Functional inactivation of p53 by human T-cell leukemia virus type 1 Tax protein: mechanisms and clinical implications

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Human T-cell leukemia virus type 1 (HTLV-I) has been implicated with the etiology of adult T-cell leukemia/lymphoma (ATL) and certain other clinical disorders. Although the leukemogenic mechanism of HTLV-1 is not fully understood yet, the viral Tax protein is widely regarded as a key factor in this mechanism. Tax can modulate the synthesis or function of many regulatory factors which control a wide range of normal and oncogenic cellular processes and therefore, it acts as a potent oncoprotein. In the last few years, special attention has been attracted to Tax interference with the transactivation function of p53, a tumor-suppressor protein that is involved in regulation of the cell-cycle and apoptosis and in maintaining the cellular genome integrity. p53 is mutated in ~60% of all human tumors. In contrast, mutant p53 is found in only small percentage of ATL patients. Nevertheless, p53 is inactive in the leukemic cells of most ATL patients and in most HTLV-1 transformed cells. By inactivating p53, Tax can immortalize the HTLV-1-infected cells and destabilize their genome. Consequently, such cells can progress toward the ultimate leukemic state by a stepwise accumulation of oncogenic mutations and other types of chromosomal aberrations. Furthermore, since p53 exists in most ATL patients in its wild-type form, its reactivation by therapeutic drugs might be an effective approach for ATL therapy. Several mechanisms have been proposed so far for Tax-induced p53 inactivation. Understanding the exact mechanism of this Tax effect is essential for designing effective means for this therapeutic approach. In this review article, we discuss the various mechanisms proposed for Tax interference with p53 functions and their clinical and therapeutic implications.

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is phylogenetically a descendant of the simian T-lymphotropic virus type 1 (STLV-1) (1,2). It is the first retrovirus to be linked with the etiology of human malignancy. After the discovery of interleukin 2 (IL-2) which enabled long-term growth of human T-cells in culture by Gallo and his colleagues (3–6), HTLV-1 was detected in 1979 in fresh leukemic cells of American patients with cutaneous T-cell lymphoma and reported in 1980 (7). Latter, in 1982 this virus was discovered also in Japan by Yoshida’s group (8). In 1977 epidemiological studies conducted by Uchiyama and his colleagues (9,10) revealed an unusual clustering of adult T-cell leukemia/lymphoma (ATL) in some areas of Japan and they recognized this malignancy as a distinct clinical entity, that manifests in adult life by presentation of skin lesions, lymphadenopathy, hepatosplenomegaly, elevated counts of white blood cells and presence of abnormal lymphoid cells with T-helper phenotype. The geographical clustering of this malignancy pointed to a transmissible etiological agent, which turned out to be HTLV-1. Few years later HTLV-1 was also associated with the etiology of a neurological syndrome called tropical spastic paraparesis (TSP) or HTLV-1 associated myelopathy (HAM) (11,12) and afterwards it was also linked with certain additional clinical disorders (13,14). Interesting descriptions of HTLV-1 evolution, the history ATL recognition, and HTLV-1 discovery and its molecular biology and pathogenicity can be found in a number of recent review articles (15–27).

It is currently estimated that HTLV-1 affects ~20 million people in the world, 5–10% of whom develop an HTLV-1 related disease, usually 10–40 years after the initial infection (13,16). Despite the intensive studies focused on this virus, its pathogenic mechanism is still not fully understood. However, the viral Tax protein is widely regarded as a key factor in this mechanism because of its capacity to stimulate or repress the synthesis or function of many regulatory factors involved in a wide range of normal and pathologic cellular processes (16,17,19,27).

