many viral oncoproteins such as adenovirus E1A, HPV-16 E7 and SV40 large T antigen impair cell cycle phase progression (37). Normally, the transition from one phase of the cell cycle to the next is regulated at "checkpoints" which are, in part, governed by cyclin-dependent kinases (CDKs) assembled with partner cyclins. CDK-cyclin complexes are regulated by phosphorylation/dephosphorylation mediated through CDK-activating kinases (CAK) and phosphatases. They can also be regulated through physical binding with CDK inhibitory proteins (CKIs; reviewed in 34, 38-43).

D- and E-type cyclins control cell cycle transition from G1 to S (reviewed in 40, 41, 44). Cyclin D-CDK complex phosphorylates the retinoblastoma tumor suppressor gene product (pRb; 45, 46). Hypophosphorylated pRb binds members of the E2F transcription factors and inactivate E2F-function. E2F normally serves for the expression of genes (such as dihydrofolate reductase, DNA polymerase α, and cyclins) which are critical for S phase events (reviewed in 44, 47). Hyperphosphorylation of pRb disables its E2F-binding. E2F is thus released to activate cellular gene products which influence the passage of cells from G1 into S (48, 49). Thus, regulation of pRb phosphorylation by cyclin D- CDK and CKIs such as p16INK4a, p21CIP1, and p27Kip1 are critical in controlling overall cellular metabolism (reviewed in 43).

In principle, there are three straightforward ways through which viruses can overcome pRb-dependent cell cycle control. First, viruses could affect events upstream of pRb by targeting CDKs or CKIs. Second, virally-encoded proteins such as adenovirus E1a, HPV E7 or SV40 large T antigen can influence pRb either through physical binding (50-52) or through protein destabilization (53, 54). Third, viruses could act downstream of pRb by directly targeting E2F. Consistent with this last mechanism, ectopic expression of E2F has been shown to drive cells from G1 into S (55, 56).

The outcome of the cellular response to virus-mediated cell cycle subversion can come in two forms. Cells can respond to dysregulation by committing apoptosis. Indeed, it has been observed that when factors such as E1A, E7 or E2F1 are overexpressed, apoptosis can be induced through a p53-mediated pathway (57-60). On the other hand, it is equally evident that viruses have evolved strategies to cope with such cellular responses. Thus, many viral transforming proteins can suppress p53-function, thereby allowing virally-infected cells to evade apoptosis (37).

Cell cycle regulation in HTLV-I transformed cells is clearly dysregulated. Studies from us and other have shown that Tax accelerates G1 to S and S to G2/M progressions (61-64). One consequence of such accelerated progression is that genetic mistakes made in one phase of the cell cycle are not afforded time to be corrected before the mistakes are imprinted in the next phase of the cell cycle. Hence, in Tax-expressing cells, the failure to orderly repair ambiently generated genetic lesions leads to large accumulations of micronuclei (65, 66) and multinucleated cells (67), hallmarks of extensive genomic damage. Possibly then, transformation by HTLV can be explained by the cumulative burden of unrepaired genetic lesions accrued over multiple cell division cycles. The threshold of burden needed to tip a normal cell to a cancerous cell may thus determine the duration of latency prior to overt manifestation of leukemia.

