Human T-lymphotropic virus proteins and post-translational modification pathways

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Abstract

Cell life from the cell cycle to the signaling transduction and response to stimuli is finely tuned by protein post-translational modifications (PTMs). PTMs alter the conformation, the stability, the localization, and hence the pattern of interactions of the targeted protein. Cell pathways involve the activation of enzymes, like kinases, ligases and transferases, that, once activated, act on many proteins simultaneously, altering the state of the cell and triggering the processes they are involved in. Viruses enter a balanced system and hijack the cell, exploiting the potential of PTMs either to activate viral encoded proteins or to alter cellular pathways, with the ultimate consequence to perpetuate through their replication. Human T-lymphotropic virus type 1 (HTLV-1) is known to be highly oncogenic and associates with adult T-cell leukemia/lymphoma, HTLV-1-associated myelopathy/tropical spastic paraparesis and other inflammatory pathological conditions. HTLV-1 protein activity is controlled by PTMs and, in turn, viral activity is associated with the modulation of cellular pathways based on PTMs. More knowledge is acquired about the PTMs involved in the activation of its proteins, like Tax, Rex, p12, p13, p30, HTLV-I basic leucine zipper factor and Gag. However, more has to be understood at the biochemical level in order to counteract the associated fatal outcomes. This review will focus on known PTMs that directly modify HTLV-1 components and on enzymes whose activity is modulated by viral proteins.

INTRODUCTION

Infection by human T-lymphotropic virus type 1 (HTLV-1) can cause a fatal hematopoietic malignancy (adult T-cell leukemia/lymphoma, ATLL) and a debilitating neurological disorder (HTLV-1-associated myelopathy/tropical spastic paraparesis, HAM/TSP).

Post-translational modifications (PTMs), like phosphorylation[1,2], ubiquitination[2,3], SUMOylation[4] and others[5], determine the fate of many cellular proteins altering their conformation (charge or hindrance), subcellular localization[6,7], and cell-cycle-related function[8]. PTMs control also gene and viral expression. Such epigenetic control is governed by PTMs on histones, devoted to the organization of chromatin together with the ATP-dependent chromatin remodeling complexes. In particular, LTR silencing is a mechanism used by cells to repress viral transcription. Histone deacetylation and methylation enforce the promoter silencing condensing the chromatin[9].

Recently, Matsuoka and collaborators[10] found that 68 % of fresh ATLL cell lines contained methylated HTLV-1 LTR and 14 % had fully methylated LTR, suggesting a correlation between the development of leukemia, the reduction of Tax expression and genetic (*tax* gene mutations) and epigenetic (LTR histone deacetylation and methylation) modifications. This evidence is coherent with the over-expression of methyltransferase SUV39H1, which silences HTLV-1 LTR, in transformed and latently infected cell lines[11].

In HTLV-1 infected cells the proviral LTR binds Tax, cyclic AMP response element binding protein (CREB), CREB-2, activating transcription factor (ATF)-1, ATF-2, c-Fos, c-Jun (transcription factors), CREB-binding protein (CBP), the histone acetyl transferase (HAT) p300 (both co-activators) and histone deacetylases (HDACs)[12]. The presence of HATs at the viral LTR coincides with histone (H3 and H4) acetylations at three regions within the proviral genome[12]. Viral transcription is subsequent to p300/Tax binding and following p300-dependent histone acetylation on their N-terminal tails[13,14]. Unexpectedly, the use of a HDAC inhibitor, the depsipeptide, in a murine ATLL model inhibits the tumor growth[15]. Also the chromatin remodeler Brahma-related gene 1 is required for Tax transactivation and acts with the PBAF complex for the nucleosome remodeling[16], where the subunit Baf53, suppressor of transcription, may be phosphorylated and hence displaced from the LTR, activating the transcription (as proposed by Kashanchi F. and collaborators[17]).

In addition to the epigenetic control of viral transcription, PTMs regulate viral protein functions by modifying their subcellular localization, stability, and network of interactions[18-20]. Furthermore, viral proteins recruit and alter the function of enzymes responsible for PTMs, like for example ubiquitin ligases and kinases[21-23], altering cellular pathways controlled by PTMs. Gathering all HTLV-1-related reports in a review become more important now, in the light of the growing interest of the scientific community in the field (15th International Conference on Human Retrovirology, HTLV and Related Viruses, Leuven, Belgium, 2011[17]).

HTLV-1 encodes structural and enzymatic viral gene products, the positive regulatory proteins Tax and Rex along with viral accessory proteins such as p12, p13 and p30[24-27]. In addition the virus encodes the HTLV-I basic leucine zipper factor (HBZ) protein within the minus strand[28,29]. These proteins are subjected to PTMs, as any component of the cellular environment, with crosstalk between different PTMs in the same protein[2]. Here, a compendium of the known viral protein PTMs, relevant to the viral function, is presented together with the set of known PTMs directly activated or inhibited by HTLV-1 components. Effects not obviously attributable to single viral proteins are described first.