In natural human infection HTLV-1 targets mainly peripheral mature CD4+ helper T-cells (28–30), although there are indications that other cells in different compartments, like peripheral CD8+ T-cells, bone marrow monocytes, and others, may also be infected. Mature peripheral CD4+ T-cells are normally non-replicating cells, unless they are activated by specific antigens or by mitogenic agents. Therefore, these cells are less accessible to mutagenesis. By constitutive induction of regulatory factors involved in activation of T-cell replication, the viral Tax protein can set infected T-cells into a continuous uncontrolled replication. Normally, replicating cells are guarded by a mitotic spindle checkpoint, which verifies proper chromosomal segregation during mitosis (31–33). Disruption of this checkpoint leads to chromosomal aneuploidy, which is frequently observed in cancer cells (34). HTLV-1 Tax protein has been shown to abrogate the mitotic checkpoint function (17,27) and lead to miscounted chromosomes in HTLV-1 transformed T-cells and leukemic ATL cells (35–37). Furthermore, the enhanced uncontrolled replication...
renders the HTLV-1-infected T-cells more accessible to mutagenic and DNA damaging factors. Most cells respond to DNA damage by activating several DNA repair mechanisms. However, Tax has been shown to interfere with most DNA repair mechanisms, thus further intensifying the genome instability of these cells (16,38). Usually, cells that suffer from mitotic checkpoint dysfunction, or cannot repair damage imposed on their DNA, enter into cell-cycle arrest or apoptosis. In contrast, HTLV-1-infected cells are protected by Tax from both of these responses (16). With these pleiotropic activities, schematically summarized in Figure 1, Tax acts as a potent oncoprotein capable of transforming cultured animal cells, inducing tumors in transgenic mice and immortalizing or transforming human primary T-cells (16,17). This oncogenic potency of Tax is thought to initiate the leukemic process leading to ATL (16).

In the last few years, special interest has been focused on Tax interference with the transcriptional transactivation function of the tumor suppressor p53 protein (26,39). This protein is involved in regulating the cell-cycle (40), apoptosis (41,42) and DNA repair (43), so that interference with these p53 functions might be one of the mechanisms by which Tax can immortalize virus-carrying T-cells of HTLV-1-infected individuals and destabilize their genome. Such cells can, then, progress toward the ultimate ATL state through a stepwise accumulation of oncogenic mutations and other chromosomal aberrations. Therefore, functional p53 inactivation is regarded as one of the important activities of Tax that account for its oncogenic potential. Several different mechanisms have been proposed for Tax inhibition of p53 transcriptional function. In this review we discuss these mechanisms and their implications on Tax oncogenicity and on possible therapeutic or preventive approaches for ATL.

Structure, function and regulation of p53

p53 consists of 393 amino acids which include four functional domains (i) the N-terminal transactivation domain, spanning between amino acids 20 and 60 that can bind to the co-activators p300/CBP (CREB-binding protein) and the p300/CREB-binding protein associated factor (p/CAF), which are required for p53 transcriptional function; (ii) the central DNA-binding domain, spanning between amino acids 102 and 292, which is responsible for p53 sequence-specific DNA binding; (iii) the tetramerization domain that spans between amino acids 324 and 355 and (iv) the C-terminal domain, which is responsible for p53 nuclear localization (44). In addition, p53 can physically bind to other regulatory proteins and abrogate or modify their functional activities (45–47).

While attention has been mostly concentrated on the transcriptional transactivation function of p53, it is important to note that a substantial number of p53 biological effects result from its capacity to also suppress transcriptional gene expression (48). p53 can suppress gene expression in several different ways. Certain genes are suppressed by interference with the function of their transcriptional activators, whereas in certain other instances p53 interferes with the basal transcriptional machinery, or recruits histone deacetylases to remodel the chromatin conformation at the target promoters (48).

p53 level and activity are regulated by a wide variety of factors at the levels of transcription, translation, protein turnover, cellular compartmentation and protein–protein interactions (49–51). More recently, attention was focused on p53 regulation by multisite post-translational modification like phosphorylation, acetylation and glycosylation, which affect its activity, stability and interaction with other proteins (52–55). Under normal physiological conditions, p53 is kept at a low level and an inactive state. Its negative regulation is mediated mainly by MDM2 (56,57), a 90 kDa oncoprotein, which binds p53. The expression of the MDM2-encoding gene is, actually, activated by p53 itself, through a p53-responsive element residing in its promoter (58). In turn, the encoded MDM2 protein affects p53 level and activity in the following three ways: (i) it ubiquitinates p53 and leads to its degradation by 26S proteosomes (59,60), (ii) it inhibits p53 transactivation function by preventing its interaction with p300/CBP and other basal components of the transcription machinery (52–55). This p53–MDM2 auto-regulatory loop is disrupted also by NADPH quinine oxidoreductase...
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1 (NQO1), which binds to p53 and protects it from degradation, thus, leading to its stabilization and accumulation (65,66).

