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SETTING THE STAGE FOR TRANSFORMATION: HTLV-1 TAX INHIBITION OF p53 FUNCTION

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1. ABSTRACT

Human T-lymphotropic virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia and tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM). Although the precise mechanism of HTLV-1 oncogenesis remains unclear, the pathogenesis has been linked to the pleiotropic activity of the viral transcriptional activator protein Tax. Tax has been shown to regulate viral and cellular gene expression and to functionally interfere with proteins involved in cell-cycle progression and DNA repair. This review will concentrate on the ability of Tax to promote cellular proliferation through activation of the NF-κB pathway while inhibiting the cell-cycle checkpoint and apoptotic function of the tumor suppressor gene p53.

2. INTRODUCTION

The human T-lymphotropic virus type 1 (HTLV-1) is a complex retrovirus belonging to the family of Delta-retroviruses. HTLV-1 is associated with two fatal human diseases: adult T-cell leukemia (ATL) and the neurodegenerative disease tropical spastic paraparesis /HTLV-1 associated myelopathy (TSP/HAM) (reviewed in (1-4)). ATL is an aggressive lymphoproliferative disease which can be classified into distinct clinical subtypes: pre-ATL, the acute form, the sub-acute or smoldering form, the chronic form, and ATL lymphoma (5,6). HTLV-1 is endemic in Southern Japan, the Caribbean basin, inter-tropical Africa, the Middle-East, South-America, and Papua New Guinea (5). It is estimated that 20-30 million people worldwide may be infected with HTLV-1. Of those, about 4% will go on to develop disease after a latency of 20 or more years (7,8).

In vivo, HTLV-1 infects CD4+ peripheral T-cells but has also been detected, to a lesser extent, in CD8+ T-cells (9-11). Unlike typical transforming retroviruses, HTLV-1 does not encode a cellular oncogene or disrupt cellular gene regulation by insertional mutagenesis. While several viral proteins act in concert to allow infected cells to avoid immune regulation, modulate anti- and proapoptotic signals, and increase T-cell responsiveness to extracellular stimuli, the viral Tax protein is the major viral oncoprotein (12-15).

3. TAX THE VIRAL ONCOPROTEIN

The long latent period that precedes the onset of ATL suggests a multistep mechanism of leukemogenesis (16). Two major ways in which viral oncoproteins affect the passage of cells through individual phases of the cell-cycle are by (1) increasing the expression level of growth promoting genes and (2) by altering the function of cell-cycle regulatory proteins.

Several studies have established that Tax expression is both necessary and sufficient to establish the transformed phenotype. Examination of malignant cells from ATL patients, which carry defective HTLV-1 proviral genomes, suggests the genomes preferentially retain the 3' end of the proviral genome encoding the Tax protein (17,18). Several other lines of evidence more directly demonstrate the importance of Tax in cellular transformation. Using a *Herpesvirus saimiri* vector, expression of the 3' portion of the HTLV-1 genome containing the Tax gene was able to transform T-cells resulting in a phenotype similar to HTLV-1 transformed cells (12). Mutation of the start codon of Tax eliminated cellular transformation (19). Rodent fibroblastic cell lines expressing Tax form colonies in soft agar and tumors in nude mice (14). Tax also immortalizes rat embryo

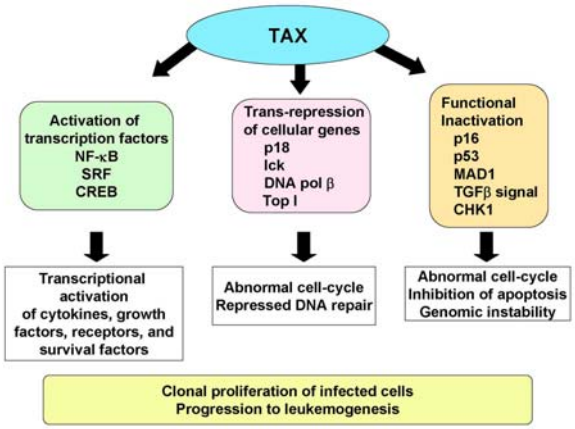

Figure 1. The pleiotropic effects of Tax. This is a summary of the activity of Tax within an infected cell that leads to uncontrolled cell proliferation. (adapted from Yoshida, 2001)

fibroblasts and cooperates with *ras* in cellular transformation (20). Subsequently, Smith and Greene found that Tax alone could transform Rat2 cells (13). In addition, Tax expression allows factor independent growth of established murine T-cell lines (21).