3. HTLV-I TAX AND CELL CYCLE

3.1. Tax accelerates G1 to S progression through inhibition of p16INK4a-activity

p16INK4a inhibits the activity of the G1-specific CDKs, CDK4 and CDK6 (43). To understand better the proliferation of ATL cells, we and others (68, 69) searched for functional interactions between Tax and p16INK4a. Tax was found to bind p16INK4a directly. Binding by Tax abrogated the inhibitory effect of p16INK4a on CDK4 kinase activity. In Tax-expressing cells, a decrease in p16INK4a - CDK4 complex was correlated with an increase in Tax- p16INK4a complex. Indeed, the growth-arresting effects of p16INK4a in U2OS cells can be reversed by Tax over-expression (69). Thus, through direct protein-protein binding Tax inhibits p16INK4a-function, thereby indirectly activating CDK4-cyclin D function (68, 69). Interestingly, similar direct protein-protein interaction was also observed for Tax and p15INK4b. By contrast, Tax affects a third CDK-inhibitor (p18INK4c) through a transcriptional repression mechanism (70). Taken together those results suggest a theme of functional interplay between Tax and CDK-inhibitors which results in a net increase in G1-cyclin/ CDK kinase activity leading to Rb-phosphorylation and E2F-release.

3.2. Targeting of cyclins by Tax

Despite the findings described above, a cell line lacking p16INK4a shows, nevertheless, a large Tax-effect on G1 to S cell cycle progression coupled with E2F activation. This suggested that there is additionally a CDK-inhibitor-independent pathway utilized by Tax. Indeed, using a Jurkat-derived cell line (JPX9 T-cell), in which the p16INK4a gene is deleted and Tax expression can be induced, we found that in the absence of serum Tax-expression drove a significant proportion of cells from G0/G1 into S. In vitro kinase assay with pRb as substrate showed that in the absence of p16INK4a Tax increased CDK4-associated and cyclin D3-associated kinase activities by 6.5- and 3.5- fold, respectively. A similar Tax-effect on CDK6 activity was also observed which was accompanied by concomitant increases in hyperphosphorylated pRb and E2F2-function. These findings support the notion that a p16INK4a-independent route exists for Tax to influence G1 cyclin-CDK activity.

The direct effect of Tax on cyclin D was separately confirmed using C2C12 myocytes. Here, Tax

expression inhibited muscle-cell differentiation by increasing cyclin D1 activity which resulted in hyperphosphorylation of Rb. In this myocyte model, the activities of D-cyclins and Rb contribute importantly to cellular differentiation. Compatible with this principle, ectopic expression of cyclin D2 and D3 in 32D cells was found to inhibit differentiation (71). Overexpression of cyclin D1 also prevented MyoD-activated expression of muscle specific genes (72, 73), and the functional state of Rb is essential for maintaining the post-mitotic state of differentiated myotubes (74, 75). Indeed, here, myogenic differentiation correlates with increased amounts of hypophosphorylated Rb (74-77). Our finding that Tax prevents myocyte-differentiation is consistent with the thought that this viral oncoprotein has, at least, two effects: inactivation of CKIs (i.e. p16INK4a) and direct activation of cyclin D-CDK through a CKI-independent route (63).

In Tax-expressing cells, no significant changes in the steady-state levels of cyclin D3, CDK4 or CDK6 mRNA were seen, ruling out a transcriptional mechanism. Previously, both adenovirus E1A and human papillomaviruses E7 oncoproteins have been found to bind cyclin E-p33 CDK2 and/or cyclin A-p33 CDK2 (78-81). In a similar vein, we found that Tax directly bound to and enhanced the phosphorylation of cyclin D. Phosphorylation of cyclins has been reported for cyclin D1 and cyclin E. In both instances, the phosphorylated forms appear when the cyclins were actively complexed to cognate CDKs (45, 82). Sherr and co-worker have suggested that phosphorylation of cyclin D1 on threonine 286 might be an autoregulatory mechanism that modulates degradation via the ubiquitin-proteasome pathway (83). It is currently unclear to us whether the Tax-induced hyperphosphorylation of cyclin D leads into a similar pathway.