HTLV-1 INFECTION PROMOTES PTMS

Cerebrospinal fluid in HAM/TSP

The only documented PTM difference in the cerebrospinal fluid between asymptomatic carriers and HAM/TSP patients is enhanced Tau-protein phosphorylation (Tau is an abundant CNS molecule involved in stabilizing microtubules) and an increase in axonal ubiquitination[30]. Valenzuela and collaborators recently found that cyclin-dependent kinase (Cdk)5 prevents Tau hyper-phosphorylation caused by HTLV-1-infected cell secretions[31], but further studies are needed to understand the relevance of those modifications.
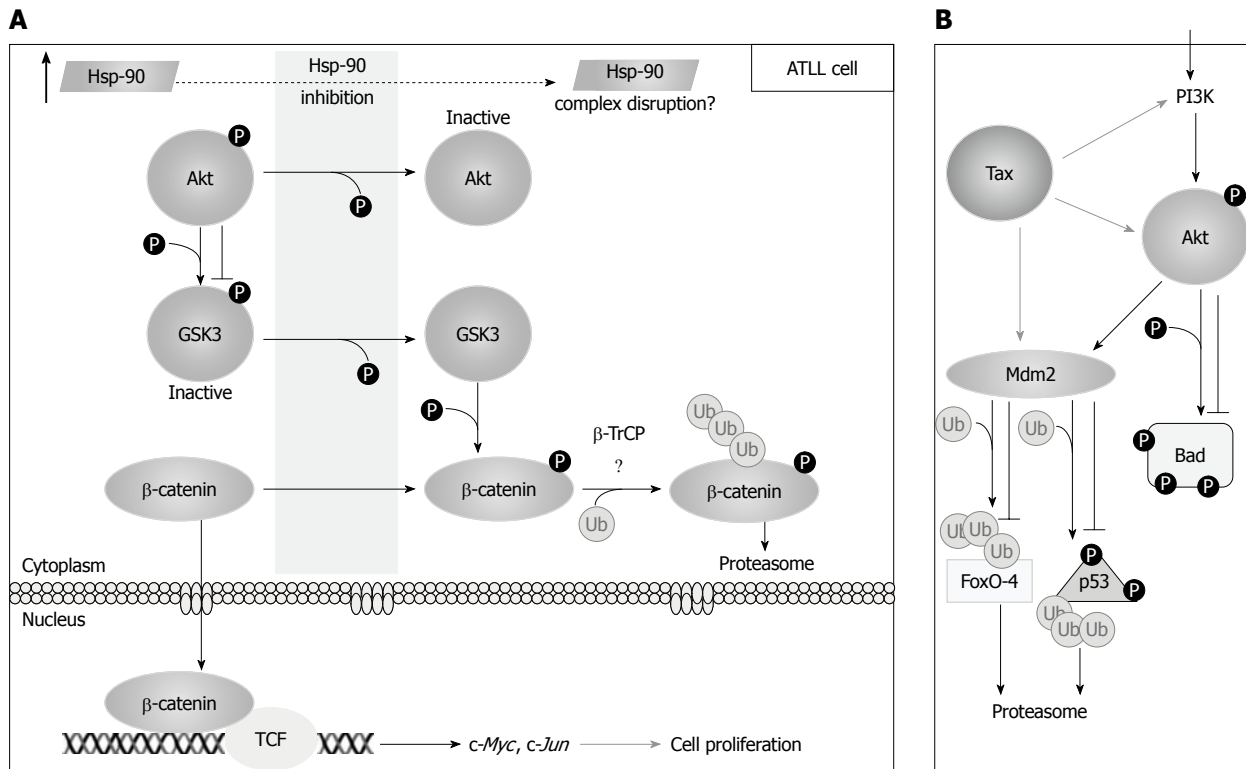
Heat shock protein 90, Akt signaling and Lyn

Heat shock protein 90 (Hsp-90), a molecular chaperone, is overexpressed in HTLV-1-infected cells and primary ATLL cells[32] and is hypothesized to be involved in the stabilization of viral proteins. Interestingly, Hsp-90 inhibition, in ATLL cells, induces cell death and promotes de-phosphorylation and inactivation of Akt with subsequent GSK3 activation, which phosphorylates β-catenin for ubiquitination[33]. In turn, ubiquitinated β-catenin is targeted for degradation, probably by β-transducin repeat-containing protein (β-TrCP)[34]. Otherwise, β-catenin enters to the nucleus and forms with T-cell transcription factor an active complex on target genes, such as c-*Myc* and c-*Jun*[33], inducing cell proliferation (Figure 1A).

Collectively these results suggest an involvement of Hsp-90 overexpression in maintaining activation of Akt signaling, hence promoting cell survival[35]. Interestingly, within the setting of B-cell lymphocytic leukemia (BCLL), Hsp-90 stabilizes and activates constitutively Lyn[36], a member of the Src family kinases (SFK) downstream the T cell receptor (TCR) pathway and upstream Akt pathway. Active Lyn appears to determine the activation of Janus family tyrosine kinase 3 (Jak-3) and signal transducer and activator of transcription 5 (Stat-5)[37], important during HTLV-1 infection (see relevant section below). The disruption of the complex containing activated Lyn and Hsp-90 revealed to be a potential pharmacological target for BCLL therapy[36]. This notion could be transposed in the setting of ATLL, considered that Lyn replaces Lck in HTLV-1 infected T cells[37]. It will be of great interest for therapy, to corroborate experimentally the hypothesis that Hsp-90 and Lyn sinergically cooperate at the onset of ATLL to promote T cell activation and proliferation.

TCR signaling

TCR pathway promotes T cell proliferation. Upon TCR engagement, Lck and Fyn phosphorylate immunoreceptor tyrosine-based activation motifs, which act as docking sites for Syk-family kinases, primarily ZAP-70 in T cells. Activated ZAP-70 can in turn phosphorylate the linker for activation of T cells (LAT), thus triggering a number of cascades, culminating in T-cell activation[38].

The signal transduction is not efficient in HTLV-1-infected cells for the progressive decrease in CD3 expression[39]. While CD3 is reduced, the TCR pathway is not inactivated. Recently, authors provided evidences that, in HTLV-1-infected cells, a switch occurs between Lck and Lyn expression, effector typically expressed in B cells[37,40]. Furthermore, FynB and Syk, poorly represented in T

Figure 1 Diagrams representing Akt signaling alterations in adult T-cell leukemia/lymphoma cells under treatment with heat shock protein 90 inhibitors (A) and Tax contribution on Akt signaling (B). A: In adult T-cell leukemia/lymphoma (ATLL) cells, geldanamycin-derivates inhibition of heat shock protein 90 (Hsp-90) promotes Akt inactivation and hence GSK3 activation for β-catenin degradation, and cell death; B: Activation of Jak/Stat or T cell receptor signaling or Tax expression promote phosphatidylinositol-3-kinase (PI3K) activation, Akt activation and Mdm2 activation with subsequent inactivation of Bad and degradation of forkhead box O-4 (FoxO-4) and p53. Question marks represent hypothesis; P: Phosphorylation; Ub: Ubiquitin; black thin arrows represent activation, grey thin arrows represent enzymatic reactions; dashed arrow, inhibition; protein overexpression in infected cells is represented by thick arrow.

cells, are overexpressed, where ZAP-70 is absent[41]. It is still unclear why the T cell is prompted to express effectors present in B cells, namely Lyn, FynB and Syk, but, intriguingly, it suggests a possible parallelism between ATLL and BCLL.