In transgenic mice, Tax protein expressed from the HTLV-1 LTR gave rise to animals that developed neurofibromas and mesenchymal tumors (22-24). In addition, arthritis and thymic atrophy have been observed (25). To investigate Tax's leukemic potential, Grossman et al. (26) targeted Tax to the mature T-lymphocyte compartment by developing mice which express Tax under the control of the granzyme B regulatory element. While these mice did not develop CD4+ T-cell leukemia, they did develop large granular lymphocytic leukemia of natural killer (NK) cell and cytotoxic T lymphocyte (CTL) nature (26).

3.1. Tax regulates gene expression

Tax is a 40kDa phosphoprotein that is predominately nuclear but has been shown to shuttle between the nucleus and cytoplasm (27). Tax lacks a cellular homologue (28), but has been shown to transactivate or transrepress the expression of a wide number of cellular genes. These include cytokines, growth factors, cellular receptors, cell-cycle regulators, DNA repair proteins, or proteins which regulate apoptosis (1,3,29-35). Tax does not bind DNA directly but acts through cellular transcription factors, CREB, NF-κB, and SRF (1,34). With the advent of DNA microarray technology, profiles of HTLV-1 infected cells and Tax expressing cells have allowed a more complete list of HTLV-1/Tax regulated genes (36-39). It's these pleiotropic actions of Tax that predict its central role in leukemogenesis (Figure 1).

3.2. Tax activation of the viral LTR

Tax was originally identified as a transcriptional activator for viral gene expression. Tax drives viral gene expression from three imperfect 21 base pair repeat enhancer elements located within the U3 region of the HTLV-1 long-terminal repeat (LTR). Each Tax-responsive element (TRE) contains a core CREB/ATF binding site flanked by 5'G- and 3'C-rich residues (40,41). Tax efficiently activates the LTR by forming a Tax/CREB/TRE complex where binding of Tax to CREB enhances CREB homodimer formation (42-45). Tax is believed to contact the G-C-rich flanking sequences of the DNA, which results in a conformational change in Tax allowing the exposed C-terminal region of Tax to recruit the co-activators CBP/p300 and PCAF (46,47). Unlike CREB-mediated transcription, Tax-mediated transcription occurs in the absence of CREB phosphorylation. The ability of Tax to activate transcription via CREB/ATF sites is context specific, since transcriptional activation of cellular promoters that contain CREB sites is not seen (43,48,49).

3.3. NF-κB activation

Tax works at several levels to maintain constitutive activation of the NF-κB pathway (50). Although a direct interaction between Tax and different members of the NF-κB family have been reported (51-56), the primary action of Tax in activating NF-κB has been shown to occur through interaction with IKKγ in the IKK signalsome which includes IKKα, IKKβ, NIK, and MEKK1 (57-59). The mechanism by which Tax stimulates the IKK complex through IKKγ is not clearly understood. A more comprehensive discussion of this topic can be found in reviews on Tax activation of NF-κB (35,50,60,61).

Although other pathways have been implicated (13,62), strong evidence supports the activation of the NF-κB family of eukaryotic transcription factors by Tax as playing a critical role in HTLV-1 induced leukemia (21,63,64). This is not surprising since the NF-κB family plays an important role in the regulation of immune responses, embryonic and cellular development, apoptosis, cell-cycle progression, inflammation, and oncogenesis.