Another important player that negatively regulates p53 is MDMX, which is structurally related to MDM2. Since its discovery, about 10 years ago (67), a large body of biochemical data has accumulated on the functions of MDMX, often leading to conflicting molecular models. Nevertheless, virtually all these data point toward a critical role of MDMX in the regulation of the p53–MDM2 network (68).

Cyclooxygenase-2 (COX-2) has been recently also implicated in p53 regulation. It has been found that p53 upregulates COX-2 expression which, in turn, binds to p53. This binding does not affect the turnover of p53 nor its subcellular distribution, but it interferes with p53 transcriptional and apoptotic-inducing functions (69). In addition, Jun-N-terminal kinase (JNK) and poly(ADP-ribose) polymerase-1 (PARP-1) (62) have been linked with the regulation of p53 steady state level.

DNA damage and certain other stress conditions upregulate the level and/or activity of p53 through a variety of other cellular factors affecting its expression or post-translational modifications (70), which include its phosphorylation and acetylation at specific residues (52). It has been shown that in stress conditions, p53 is phosphorylated at several specific serine residues. However, Saito et al. have noted that the exact set of the phosphorylated serine residues may vary between different stress-inducing factors (71). These authors suggest that p53 phosphorylation is regulated through a complex cascade involving both, activation of secondary effector protein kinases as well as intermolecular phosphorylation site-interdependencies which, together, avoid inappropriate p53 inactivation by conducting signal amplification and convergence of signals from multiple stress pathways. On the other hand, Dohony et al. (72) have shown that phosphorylation of serine-37 is primarily important for p53 activity in response to DNA damage.

p53 phosphorylation disrupts its interaction with MDM2, thus leading to its stabilization and accumulation in the nucleus (52,73). It also enables the binding of p53 to CBP, p300 and p/CAF (73–75), which acetylate it at certain specific lysine residues (73,75). In addition, recent studies (76,77) have demonstrated that DNA damage induces phosphorylation of certain serine residues of MDMX and that this phosphorylated protein is selectively bound and degraded by MDM2 prior to p53 accumulation and activation. Based on these observations, it has been suggested that in response to DNA damage the ubiquitine E3 ligase activity of MDM2 is shifted from p53 to it, inhibiting its interaction with MDM2 (84–86) or by functional inhibition of ubiquitine, proteosomes and other cellular effectors that downregulate p53 level or function (87). Furthermore, drugs are presently designed to reactivate w.t. p53 functionality in tumors harboring inactive p53 mutants, by restoring their w.t. conformation (88–90). Molecular reagents capable of introducing w.t. p53 into tumors with deletion of the two p53 alleles or with two mutated p53 alleles, are also currently under intensive research (91–94).

p53 suppression as an oncogenic strategy of tumor viruses

Several tumor-inducing human viruses, including certain small DNA viruses (adenoviruses, polyomaviruses, papillomaviruses 16 and 18, and hepatitis B and C viruses), large DNA viruses (cytomegalovirus, herpes virus 6 and 8, Epstein-bar virus) (95) and human retroviruses (HTLV-1 and HTLV-2) (96), adopted various mechanisms for p53 downregulation by their virally encoded oncoproteins as part of their oncogenic potential. This review will focus on the various mechanisms proposed for p53 downregulation by the HTLV-1 Tax oncoprotein.

p53 status in ATL and HTLV-1 transformed cells

In contrast to most other human tumors, the overall frequency of mutated p53 in ATL patients is low. However, mutations in p53 and several other cell cycle and apoptosis regulating factors are found at a markedly higher frequency in patients with acute/lymphomatous ATL than in patients with chronic/smoldering ATL. This led to a postulation that mutations in these proteins are rather associated with the progression to the more severe stages of ATL than with the initial clinical stages of this malignancy (97,98). Yet, the leukemic cells of most ATL patients and HTLV-1 transformed cells contain elevated levels of functionally inactive w.t. p53 protein (26,39,99–102). It has been demonstrated that the HTLV-1 Tax oncoprotein alone is sufficient for abrogating the transactivating function of p53 and for its stabilization without direct binding between these two proteins (26,39,103–105). Several different mechanisms have been proposed, so far, for Tax-induced p53 inactivation, which are probably cell-type dependent (26,106).