Downstream ZAP-70/Syk and LAT, activated Ras triggers many Ser/Thr kinases, including ERK, JNK and MAPKp38[42], members of the mitogen-activated protein kinase family (MAPK). MAPK are activated in response to DNA damage[43] and decide the fate of the cell, promoting either repair of the damage or, if the damage is too severe to be repaired, apoptosis. Yamaoka and collaborators recently showed that protein geranylgeranylation is responsible for ATLL cells viability[44]. This contribution of the geranylgeranylation to the ATLL cell survival could be correlated with PTM geranylgeranylation of Ras, a necessary step for its translocation to the plasma membrane, where it is functionally active[45]. In Tax-expressing cells, Tax modulates and increases the phosphorylation (activation) of Ras and ERK kinases, thus facilitating cell proliferation[46]. In this context, Tax protects from apoptosis cooperating with Ras in activating ERK signaling[47]. Furthermore, Tax physically interacts, through its PDZ binding motif, and blocks the function of Erbin, negative regulator of the Ras-Raf-ERK pathway, enhancing the cell proliferation and the protection from apoptosis[48].

Collectively, these results indicate that during HTLV-1 infection TCR response to stimuli is hampered and, at the same time, downstream effectors not belonging to T cells are upregulated, promoting constitutive activation of T cells. Interestingly, p12, p13, p30 and Tax possess putative SH3 binding motifs, whose possible interaction with SFK is not fully explored. Further studies on viral TCR perturbation could guarantee access to new therapeutic approaches.

Jak/Stat

Jak/Stat activation appears to be associated with the replication of primary ATLL cells[49]. In particular, it has been previously shown in patients that more than 65% of T cells possess constitutive DNA-binding activity of one or more Stat proteins and this activity seems to persist over time[49].

Interleukin (IL)-2 stimuli rapidly induce tyrosine phosphorylation of intracellular substrates, including its receptor IL-2Rβ, Jak-1 and -3 and Stat elements[50]. HTLV-1-immortalized cell lines often exhibit constitutive Jak/Stat phosphorylation and activation[51], differently from HTLV-2-infected cells[52]. In T cells immortalized with a molecular clone of HTLV-1, the Jak/Stat signaling during IL-2 stimulation seems to promote HTLV-1 transmission[53].

Interestingly, in a rabbit HTLV-1-infected cell line, bearing truncated forms of p13 and p30 (RH/K34, see

relevant section below), the Jak kinase Tyk2 and Stat3 are constitutively phosphorylated and activated[54]. Surprisingly, in other cell lines, HTLV-1-infected 293T and HeLa cells, phosphorylation and activation of Tyk2 and of Stat2 are reduced, for the reduction of the IFNα signal transduction[55]. In human T cells, inhibition of Jak2/Jak3 does not seem to block the proliferation of HTLV-1-transformed and IL-2-independent cells, Hut102B2 and MT-2[56], since the Jak/Stat pathway seems functionally redundant for proliferation. In contrast with that finding, Waldmann and collaborators recently demonstrated that CP-690,550, a specific inhibitor of Jak3, suppresses proliferation of peripheral blood mononuclear cells (PBMCs) from ATLL and HAM/TSP patients, promoting the drug as an effective therapeutic agent in patients whose cells require Jak3/Stat5 stimulation by γc cytokines (IL-2, IL-9, and IL-15) to proliferate[57].

In turn, the suppressor of cytokine signaling (SOCS), inhibitor of Jak/Stat signaling, is upregulated in HTLV-1$^+$ CD4$^+$ T cells from HAM/TSP and asymptomatic carriers, and correlates with viral replication[58]. In particular, SOCS-1 inhibits IFN expression and IFN-stimulated gene expression[58]. SOCS adaptors are bound to cullin-2 and -5 through elongin C and elongin B proteins to constitute ubiquitin ligase complexes[59]. At the 15th International Conference, Harhaj E. W. and collaborators showed that, in the presence of Tax, the expression and the stability of SOCS-1 are highly increased[17]. The independence from cytokines in infected cells could be associated to the inhibition of the upstream effectors through a mechanism requiring ubiquitination that should be further investigated.

Cell cycle

The cell cycle is controlled by a cyclic series of activation and repression though phosphorylation cascades and is profoundly altered during HTLV-1 infection[60]. HTLV-1-infected, as well as ATLL and HAM/TSP cells, exhibit overexpression of the Cdk inhibitor p21/Waf1[61]. p21/Waf1 interacts specifically with the Cdk2/cyclin A complex and reduces the phosphorylation level of the retinoblastoma protein (Rb), a tumor suppressor, and prevents the release of E2F-1, transcription factor required for G1 and S phases[62].

p27/Kip1 is an other Cdk inhibitor and blocks the Cdk2/cyclin E complex. p27/Kip1 upregulation arrests the cell cycle in G1 upon serum starvation[63]. Interestingly, HTLV-1-transformed cells exhibit a higher level of the p27/Kip1 than the HTLV-1-immortalized cells[63].

Additionally, Aurora kinases are overexpressed in HTLV-1 positive cells. Aurora kinases play an important role in cell cycle control, since their inhibition arrests mitosis and serves to induce apoptosis[64]. Aurora A is overexpressed in HTLV-1-infected and ATLL cells. siRNA knockdown experiments and chemical inhibition of Aurora A has been reported to lead to growth suppression in HTLV-1-infected cell[65]. The ubiquitin ligase CHFR regulates the turnover of Aurora A, and in infected cells

CHFR level is reduced by methylation and subsequent silencing of its promoter[65]. Aurora B is overexpressed in HTLV-1-infected cells and its specific inhibition induces apoptosis only in HTLV-1-infected cells compared to non-infected, through overexpression of p21/Waf1 and p53[66].

The overexpression of Cdk inhibitors is concurrent with the upregulation of Aurora kinases, with the p30-related delay of entry into S phase and the Tax-related delay of S-G2-M transition (Figure 2). The contrasting results on the cell cycle alterations attributed to HTLV-1 are far from being understood and evaluated for therapy purposes.