An early study showed that while antisense oligonucleotides to Tax had no effect on tumor growth, antisense to NF-κB blocked Tax induced tumor growth (65). More recently, using the infectious molecular clone of HTLV-1, Robek et al. (63) demonstrated that mutants in Tax that could not activate the NF-κB pathway could not immortalize human T-lymphocytes. In contrast, Tax mutants that failed to bind p300/CBP or activate the HTLV-1 LTR still allowed lymphocyte immortalization (63,64). Similarly, inhibition of apoptosis induced by factor withdrawl in mouse CTLL cell lines correlates with the ability to activate NF-κB and induction of the anti-apoptotic, NF-κB-regulated gene, Bcl-x_L (21,66). Further, inhibition of NF-κB activity by expression of the NF-κB2 precursor abrogated Tax-mediated transformation of rat fibroblasts without affecting viral LTR activation (67). Using Tax-transgenic mice, Portis et al. (68) demonstrated that sodium salicylate and cyclopentenone prostaglandins, inhibitors of NF-kB activity, blocked spontaneous proliferation of Tax transgenic mouse spleen cells. In addition, Tax-induced tumor cells resistant to irradiation-

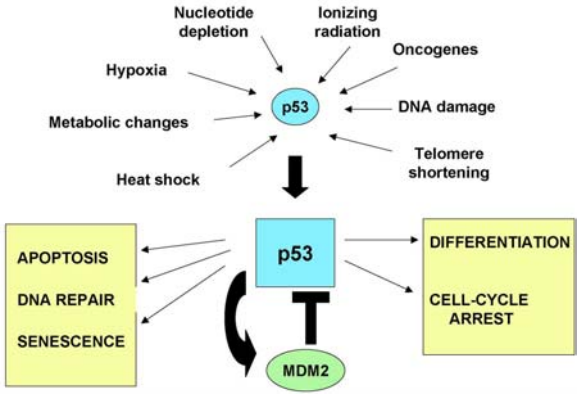

Figure 2. The p53 response. Diagrammatic representation of the central role p53 plays in the coordination of the cellular response to a variety of stresses.

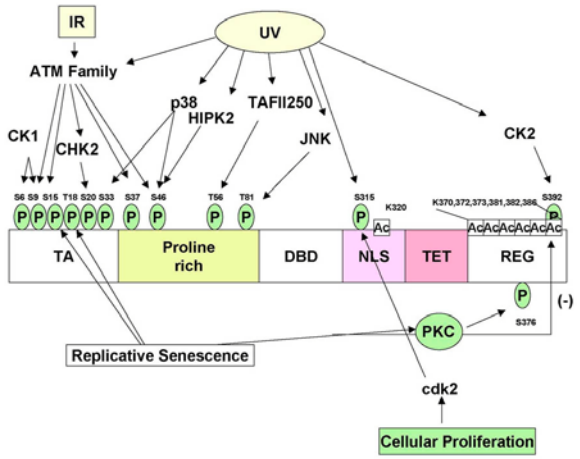

Figure 3. p53 modifications. The human p53 protein has distinct functional domains TA, transactivation; Proline rich; DBD, DNA binding domain; NLS, nuclear localization sequence; TET, tetramerization domain; REG, regulatory domain. This schematic diagram depicts the phosphorylation (P) and acetylation (Ac) sites of the p53 protein upon DNA damage or during proliferation and senescence. S, serine; T, threonine; K, lysine; IR, ionizing radiation; UV, ultraviolet radiation. The putative kinases are indicated. ATM, Ataxia telangiectasia mutated; CK1 and CK2, casein kinase 1 and 2; p38, p38 mitogen-activated kinase; HIPK2, homeodomain-interacting protein kinase 2; JNK, Jun-terminal kinase; CDK2, cyclin-dependent kinase 2; PKC, protein kinase C. *In vitro*, PCAF acetylates p53 at K320 while CBP/p300 acetylates K370, K372, K373, K381, K382, K386. These same C-terminal residues acetylated after DNA damage are also the residues ubiquitinated by MDM2.

induced apoptosis became sensitive in the presence of sodium salicylate and prostaglandins. Finally, our studies suggest a link between NF-κB activation and Tax-mediated inhibition of the tumor suppressor, p53 (69).