Tax inhibits p53 function by its constitutive phosphorylation at specific serine residues

Brady’s group (107) analyzed the status of p53 protein in several HTLV-1 transformed cell lines and found it to be hyperphosphorylated at serine-15 and serine-392, whereas no such phosphorylation was detected in non-infected control cells. It was noted that this phosphorylation had no effect on the sequence-specific DNA binding of p53 despite its conformational changes detected by chymotryptic digest. However, the DNA-bound phosphorylated p53 failed to interact with the basal transcription factor TFIIID, and this failure proved to result from the serine-15 phosphorylation.
Furthermore, this phosphorylation also prevented the interaction of p53 with MDM2 and led, in this manner, to p53 stabilization. Interestingly both impairments could be restored by specific phosphorylation at serine-37. This finding indicates that p53 interactions with TFIID and with MDM2 are both regulated by the phosphorylation status of these two serine residues. Notably, however, in contrast to this observation, Ariumi and his colleagues (105) found in their experiments that phosphorylation of serine-15 was not the major cause of Tax-mediated p53 functional inactivation and that this inactivation was actually exerted through a cAMP response element binding protein (CREB)-associated pathway.

Notably, the above-mentioned indications for conformational changes in p53 protein of the HTLV-1-infected cells are inconsistent with earlier data reported by Brady’s group (101) and other laboratories (104,108), demonstrating that p53 of several different HTLV-1 transformed or immortalized cell lines preserves its w.t. conformation. This discrepancy might be attributed to the different methods used to determine p53 conformation. In the earlier studies p53 conformation was assessed by testing its recognition by different anti-p53 monoclonal antibodies (101,104,108).

These studies demonstrated that p53 of the HTLV-1-infected cells could be immunoprecipitated by the pAb1801 antibody, which recognizes both w.t. and mutated p53 conformations, and by pAb1620 antibody, which recognizes only the w.t. form, but not by the pAb240 antibody, which recognizes only mutated p53 conformation. Brady’s group further substantiated this notion by and by RT–PCR sequence analysis of the p53-encoding mRNA (101).

Role of NF-κB in Tax-induced p53 functional inactivation

Activation of NF-κB accounts for a substantial part of Tax oncogenic potential, since transcription factors of the NF-κB family are involved in a wide variety of normal and oncogenic cellular processes (16,109). It was, therefore, of interest to elucidate whether these transcription factors are involved also in Tax-induced p53 functional suppression. Using different Tax mutants, Brady’s group has shown in one of their studies (110) that p53 suppression can be induced by the TaxM47 mutant, which activates NF-κB pathway but not the CREB/ATF (activating transcription factor) pathway, and by Tax(V89A), which does not bind CBP and p300. However, no such suppression is induced by TaxM22, which activates the CREB/ATF pathway, but not the NF-κB pathway. In addition, they have shown in this study that p53 inactivation by w.t. Tax is abrogated by a negative dominant IκBα mutant but not by overexpression of p300 or CBP. These data indicate that
Tax exerts its p53 inactivating effect via an NF-κB associated pathway, whereas neither the CREB pathway nor the p300/CBP co-activators are involved in this effect. Furthermore, the authors have demonstrated in this study that NF-κB pathway was involved also in the above-mentioned Tax-induced p53 phosphorylation at serine-15 and serine-392. Subsequent experiments revealed that Tax could not inhibit p53 function in p65(Re1A)-knocked out mouse embryo fibroblasts (p65-KO MEF), although it could interact, in these cells, with the IKKγ/NEMO subunit and activate the IKKα and IKKβ subunits. These findings suggest that p65(Re1A) activation is essential for p53 inactivation by Tax. This notion was further substantiated by showing that ectopic expression of p65(Re1A) restored Tax ability to inactivate p53 in these p65-KO cells. However, p65(Re1A) mutant lacking the C-terminal transactivation domain was incapable of this restoration, indicating that p65(Re1A) transcriptional function was essential for this effect. Several studies have demonstrated that p300/CBP are required for p53 transcriptional activity (111–115) and that 65(Re1A) inhibits the transcriptional function of p53 by sequestering these co-activators (116–119). Although these findings can provide a reasonable explanation for the above described p65(Re1A)-mediated p53 inactivation by Tax, they are strictly contrasting the finding of Brady’s group that CBP is not involved in Tax-induced p53 inactivation (110). Furthermore, Brady’s group has ruled out the possibility that the above-noted p65(Re1A)-induced p53 functional suppression resulted from inhibition of p53 formation by demonstrating that in p65-KO MEF, p65(Re1A) actually stimulates the transcriptional expression of the p53-encoding gene (110). Other studies have demonstrated an activation of the p53 gene expression by p65(Re1A) in several other cell lines as well (120–123). It seems reasonable to speculate that this transcriptional stimulation partially accounts for the elevated p53 level detected in HTLV-1-infected cells. However, it does not explain the critical requirement of p65(Re1A) transcriptional function for Tax-induced p53 phosphorylation and its inactivation.