## HTLV-1 P40, THE TRANS ACTIVATOR OF TRANSCRIPTION TAX

Several pathways are activated or repressed after HTLV-1 infection and single viral proteins act to mediate with specific PTMs and interactions these downstream effects. The most important is the transactivator of transcription, Tax. In Figure 3 is represented a model for Tax localization depending on its PTMs.

Tax is a pleiotropic protein with a predominately nuclear subcellular localization that performs multiple functions *via* protein-protein interaction. Several Tax PTMs were described, including ubiquitination[67], SUMOylation[67], phosphorylation[68-70] and acetylation[71]. Probably, different Tax species coexist at any time during the cell cycle and in different locations, depending on the cell type (see for example its ubiquitination state in[72]).

Recently, Kfoury *et al*[67] showed by live confocal microscopy, that Tax molecules shuttle from SUMO-enriched nuclear bodies to ubiquitin-enriched perinuclear centrosomal aggregates close to *cis*-Golgi (Figure 3). Those molecules are likely PTM Tax variants, considered that the SUMOylation and ubiquitination-defective Tax K4-8R mutant is not recruited to these subcellular compartments[67].

Biochemical coupling of the transcription factor nuclear factor κB (NF-κB) to antigen and co-stimulatory receptors (CD3 and CD28, respectively) is required for the temporal control of T-cell proliferation and is modulated by viral proteins[38]. Tax activates NF-κB by interacting and constitutively stimulating the activity of the I-κB kinase (IKK)[73], which usually is transiently activated through the TCR/CD3 and CD28 engagement. Tax SUMOylation sends the IKKγ (known as NEMO) to the centrosome[67] and is involved in the formation of Tax-related SUMO-enriched nuclear bodies and in the recruitment therein of RelA (known as p65), resulting in transcriptional activation[74,75]. On the contrary, ubiquitin addition appears to modify Tax in a proteasome-independent manner from an active to a less-active transcriptional form[76], even though Tax ubiquitination is critical for the relocalization of IKK complexes to perinuclear hot spots coinciding with Golgi[77]. Counterintuitively, Nasr *et al*[74] have shown that Tax ubiquitination and SUMOylation are dispens-
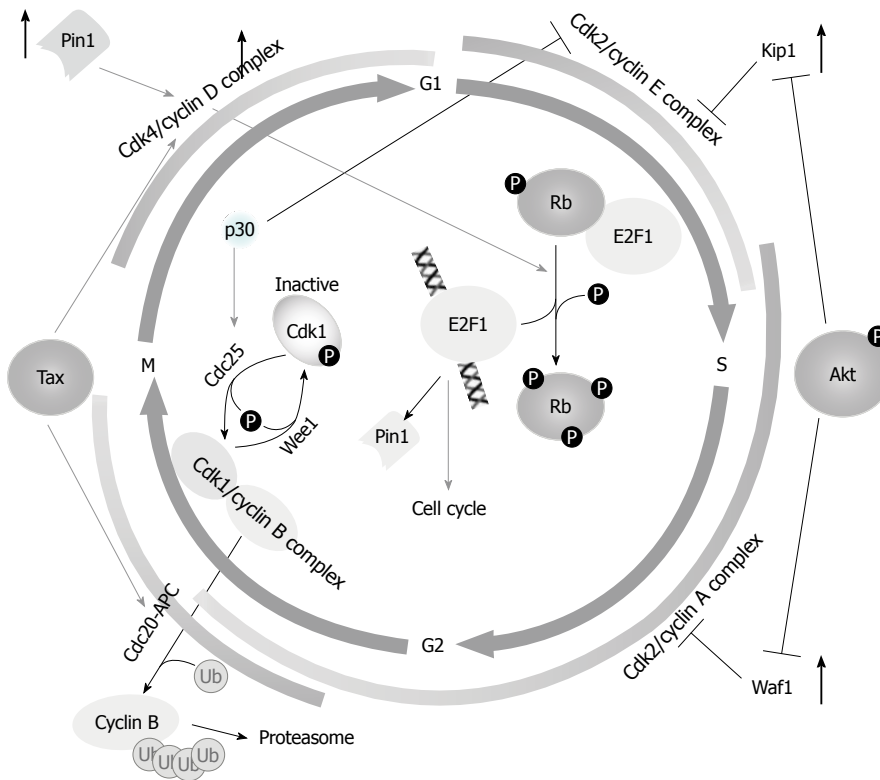
Figure 2 Cell cycle deregulation in infected cells and Tax-expressing cells. G1 progression begins with cyclin D expression, formation of the Cdk4/cyclin D complex that in turn phosphorylates retinoblastoma protein (Rb), inducting E2F1 transcription. Cyclin D is upregulated by Tax and by Ras-Raf-ERK stimulation. It is stabilized by interaction with Pin1, overexpressed in infected cells. G1/S phase transition, promoted by the Cdk2/cylclin E complex is antagonized by p30 and by over-expressed p27/Kip1. Overexpressed p21/Waf1 inhibits Cdk2/cyclin A-promoted S/G2 transition. In turn, overactivation of Akt inhibits p27/Kip1 and p21/Waf1 functions. Tax promotes, through cdc20-associated anaphase promoting complex (Cdc20-APC) activation, cyclin B degradation. At the same level acts p30, that activates Cdk1 by Cdc25-dependent de-phosphorylation. Cyclic stability of Cdk/cyclin complexes are represented with fading arcs; P: Phosphorylation; Ub: Ubiquitin; black thin arrows represent activation, grey thin arrows represent enzymatic reactions; protein overexpression in infected cells is represented by thick straight arrows.