4. THE TUMOR SUPPRESSOR p53

Eukaryotic cells have developed signaling pathways to coordinate cell-cycle transitions and ensure faithful replication of the genome before cell division. These regulatory pathways are termed cell-cycle checkpoints (70). Checkpoints exist in cells that will interrupt cell-cycle progression when damage to the genome or spindle is detected, or when cells have failed to complete an event. When a checkpoint is triggered, cells arrest transiently to allow for the repair of cellular damage or alternatively, if damage is irreparable, signal pathways lead to programmed cell death. The ability of normal cells to undergo cell-cycle arrest or apoptosis is critical for the maintenance of genomic integrity. Defects in cell-cycle checkpoints can result in gene mutations, chromosome damage, and aneuploidy, all of which may contribute to tumorigenesis.

Arguably the most frequently detected alteration in human cancer is inactivation of the tumor suppressor, p53. In fact, mutation of p53 is associated with approximately 50% of all human cancers. In addition, p53 is a frequent target for inactivation by viral transforming proteins such as SV40 large T-antigen, HPV E6, hepatitis B X-antigen, and adenovirus E1A and E1B (71). While Tax has been shown to affect several cell-cycle checkpoints such as G1/S and DNA repair (1,72), this review will focus on the role of Tax in p53 inhibition.

4.1. p53 function

The p53 protein belongs to a family of related proteins that includes two other members, p63 and p73 (73,74). While the proteins are all structurally and functionally related, p63 and p73 have clear roles in development, whereas p53 seems to have evolved to prevent tumor development and has earned the name "cellular gatekeeper".

Several stress signals can activate p53, triggering a variety of responses including cell-cycle arrest, differentiation, DNA repair, apoptosis, or senescence (Figure 2). There are five recognized domains in p53: the N-terminal transcriptional activation, proline rich, sequence specific DNA-binding, tetramerization, and basic regulatory domains (Figure 3). p53 functions as a tetrameric, sequence specific DNA-binding transcription factor that controls the expression of an array of gene products in response to diverse stress stimuli.

p53 is extensively phosphorylated, and modification at several residues has been specifically associated with the ability of p53 to respond to certain stress signals (Figure 3). There are numerous phosphorylation sites in the N-terminal domain and phosphorylation of serines 15, 20, 37 and threonine 18 have been shown to regulate p53/MDM2 binding *in vitro*. The kinases signaling to p53 include casein kinase 1 and 2, ataxia telangiectasia kinase (ATM), ATR (ATM/Rad3 related kinase), CHK1 and 2, jun N-terminal kinase (JNK), and DNA-dependent protein kinase (DNA-PK) (reviewed in (75,76)). Several of these kinases have also been shown to phosphorylate MDM2 *in vitro* within the p53-binding domain further suggesting a regulatory role for these modifications ((77) and references therein).

Under damage or stress conditions, the phosphoacceptor sites are modified through a regulated kinase cascade *in vivo* ((71,78-82) and references therein). It is the complexity and combination of the phosphorylation sites that appears to dictate the fate and function of p53. For example, the phosphorylation pattern of p53 differs during the cell-cycle and coincides with the ability of p53 to associate with regulatory proteins including p300 and MDM2 (75,83,84). In addition, *in vitro* protein-protein interaction assays demonstrated that phosphorylation at serine 15 alone inhibited the interaction of p53 with TBP (85). In contrast, phosphorylation at serine 15 and 37 did not inhibit TBP binding, suggesting that the combination of phosphorylated sites is important.

In addition to its complex phosphorylation pattern, p53 is acetylated on at least 3 lysine residues, 320, 373, and 382 (86-89). Additional C-terminal lysines have also been identified as potential acetylation sites. Acetylation of p53 has been implicated in transcriptional regulation by p53 and its association with basal transcription machinery (86-89). In addition, the association of p53 with deactylases has implications for regulating both p53 transcriptional activation and repression functions (82,90).

4.2 p53 in HTLV-1 infected cells

HTLV-1 infection is associated with stimulation of G1- to S-phase progression (91). Because p53 plays such a key role in G1- to S-phase transition, several groups examined the status of p53 in HTLV-1 infected cells. Early observations found that p53 was wild-type in sequence but stabilized in most HTLV-1 infected T-cells (92,93). For those cells having p53 mutations, they appeared to correlate with late stages of disease (94,95). In 1996, Cereseto et al. demonstrated that p53 was transcriptionally impaired in ATL cells. In addition, these cells failed to undergo G1 arrest after induction of DNA damage (96).