In contrast to our findings, others have found increased cyclin D2 expression (84-87) in HTLV-transformed cells. Using transient transfection assays, it was shown that HTLV-1 Tax transcriptionally up-regulated the cyclin D2 promoter and that this effect depended on intact CREB/ATF and NF-Kb signaling pathways (85-87). Of interest, Santagio et al. observed that in mock-infected cells cyclin D2 associated only with CDK6, but in HTLV-1 infected cells this cyclin associated instead with CDK2, CDK4 and CDK6 (86). In addition to increased cyclin D2 levels, Iwanaga et al. observed elevated cyclin E, cdk6, CDK4, and CDK2 in T-cells expressing Tax. Separately, Lemasson et al., using transient transfections of E2F1 promoter-reporter, also described the transcriptional activation of E2F1 by Tax (88). It remains to be determined whether cell type differences explain some of the variant findings.

Interestingly, we recently found that Tax paradoxically repressed transcriptionally the expression of an S-phase cyclin, cyclin A (89). The cyclin A promoter is a TATA-less promoter with upstream CREB/ATF sites. Using gel shift assays, we showed that Tax altered the formation of protein-DNA complexes at the ATF sites. Moreover, a Tax mutant protein inactive in the CREB-signaling pathway failed to repress the cyclin A promoter. These results suggest that Tax could inhibit cyclin A expression through protein-protein interaction with CREB/ATF factors. Alternatively, others have reported inhibition of cyclin A-CDK2 activity by Tax via the induction of p21 waf1 (62). Although the biological implications of cyclin A repression remain to be fully addressed, this example further illustrates the diverse approaches used by the HTLV-I oncoprotein to disrupt normal cell cycle progression and control.

3.3. Deregulation of checkpoints for DNA aberrations

Expression of Tax in mammalian cell induces the formation of multinuclei/micronuclei which is consistent with a perturbation in chromosomal segregation and orderly nuclear division. During mitosis, a spindle checkpoint regulates nuclear division by halting progression into anaphase until all chromosomes are aligned properly on a bipolar spindle (90). Seven spindle checkpoint proteins including those responsible for a mitotic arrest deficient phenotype (MAD) have been characterized. Amongst the spindle checkpoint factors, the MAD1 and MAD2 proteins form heterodimers which enforce metaphase arrest in response to spindle damage. Interestingly, human MAD1 was recently revealed to be a Tax-binding protein (67). The checkpoint function of MAD1 was found to be inactivated when it was bound by Tax.

Tax-MAD1 interaction potentially explains the frequent observations of multinucleated giant cells and aneuploidy in ATL. On the other hand, ATL cells also contain clastogenic DNA-damage which cannot be accounted for by a defect in the mitotic spindle checkpoint. To explain clastogenic lesions, several investigators have investigated Tax – p53 interaction. p53 is involved in both G1/S and G2/M checkpoint responses to DNA damage (reviewed in 41), and it is the most frequently mutated gene in human cancers. More than 50% of cancers have loss-of-function p53 mutants. Curiously, the great majority of HTLV-1 transformed T-cells have wild type p53 alleles (91). This suggests that loss of p53 function in ATLs largely occurs through a mechanism other than genetic mutation. Interestingly, elevated expression of p53 is seen in many HTLV-1 infected and Tax-immortalized cells (26, 92). Based on intensive studies from several laboratories, the mechanism used by Tax to inhibit p53 function is thought to likely occur through a sequestration of the p300/CBP coactivator (93, 94). Our findings using several Tax mutant proteins confirm this mechanism for inhibiting p53 function. Interestingly, we also found that Tax failed to repress p53 function in ATM-knock out cells, suggesting an additional functional requirement for the ATM-kinase in oncoprotein-tumor suppressor interplay (30).

It should be pointed out that other possible explanations exist for the high rate of genetics lesions in HTLV-I transformed cells. For example, DNA-repair enzymes could be directly targeted by Tax. Indeed, Tax has been shown to repress the expression of DNA polymerase β (17), a cellular enzyme involved in host cell DNA repair, and to inhibits topisomerase I activity (95), a protein involved in transcription and genomic stability. Furthermore, Tax activates the expression of PCNA gene (cellular proliferating cell nuclear antigen) (96); this activity correlates with a reduction in nuclear excision repair (NER). Details on these activities of Tax are summarized elsewhere (97).