able for its transcriptional activation of CREB-dependent genes. Indeed, Tax mutants for Lys-85 or Lys-88, poorly contributing to Tax ubiquitination[72], are unable to recruit CBP/p300[78].
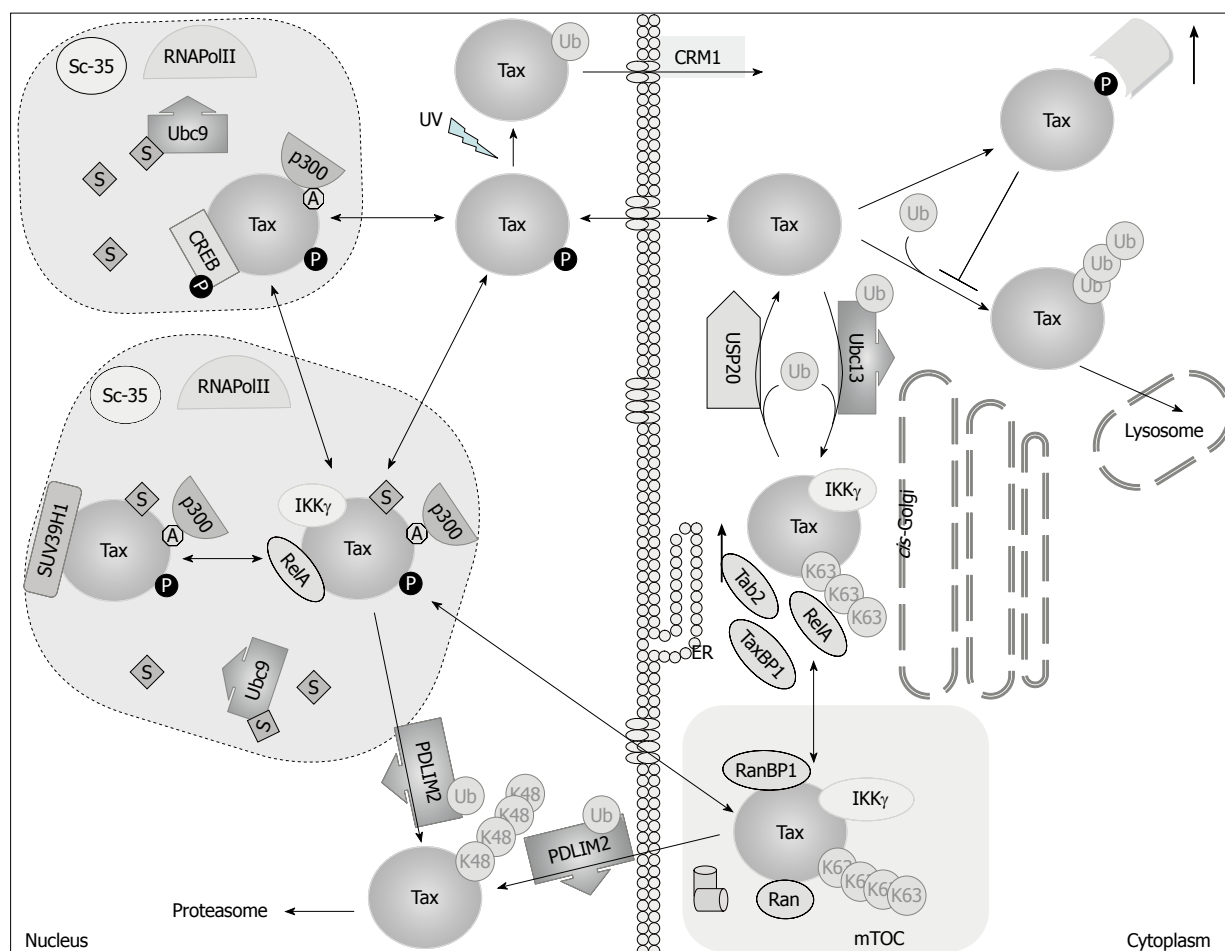
Tax ubiquitination

Only recently, it was demonstrated that Tax ubiquitination contributes to viral transcriptional activity, as a prerequisite to recruit IKKγ in the centrosome (known as MTOC, microtubule-organizing center) and in SUMO-enriched nuclear bodies[67] (Figure 3). Ubiquitination is also involved in Tax binding with IKK complex[79] and the upstream transforming growth factor-β-activated kinase 1 (TAK-1) binding protein 2 (TAB2[80]). The authors showed also that overexpression of TRAFs strongly induces Tax ubiquitination[80] (TNF receptor-associated factors, adapter proteins that mediate cytokine signaling[81]).

Two forms of ubiquitinated Tax coexist. The cytoplasmic (Lys-63-branched and Lys-48-branched poly-ubiquitinated, K63 and K48) Tax is stable, as its amount is only modestly increased by proteasome inhibition. The nuclear K48 poly-ubiquitinated Tax is not stable and only under proteasome inhibition is stabilized[82]. Ubiquitin-mediated proteolysis is highly selective and tightly regulated. The specificity of the process relies on

the ubiquitin-protein ligases, which directly bind both the substrate and the ubiquitin-conjugating enzymes[59]. Tax K48 poly-ubiquitination involves PDLIM2, an ubiquitin E3 ligase with PDZ and LIM domains[82]. PDLIM2, without the involvement of Tax PDZ binding motif, binds to and recruits Tax from both the cytoplasm and the Tax-related nuclear bodies into the nuclear matrix where the poly-ubiquitinated Tax is degraded by the proteasome[82] (Figure 3). Interestingly, Xiao G. and collaborators have shown that PDLIM2 expression is epigenetically down regulated in primary ATLL cells[17].

Furthermore, it was demonstrated that Tax is mainly K63 poly-ubiquitinated and that this ubiquitination depends on the interaction with the ubiquitin-conjugating enzyme Ubc13, which proved critical for interaction with IKKγ and canonical and non canonical NF-κB activation[83] (Figure 3). Also the NEMO-related protein Optineurin has been shown to interact with ubiquitinated Tax in HTVL-1-infected cells and to promote with Tax1BP1 the NF-κB activation[84]. Interestingly, the Tax mutant M22 is deficient in NF-κB activation, is less ubiquitinated and does not interact with Ubc13[72,83]. Consistently with this results, the ubiquitin-specific peptidase USP20 inhibits Tax transactivation activity, promoting its de-ubiquitination[85] (Figure 3).