Biochemical studies of p53 in HTLV-1 infected cells demonstrated that p53 existed primarily as tetramers (85), which reside in the nucleus. Using biotinylated oligonucleotides or gel mobility shift assays, it was shown that p53 from transformed cells can bind DNA in a sequence-specific manner (85,93,97). Interestingly, in the transformed cells, p53 was found to be hyperphosphorylated at serines 15 and 392 by phosphopeptide mapping (85). This observation was of significant interest since, as discussed above, the pattern of p53 phosphorylation is significantly altered in response to stress (78,79,82) and in human tumors (98). Moreover, the phosphorylation of p53 is predicted to alter its conformation and its association with other factors. This may explain why p53 is inactive in tumor cells despite its wild-type genotype (85,99).

Viral oncoproteins such as SV40 large T-antigen or adenovirus E1B (71) have been shown to directly bind to p53, inactivating its function. Several groups have shown by immunoprecipitation or *in vitro* pull-down assays that Tax protein does not bind to p53 (85,97,100). It appears more likely that Tax inhibits p53 function through an indirect mechanism. The indirect mechanism, however, results in a similar phenotype to E1B since p53 in the transformed cells does not interact with the basal transcription factor TFIID (85). Interestingly, decreased TFIID binding correlated with phosphorylation of p53 at serine 15 (83,85) suggesting that Tax regulates p53 through modulation of upstream kinase activity or specificity.

In normal non-stressed cells p53 has a very short half-life due to a negative feedback loop mechanism in which MDM2 protein plays a key role (101,102). Transcription of MDM2 is upregulated by p53. In turn, MDM2 directly binds to p53 and functions as a ubiquitin E3 ligase that promotes the conjugation of ubiquitin to p53 resulting in its proteasome-mediated degradation. The importance of this negative feedback loop is illustrated by the result that MDM2 null mice are not viable unless crossed to p53 null mice (103). Consistent with the stabilization of p53 in HTLV-1 infected cells, MDM2 binding to p53 was not detected (85). In related studies, Takemoto et al. found that p53 stabilization and functional impairment in HTLV-I transformed cells occurred in the absence of genetic mutation or alteration of the p14 ARF-MDM2 loop (104).

5. MECHANISMS OF TAX-MEDIATED p53 INHIBITION

Several groups have shown that of the HTLV-1 encoded proteins, expression of Tax protein alone is sufficient to inhibit p53 transcriptional activity. Transient transfection assays demonstrated that Tax could inhibit p53 activity on reporter constructs (97,105,106). Similar observations were reported in stable T-cell lines expressing only Tax (107).

The mechanism by which Tax inhibits p53 function is not completely understood, but much progress has been made. There is general agreement that Tax-mediated p53 inhibition is not through direct binding of Tax, altering p53 sub-cellular localization, or disrupting DNA-binding. However, there is controversy as to whether Tax uses the NF-κB or CREB/ATF pathway to inhibit p53 function. Our studies point to the activation of the NF-κB pathway as being important for Tax-mediated p53 inhibition (69,108). In other studies, Tax activation of the CREB/ATF pathway appears important (97,100,109,110). A partial resolution of these apparently discrepant results was offered by Pise-Masison et al., who reported that the mechanism was cell-type dependent and depended largely on the intracellular pool of CBP/p300 (109). More recently, an indirect mechanism of p53 inactivation by Tax has also been proposed because of the interaction of Tax with the hTid-1 protein that is a human homologue of the *Drosophila* tumor-suppressor protein Tid56 (111).