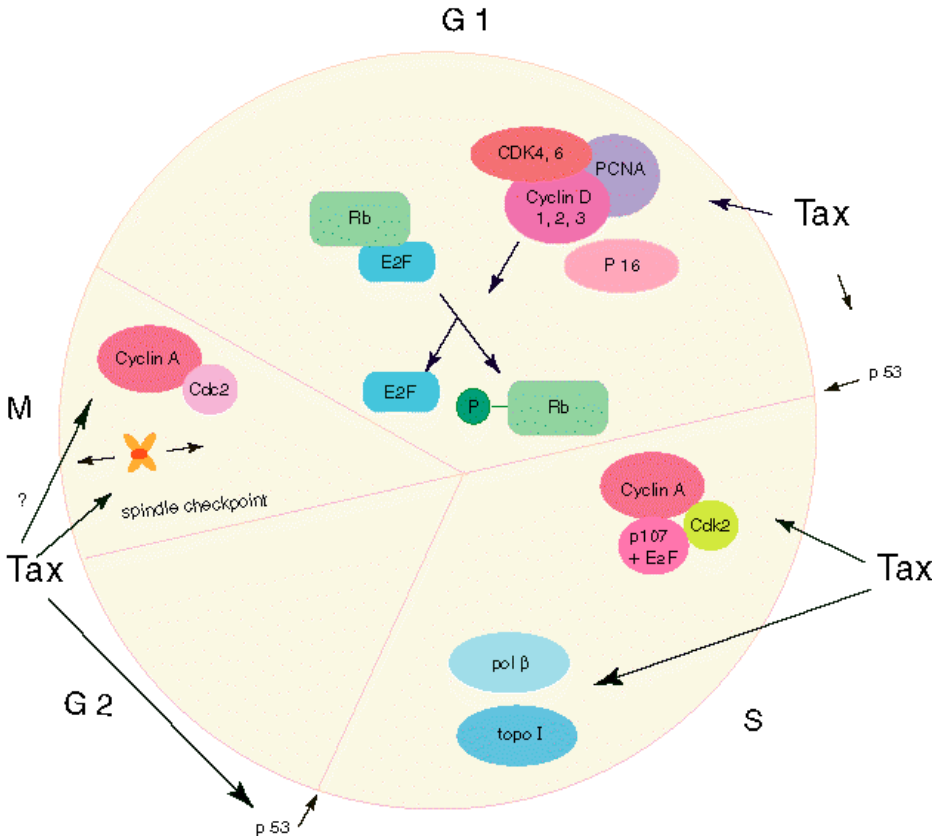

Figure 1. HTLV-I Tax oncoprotein affects multiple components of the cell cycle machinery. Tax is shown to affect cyclins and CDKs in G1, S, and M phases of the cell cycle. In addition, Tax also affects the functions of p53, DNA-polymerase β, topoisomerase I, as well as the mitotic spindle assembly checkpoint.

4. PERSPECTIVE

Here we have reviewed in a non-exhaustive fashion how the HTLV-I Tax oncoprotein can affect controls in the G1, S, and M phases of the cell cycle. Tax exerts cellular dysregulation through targeting cyclin-CDK components including cyclin D, cyclin A as well as CKIs such as p16INK4a. In addition to the cyclin-CDK axis, Tax can disturb at least two critical checkpoints through interactions with MAD1 and p53. Finally, Tax can directly affect enzymes/factors (e.g. human pol-β, topoisomerase I, and PCNA) which are normally utilized by the cell for repair of genetic lesions. The multifaceted activities of the Tax protein illustrate how a relatively small retrovirus has evolved functions to capably deactivate the multi-layered host defenses against pathological transformation (see figure 1). Understanding the workings of the Tax oncoprotein has revealed for us the many cellular intricacies in normal cell cycle control.

5. ACKNOWLEDGEMENTS

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