Figure 3 Diagram representing Tax localization and function controlled by protein post-translational modifications. After translation, Tax could be phosphorylated at Ser-160 and hence stabilized by Pin1, preventing its poly-ubiquitination with subsequent lysosomal degradation (top right). Tax is K-63 poly-ubiquitinated by Ubc13, promoting its relocation to a region between endoplasmic reticulum (ER) and *cis*-Golgi, where it interacts with I-κB kinase (IKK)γ, the overexpressed Tab2 and RelA. UPS20 can de-ubiquitinate Tax (centre). From *cis*-Golgi, Tax shuttles to MTOC, where it interacts with IKKγ, RanBP1 and Ran. From MTOC, Tax shuttles to Tax-related nuclear bodies containing Sc-35 and RNAPolII. There, it is acetylated by p300 and SUMOylated, probably by Ubc9 (left). In nuclear bodies, Tax interacts with p300, IKKγ, RelA and the methyltransferase SUV39H1. Furthermore, Tax interacts also with phosphorylated cyclic AMP response element binding protein (CREB). PDLIM2 has proven to cause K48 poly-ubiquitination of Tax, promoting its nuclear proteosomal degradation after translocation from nuclear bodies and MTOC (bottom left). Tax displays its nuclear functions when Ser-300 or Ser-301 are phosphorylated (nucleus). After UV treatment, mono-ubiquitinated Tax is exported to the cytoplasm through CRM1 (top). Thin arrows represent relocation or modification; P: Phosphorylation; S: SUMO1; A: Acetylation; Ub: Ubiquitin; K48 or K63: Chains of ubiquitin; UV: Ultraviolet light exposure; protein overexpression in infected cells is represented by thick arrows.

Additionally, UV irradiation has been demonstrated to induce Tax mono-ubiquitination at the SUMOylatable Lys-280 and Lys-284 residues, which promotes Tax dissociation from nuclear bodies and Tax nuclear export through the CRM1 pathway[86] (Figure 3).

Tax phosphorylation
Interestingly, Ser160-phosphorylated Tax is stabilized by the interaction with Pin1, a prolyl isomerase, which inhibits Tax ubiquitination and its subsequent lysosomal degradation[87,88]. Ser-160 phosphorylation happens during the mitosis, but the kinase involved is presently unknown. Remarkably, Pin1 stabilizes other phosphorylated proteins: c-Jun and c-Fos, enhancing the activator protein 1 (AP-1) transcriptional activity; β-catenin, enhancing cell proliferation; RelA, enhancing the NF-κB activity; and cyclin D1, whose transcription is promoted by the three mentioned transcription factors. In turn, Pin1-stabilized cyclin D1 is retained in the nucleus and promotes G1-S transition[89]. Pin1 overexpression disrupts the tight coupling of centrosome duplication with DNA synthesis, which leads to centrosome amplification, chromosome instability (aneuploidy) and oncogenesis both *in vitro* and *in vivo*[90]. The overexpression of Pin1 in Tax-expressing HTLV-1-transformed cells[88] is consistent with previous characterizations showing that Pin1 expression is tightly regulated and correlates with oncogenesis[89]. Finally, Tax with CREB physically associates the promoter of cyclin D1 *in vivo*, upregulates the gene and promotes G1-S transition[91]. On the other hand, Tax delays the S-G2-M transitions, favoring the degradation of cyclin A, cyclin B1 and securin. Cyclin B1 is targeted for ubiquitination by Tax-activated cdc20-associated anaphase promoting complex (cdc20-APC), an E3 ubiquitin ligase that controls metaphase to anaphase transition and, bound to Tax, becomes active before the cellular entry into mi-

tosis[92] (Figure 2). The authors proposed that aneuploidy and chromosomal instability are direct consequences of Tax activation of cdc20-APC. Thus, we can hypothesize that Pin1 and Tax work in a synergistic manner to promote tumorigenesis, path not explored for therapeutic purposes yet.

Tax is known to be phosphorilated since 1988[93]. Research attributed the serine residues were the target of this PTM[94] and useful studies on serine point mutations were performed providing evidence to support this idea[95,96]. Disappointingly, specific Tax phosphorylation patterns are associated with the particular cell line employed[97]. At the present, important phosphorylation sites of Tax are known but still some open question remains. Phosphorylation on Tax Ser-300/301 (or on Ser-304 where present) is essential for the transcriptional activation[68]. Phosphorylation on Ser-160 stabilizes Tax during the mitosis and enhances the activation of NF-κB[87]. The kinases involved are unknown and could represent a genuine target for Tax-oriented therapy. Only the pleiotropic and constitutively active CK2 (formerly known as casein kinase 2) is proven to phosphorylate Tax *in vitro*, modulating the functions associated with its C-terminus[70], which is extremely important for the binding, with subsequent inhibition, of class I PDZ-containing proteins, some of them tumor suppressors with scaffold function[98,99].

Furthermore, advanced and highly sensitive techniques, such as tandem mass spectrometry, struggle to detect Ser-300/301 phosphorylation or are entirely unable to detect Ser-160 phosphorylation, important for Tax function[69]. The coverage of N- and C-terminal tagged Tax is incomplete for technical limitations and, surprisingly, threonine phosphorylation predominates. The phosphorylated residues within Tax described to date are Thr-48, Thr-184, Thr-215, Ser-300 or 301 and Ser-336[69]. Interestingly, in functional studies, the authors showed that a phosphomimetic point mutation on the Thr-48, always found phosphorylated, reduced Tax activity on CREB and NF-κB promoters, as if Tax function was always switched off. Mutation of the predominantly phosphorylated residue Thr-215 to the phosphomimetic aspartate led to a severe reduction of Tax activity[69], suggesting again the predominant presence of inactive Tax. Lastly, discordance between peptide identification (Thr-84 peptide) and Tax sequence in Durkin's report generates ambiguity and makes it difficult to draw neat conclusions. Point mutations do not seem to clearly delineate a conclusive definition of the roles of phosphorylated Tax residues.

Due to the complexity of the Tax phosphorylation pattern, the study of multiple point mutations (all-but-one) and mutant expression in lymphocytes could contribute to the comprehension of the actual relevance of each phosphorylation for Tax function. Also the identification of kinases involved in Tax phosphorylation could suggest new therapeutical strategies.