5.1. NF-κB pathway

As seen in Tax-induced cellular immortalization/transformation, our laboratory has found a strong link between Tax's ability to activate NF-κB and its ability to inhibit p53 in lymphocytes (69) (Figure 4A). Blocking NF-κB activation by expressing a dominant

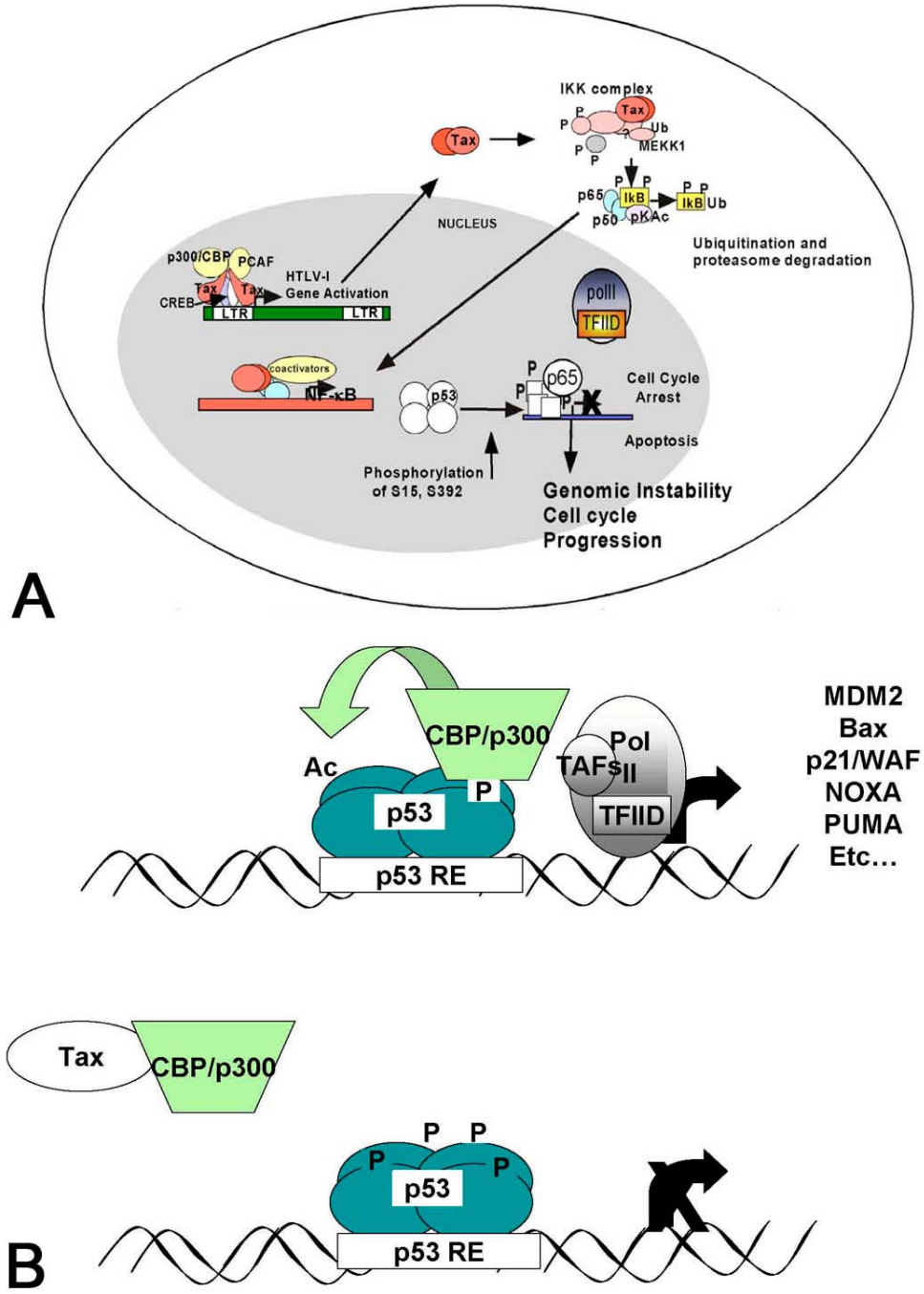

Figure 4. Mechanisms of Tax-mediated p53 inhibition. (A) Schematic representation of the nuclear and cytoplasmic functions of Tax which lead to p53 transcriptional inactivation. The Tax protein, in conjunction with CREB and the co-activators PCAF and CBP/p300, activates transcription from the viral long terminal repeat (LTR). Tax expression leads to the activation of the NF-κB pathway through the IKK signalsome. This activation leads to nuclear translocalization of the NF-κB transcription factor, which in turn activates NF-κB responsive genes involved in proliferation and cell survival. Tax expression also leads to the phosphorylation of p53 at serines 15 and 392. This phosphorylation contributes to the binding of the NF-κB subunit p65/RelA to the p53 protein. Although p53-p65 complexes are found bound to p53 responsive promoters *in vivo*, the basal transcription machinery is not recruited to the promoter. This results in the inhibition of p53 transcriptional activity. (B) Binding of the co-activators p300/CBP to p53 protein have been implicated in p53 transactivation. Once bound to p53 p300/CBP can acetylate lysine residues in the carboxy-terminal region of p53 enhancing it's sequence specific DNA-binding. The viral Tax protein is also capable of binding p300/CBP and can complete with p53 for binding resulting in inhibition of p53 transcriptional activation function.

negative IκBα protein blocks Tax-mediated p53 inhibition not only in Tax transfected cells but also in HTLV-1 transformed cells (69,112). Since expression of exogenous p300 could not rescue p53 activity, squelching of the co-activator by Tax or NF-κB appears not to be responsible for the block in p53 transcriptional activity in this system (69).

Our present evidence suggests that p65/RelA is uniquely involved in p53 inhibition. Antisense oligonucleotides to p65 but not p50 or c-Rel restored p53 activity in transformed cells (112). In addition, p50 null cells allowed Tax-mediated p53 inhibition, but p65 null cells could not support inhibition unless the p65 subunit was added back (69). In the presence of Tax protein, p65 appears to inhibit p53 transactivation function by direct interaction with p53. p65/p53 complexes were