More recently Brady’s group (124) has demonstrated that Tax induces a physical binding of transcriptionally active p65(Re1A) to p53 and that this binding requires the above-mentioned p53 phosphorylation at serine-15 and serine-392. Consistent with the transcriptional inactivation of p53, no binding of TFIID to this complex has been detected. Notably, these authors have shown that the p65(Re1A)-p53 complex represses the transcriptional expression of the MDMP2-encoding gene by binding to the p53-responsive element residing in its promoter. This repression may account for the stabilization of p53 in HTLV-1-infected cells. However, it is contrasting the data of Takemoto et al. (100). These authors could not detect MDM2 protein in HTLV-1-infected cells. However, they found that MDM2 level could be restored by treatment with a proteosome-specific inhibitor and concluded that the absence of MDM2 in these cells resulted from its enhanced proteosomal degradation rather than from inhibition of its transcriptional or translational expression.

Using IKKα−/− and IKKβ−/− MEF, Brady’s group has shown, in a subsequent study, that p53 inactivation by Tax and the associated phosphorylation of p65(Re1A) at serine-536, depend on Tax-induced activation of IKKβ (125). It is possible that this specific phosphorylation is essential for the binding of p65(Re1A) to p53 reported in their earlier study (124). Strikingly, however, in contrast to their earlier evidence that Tax-induced p53 inactivation requires a transcriptionally active p65(Re1A) (110), in this latter study they show that transcriptional activity of p65(Re1A) is actually not needed for its involvement in p53 inactivation (125).

Next, Brady’s group identified an additional player in the mechanism of Tax-induced p53 inactivation. They found that Tax-activated Akt was involved in p53 suppression (126). Akt is a serine/threonine protein kinase, which is molecularly related to both protein kinase A (PKA) and protein kinase C (PKC) and is, therefore, termed protein kinase B (PKB) (127). Blocking Akt activity prevents both, the activation of p65(Re1A) by Tax and the Tax-mediated p53 stabilization and functional inactivation, while increasing the MDM2 gene expression (126) which is, otherwise, suppressed by p53 (128). In addition, blocking Akt activity prevents also the above-mentioned IKKβ-mediated p65(Re1A) phosphorylation at serine-536 (126).

An interesting observation has been reported recently by Dreyfus et al. (129), showing that IκBα binds to p53 and inhibits its transcriptional function. Since p65(Re1A) is a transcriptional activator of IκBα gene expression (130,131), it can be speculated that Tax-activated p65(Re1A) may contribute to the Tax-induced p53 functional inactivation by elevating the level of newly formed IκBα, which can, in turn, bind to p53.

Another possible mechanism by which p65(Re1A) can contribute to the Tax-induced p53 inactivation involves its binding to 53BP2 protein (132). This protein binds to p53 and activates its transcriptional activity (133). It is, therefore, possible to speculate that the Tax-activated p65(Re1A) may be involved in Tax-mediated p53 inactivation by 53BP2 sequestration. Such a mechanism has been demonstrated, for example, to be utilized by the hepatitis C virus core protein (134).