Tax acetylation and SUMOylation
Tax acetylation on Lys-346 is dependent on Tax localiza-

tion in Tax-related nuclear bodies and on its interaction with the transcriptional co-activator p300[71] and subsequent activation of HTLV-1 promoter[13]. The prerequisite of nuclear Tax localization is phosphorylation on Ser300/301 (or on Ser-304 where present[68]). Its localization on Tax-related SUMO-enriched nuclear bodies is followed by SUMOylation (for the presence of the residues Lys-280 and Lys-284[71]) that, in turn, improves Tax acetylation. When SUMOylated Tax is acetylated by p300, its activity on integrated NF-κB promoter is enhanced[71]. In SUMO-enriched nuclear bodies, Tax colocalizes with Ubc9, an E2-SUMO conjugating enzyme with a major nuclear localization and, when ubiquitinated, promotes SUMOylation and de-ubiquitination of IKKγ[67]. One appealing highlighted hypothesis is that Tax is SUMOylated in those Ubc9-enriched bodies.

Tax-2B PTMs
A recent review described the known HTVL-1/2B Tax differences in NF-κB activation and localization, and showed how PTMs affect the two homologue proteins that share 85 % of sequence similarity[99]. HTLV-2B Tax is SUMOylated at lower levels than HTLV-1 Tax and ubiquitinated[100]. Intriguingly, Mahieux R. and collaborators showed at the 15th International Conference that ubiquitinated HTLV-2 Tax is barely detectable but still active on NF-κB pathway, promoting IKKγ and RelA nuclear relocalization[17].

Tax and NF-κB PTMs
The NF-κB pathway is correlated with the oncogenic potential of Tax. IKK activation is involved in both the canonical and non-canonical NF-κB pathways, and one of the main differences between HTLV-1 Tax and the less oncogenic HTLV-2 Tax is the ability to process p100, component of the non-canonical pathway[99]. In HTLV-1-infected T cells, p100 is actively processed, resulting in Tax-mediated p52 overproduction and NF-κB2 activation. Tax acts on p100 indirectly recruiting the IKK complex and directly binding p100, IKKα, IKKγ and the β-TrCP[59]. β-TrCP is an F-box adaptor protein, a component of the ubiquitin ligase SCF (Skp-1, Cullin-1, F-box protein), it determines the substrate specificity for ubiquitination[59] and is involved in Tax-mediated p100 processing. NIK-phosphorylated IKKα and Tax-activated IKKγ phosphorylate p100[101]. Tax increases the interaction between the adaptor β-TrCP and IKKα-phosphorylated p100, favoring p100 Cullin-1-mediated poly-ubiquitination and degradation to the active form p52[102]. Interestingly, β-TrCP is probably associated with the degradation of β-catenin[34], a protein stabilized by Pin1[89], and I-κBα[103].

In Tax-expressing cells, IKKβ is persistently phosphorylated, promoting I-κBα degradation and NF-κB activation[104], and subsequently mono-ubiquitinated by the ubiquitin ligase Ro52, in cooperation with the ubiquitin-conjugating enzyme UbcH5B[105,106]. Mono-ubiquitination redirects IKKβ to the autophagosome[106]. Moreover, Tax

interacts physically with the hypo-phosphorylated NF-κB inhibitor I-κBα, promoting its direct binding to HsN3 proteasome for degradation[107].

It is unclear whether the interaction between the TAK-1 and Tax promotes the phosphorylation and activation of IKKβ[108] or not[109]. Tax activates TAK-1 in HTLV-1-transformed T cells. Tax induces also TAB2 overexpression, a TAK-1 activator[109]. Interestingly, both HTLV-1 and -2B Tax interacts with TAB2 in perinuclear compartments close to *cis*-Golgi[110] (Figure 3). TAK-1 activity seems to be involved in JNK and MAPKp38 activation, the former to activate the ATF-2, the latter to inhibit TAK-1 in a negative loop[109]. Furthermore, during IL-2 deprivation, the viral induction promotes phosphorylation and activation of MAPKp38, in turn activating its downstream CREB and sustaining HTLV transcription in HTLV-1-infected cell[111].

Another candidate for IKK activation is the Tax interactor, MAPK kinase kinase 1 (MAP3K1, known also as MEKK1), a kinase that activates JNK and MAPKp38. MAP3K1 is part of the IKK complex, interacts with Tax and seems to activate IKK itself[104].

There are no doubts that NF-κB pathway is required for the activation and proliferation of infected cells, even though evidences suggest an inhibitory effect of HBZ on the canonical pathway[112]. The activation of the non-canonical pathway should be further explored, considered it is one of the main differences between Tax-1 and its homologue proteins, Tax2B[99].

Tax and Akt PTMs

A growing list of ubiquitin ligases recruited by Tax has been described. An example is Mdm2 ubiquitin ligase. The forkhead box O-4 (FoxO-4), a transcription factor and tumor suppressor situated downstream Akt signaling, is degraded after activation, for Tax-mediated interaction with Mdm2 and subsequent poly-ubiquitination[113].

Mdm2 is also implicated in the degradation of the phosphorylated and hence inactivated p53 tumor suppressor[114,115]. p53 is hyper-phosphorylated in HTLV-1-transformed cells and cannot interact with the transcription factor IID. Tax promotes p53 phosphorylation and inactivation, but it is unclear whether through RelA engagement[116] or through Tax/p53 competition for binding with CBP[117,118]. Also HTLV-2B Tax, but not HTLV-2A Tax, is able to neutralize p53 activity[119] (Figure 1B).

p53 inhibition and cell survival in HTLV-1-transformed cells are linked to the Tax activation of Akt[39] and phosphatidylinositol-3-kinase (PI3K)[120]. Active Akt promotes phosphorylation and inactivation of Bcl-2-associated death promoter protein (Bad), a pro-apoptotic protein[39]. Consistently, PI3K/Akt activation induces resistance to IL-2 deprivation in HTLV-1-infected cells, promoting a progressive resistance to apoptosis[121].

Tax and promoter silencing

Tax/CREB complex recruits the co-activators CBP and p300, promoting the histone acetylation and disassembly of the nucleosomes from the HTLV-1 promoter, facilitating DNA accessibility for the transcriptional machinery. The process is transcription- and ATP- independent, but needs acetyl-CoA and available histone-chaperone Nap1[122,123].