detected in HTLV-1 infected and Tax transfected cells by co-immunoprecipitation. Previous studies suggest a strong link between the ability to phosphorylate p53 at serines 15 and 392 with the ability of Tax to inhibit p53 transactivation of both reporter constructs and endogenous genes (69). Likewise, Tax-induced binding of p65 to p53 correlated with the phosphorylation status of p53 (78,85,113).

Modifications of p65 may also play a role in p53 binding and inhibition. Recent reports indicate that p65 can be both phosphorylated and acetylated and these modifications influence the protein's ability to be recruited to the transcriptional apparatus and stimulate target gene expression (114). Inducible p65 phosphorylation has been found in both the C-terminal transactivation domains and in the Rel homology domain (RHD) (114). Studies are in progress to determine the importance of p65 phosphorylation in Tax-mediated p53 inhibition. Additionally, reversible acetylation of p65 may also regulate its transactivation function (115,116). p65 has been shown to associate with HDACs, which are regulated by the phosphorylation state of p65 (117-119).

It will be of interest to identify which components of the transcription factors are associated on the active and inactive promoters. To this end, chromatin immunoprecipitation assays show that in HTLV-1 transformed cells p53/p65 bind to the MDM2 promoter. Consistent with the results of DNA pull-down assays (85), TFIID was not found on the promoter (112). In contrast, in cells in which p53 was transcriptionally active, p53/TFIID complexes but not p65 were found on the promoter. Studies are underway to fully characterize the active and inactive promoter complexes.

These studies identify a unique mechanism for p53 regulation by the p65/RelA subunit of NF-κB. The role of p65 in directly inhibiting transcriptional activity has also been proposed for the glucocorticoid receptor (GR). Two groups have recently shown that a direct interaction between p65 and GR results in mutual transcriptional inhibition (120,121). Further, while p300/CBP may function as an integrator of p65/GR physical interaction, it is not a limiting cofactor for which p65 and GR compete (120,121). Rather, similar to the proposed model for p53-p65, p65 disturbs the interaction of GR with the basal transcription machinery irrespective of the coactivator levels. Further studies are required to determine what factors govern the interaction and subsequent promoter inhibition of Tax-mediated p53-p65 complexes.