**Role of CREB/ATF in Tax-induced p53 functional inactivation**

In re-assessing the role of the CREB/ATF pathway in Tax-induced p53 inactivation, Brady’s group conducted a comparative study in various cell types (106). They found that while in human T-cells Tax inactivated p53 through an NF-κB pathway, in certain human fibroblasts this inactivation was mediated by a CREB/ATF-associated pathway which did not involve phosphorylation of p53 at serine-15 and serine-392. Moreover, in one of the tested cell lines they noted that ectopic expression of p300 but not of p/CAF restored the activity of Tax-repressed p53. The authors interpreted these data as suggesting that Tax can utilize different pathways for p53 inactivation which seem to be cell-type dependent. Miyazato et al. (135) have shown that in mouse fibroblast 53 protein inactivation by Tax was independent of NF-κB or of p300/CBP. In contrast, however, Mulloy et al. (136) have demonstrated that the CREB/ATF pathway mediates Tax-induced inactivation in both fibroblasts and human T-cells.

**p53 functional inactivation by Tax sequestration of p300/CBP**
p300, CBP and p/CAF are essential co-activators required for the transcriptional activating function of many cellular and viral transcription factors (137–139). Although Tax activates the expression of numerous genes, it does not do that by direct binding to specific DNA sequences in the promoters of these genes. Instead, it binds to other transcription factors and facilitates their sequence-specific DNA binding and enhances their transcriptional activity (16). Tax binds p300/CBP (140–143) and p/CAF (144,145). When Tax binds to transcriptional...
factors that require these co-activators, Tax facilitates their transcriptional activity by linking them to the co-activators (139,140,143–146). On the other hand, the transcriptional activity of other p300/CBP-dependent transcription factors, which do not bind to Tax, is rather suppressed by Tax due to its sequestration of these co-activators (139,147–152). As noted above, p53 transcriptional activity depends on p300/CBP (111–115), whereas Tax does not bind to p53 (105). In line with these notions, several studies have demonstrated that Tax inactivates p53 transcriptional function by p300/CBP sequestration (105,106,153,154). Interestingly, Meertens et al. have shown that Tax of HTLV-2, which is closely related to HTLV-1, utilizes CBP, but not p300, for inactivating p53 (155).

p65 and p53 regulate each other’s function through competition on p300/CBP

Several studies have demonstrated that p65 is also involved in p300/CBP mediated regulation of p53 function. It has been shown, in-fact, that both factors regulate the function of each other by competition over the limited p300/CBP cellular pool (26,39,117–119).

Conclusive comments and perspectives

ATL is an aggressive malignancy with short survival time of the patients, ranging between 6 and 24 months and it has a poor prognosis because of the leukemic cell resistance to chemotherapy. Therefore, intense research is presently conducted in many laboratories, aimed to develop novel therapeutic approaches for this malignancy. Since Tax plays a central role in ATL genesis due to its wide range of activities that contribute to its oncogenicity, Tax may serve as a target for developing new drugs with capacity of blocking these effects.

Targeted therapeutic induction of p53-mediated cell-cycle arrest and apoptosis could have been an efficient way to eliminate cancer cells. However, most human tumors carry inactive mutated p53, which render them resistant to such therapy. To overcome this obstacles, intensive effort is currently concentrated on developing small molecules and peptides that can restore the normal function onto the mutant p53 proteins (88–90). The situation with ATL is different in this respect, since the leukemic cells of most ATL patients carry wt. p53 which is functionally inactivated by Tax. The implication of this Tax-mediated p53 inactivation for ATL genesis is that in this manner Tax can immortalize the virus-harboring T-cells of the HTLV-1-infected individuals and destabilize their genome, so that these cells may progress towards the ultimate leukemic state by a stepwise accumulation of oncogenic mutations. In this review we discussed a number of different mechanisms proposed for the Tax-induced p53 inactivation, which are schematically summarized in Figure 2. Each of them includes a cascade of multiple events carried out by several regulatory factors. Blocking any one of these events alone, is sufficient for abrogating p53 inactivation by Tax. This situation is encouraging because when a sufficient number of different drugs capable of blocking different events in the p53 inactivation cascades become available they can be used for ATL treatment with various combinations, which will minimize the emergence of resistant cancerous cells to such treatment.