5.2. CREB/LTR activation pathway

In other situations p53 inactivation occurs through direct competition between Tax and p53 for recruitment of the co-activators p300/CBP (Figure 4B). CBP and p300 are highly homologous coactivators that promote gene expression by bridging DNA-bound transcription factors and the basal transcription machinery, providing a scaffold for integrating transcription factors, and by modifying transcription factors and chromatin through acetylation (122,123). Evidence indicates that interference with normal CBP/p300 function can result in a variety of diseases (122-124). CBP haploinsufficiency is the hallmark of Rubinstein-Taybi syndrome and chromosomal translocations affecting the p300 and CBP genes are the cause of congenital malformations and hematological malignancies (125). In addition, mutations in the CBP or p300 gene, accompanied by loss of the other allele, have been found in a variety of cancers (122-124).

Many factors including Tax, steroid and retinoid hormone receptors, phospho-CREB, c-Jun, c-Myb, NF-κB, TBP, and p53 have been found to interact with CBP and/or p300 (122-124). Studies have shown that Tax mutants such as K88A and V89A, which fail to interact with p300/CBP (126), failed to inhibit p53 transcriptional activity (100,106,109). *In vitro* binding assays demonstrated that Tax interferes with the recruitment of CBP to DNA-bound p53 (127) and that Tax and p53 binding to GST-C/H1-KIX was mutually exclusive (106). Similarly, in transient transfection studies, a reciprocal repression between Tax and p53 was seen (106,127). Finally, exogenous p300 could in certain cell types rescue p53 activity (109). Recent studies have also indicated that Tax can inhibit the p53 family members p73α and p73β perhaps also through p300/CBP squelching (128,129). To note, the Tax mutant M47, which is still capable of binding CBP/p300 but does not activate the viral LTR, failed to inhibit p53 activity (97,109,110). This suggests that additional factors may be involved.

It is important to note that while competition for coactivators may occur in transient transfection assays where proteins are over expressed, chromatin immunoprecipitation assays suggest that p300/CBP is present at sufficient levels to bind to both viral and cellular promoters in HTLV-1 infected cells (130). Chromatin from HTLV-I transformed Hut-102 cells was crosslinked, fragmented, precipitated with p300 antibody and the DNA subjected to PCR amplification using primers for the HTLV-I LTR or IL-15Rα promoter. The results of this study clearly demonstrate that p300 is present on both the LTR and IL-15Rα promoters (130).

6. PERSPECTIVE

Acute phase Adult T-cell leukemia carries a very poor prognosis due to the resistance of leukemic cells to

conventional or even high dose chemotherapy. In other human cancers, there is a consensus that reactivation of p53 function in cancer cells could be of therapeutic benefit. Indeed, several recent studies have defined small molecules or peptides that restore function to mutant p53 proteins illustrating the potential of this approach (131-133). As it is becoming clear that the apoptotic and cell-cycle activities of p53 are independently regulated, more targeted therapies to regulate cell death-inducing functions of p53 are being pursued. Since the majority of ATL patients harbor a wild-type p53 protein that is functionally impaired, reactivation of p53 could be potentially very beneficial in ATL treatment.

In support of this, reports have shown that inhibition of NF-κB activation with Bay 11-7082 or arsenic trioxide treatment of HTLV-1infected cells results in apoptosis (134,135). Several reports link p53 activation with arsenic induced apoptosis and cell-cycle arrest (136-140). Although no change in p53 stability or serine 15 phosphorylation was observed in arsenic treatment of HTLV-1 infected cells, p53 may be functionally active in these cells due to decreased p65 binding. It cannot be ruled out at this point that additional events may be important for full activation of p53. Thus, combination therapy may be required. With this in mind, studies using the NF-κB inhibitor PS-341, which has been successful in treatment of multiple myeloma and mantle cell lymphoma (141-144), have shown promise. Using a NOD-SCID mouse model for ATL, Tan and Waldmann found that treatment of mice with PS-341 and humanized anti-Tac was associated with a complete remission in a proportion of treated animals (145).

Constitutive NF-κB activation has been associated with breast, ovarian, prostate, and colon cancer (146-155). In addition, multiple viruses including HIV, HTLV-I, hepatitis B virus, hepatitis C, EBV, and influenza have been shown to activate NF-κB to promote viral replication, prevent virus-induced apoptosis, and mediate the immune response (50). It will be important to determine if constitutive NF-κB activation, specifically p65 expression, is a general mechanism for p53 inhibition in human cancer.

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