On the other hand, Tax and active HDAC1 form an inhibitory complex on the promoter of Src homology-2 containing protein tyrosine phosphatase 1 (SHP-1), an important negative regulator of the TCR signaling for its ability to antagonize Src kinases[124] and IL-2 signaling[125]. Remarkably, SHP-1 is inactivated in HTLV-1-immortalized (IL-2-independent) cell lines[125] but not in ATLL cells where Tax is not detectable[126]. Not surprisingly, also the TCR effector ZAP-70 is down regulated by Tax expression[41].

Moreover, Tax forms complexes also with both HDAC1 and HDAC3, promoting a negative feedback loop in which Tax transcription is down-regulated through histone deacetylation on the HTLV-1 LTR region[127]. Watanabe T. and collaborators proposed another negative feedback and demonstrated that Tax induces the histone methyltransferase SUV39H1 expression[11]. Tax tethers the enzyme to the Tax-related nuclear bodies where it represses Tax, in a dose-dependent manner, silencing the HTLV-1 LTR promoter[11].

Unexpectedly, the use of a HDAC inhibitor, the depsipeptide, in a murine ATLL model inhibits the tumor growth[15], instead of promoting it, probably for the intervention of the adaptive immunity on the desilenced cells.

Furthermore, HTLV-1 transcription increases after CD2 cross-linking and T cell activation[128]. The CD2 signaling promotes CREB phosphorylation probably with the involvement of both protein tyrosine kinases and protein kinase A[129], thereby resulting in promoting transcription. In agreement with this mechanism, it seems that Tax promotes CREB phosphorylation and interacts with phosphorylated CREB[130]. Remarkably, CREB-2 cannot be phosphorylated by PKA and is still able to interact with Tax to promote HTLV-1 transcription[131].

Tax hampers DNA damage response

In response to DNA damage, Chk2 protein kinase is phosphorylated and activated, and produces cell cycle arrest. Chk2 and DNA-dependent protein kinase form a complex with Tax in nuclear bodies[132]. Tax induces constitutive activation of this complex and hampers the response to new damage, suggesting a saturation of the DNA repair pathway caused by Tax[132]. Tax oncogenic protein exploits and manipulates many cellular pathways involving PTMs. The picture is far from complete, due to the diverse Tax performances (and variants) among different cell lines and cell cycle steps.

HTLV-1 REX PHOSPHORYLATION AND TCR PATHWAY

The phosphoprotein Rex is found predominantly in the cell nucleolus and is involved in splicing and nuclear ex-

port of viral mRNA. Rex recognizes a specific response element on the incompletely spliced mRNAs, stabilizing them and inhibiting their splicing, and utilizes the CRM1 pathway for nuclear export[133], the same used by mono-ubiquitinated Tax[86].

The major targets of Rex phosphorylation in HTLV-1-infected cells are serines and at a lesser extent threonines[134]. Rex phosphorylation at Ser-70 seems involved in the regulation of Rex function in response to extracellular stimuli, through protein kinase C activation[134]. Decreased Rex phosphorylation correlates with the reduction of unspliced viral mRNA[134].

Recently, Kesic et al[135] completely mapped the phosphorylation sites of Rex and found seven phosphorylation sites. Phosphorylations on Ser-97 and Thr-174 were demonstrated to be critical for Rex-1 function[135]. The authors mapped also HTLV-2 Rex and found that phosphorylations on Thr-164, Ser-151 and Ser-153 are functional[136]. In particular, Ser-151 phosphorylation correlates with Rex nuclear/nucleolar localization[136] and with an active Rex protein[137].

For unknown reasons, Rex expression promotes the expression of the Src kinase FynB, usually absent in T cells, thus altering the TCR pathway[41]. This switch to a SFK expressed preferentially in B cells suggests a putative activation of a B cell receptor (BCR) cascade within a T cell, raising new questions and requiring further studies.

HTLV-1 P12, TCR SIGNALING AND PTMS

Endoplasmic reticulum (ER)-localized p12 enhances p300 expression in T cells in a calcium-dependent, but calcineurin-independent manner[138], thereby negating the p30-mediated repression of LTR transcription (see below[139]).

p12 does not seem contribute to the Jak/Stat phosphorylation and activation in HTLV-1-immortalized cells[140], but promotes the phosphorylation of Stat-5 in p12-transduced PBMCs[141]. However, p12 acts downstream of TCR signaling. On one hand, p12 increases cytoplasmic calcium levels inducing the nuclear factor of activated T cells (NFAT) activation in a LAT-independent manner[142]. On the other hand, p12 inhibits the phosphorylation of LAT, Vav, and phospholipase C-γ1 upon engagement of CD3, leading to decreased TCR-induced activation of NFAT[143].

To complicate the picture, two forms of 8 and 12 KDa coexist and can be palmitoylated, as shown at the 15th International Conference by Franchini G. and collaborators. Notably, palmitoylation increases the hydrophobicity of proteins and targets them to lipid rafts[17]. Post-translational removal of the ER retention/retrieval signal located at the amino terminus of p12 yields the p8 protein, which traffics to the cell surface, promoting T cell anergy and clustering, structuring the virological synapse, ultimately increasing the virus spreading[144].

HTLV-1 P13 INTERACTS WITH SFKS

Signal transduction is based on activation of normally inactive kinases that, under external (or internal) stimuli, phosphorylate their substrates often promoting a cascade[145]. The TCR signaling uses the SFKs to transduce the signal[146]. SFKs localize in their inactive state on the inner leaflet of the plasma membrane, promptly transduce the TCR signal following recognition of immunocomplexes on antigen-presenting cells, and ultimately lead to T-cell proliferation and immune response[38].

Interestingly, p13, a mitochondrial and nuclear protein[147], binds the SH3 domain of SFKs[146]. p13/SFK forms a stable heterodimer that translocates to mitochondria by virtue of a p13 N-terminal mitochondrial localization signal[146]. As a result, the activity of SFKs is dramatically enhanced with a concomitant increase in mitochondrial tyrosine phosphorylation[146]. Contrasting reports exist as to whether p13 is able to target the inner mitochondrial membrane and to perturb the mitochondrial membrane potential[146,148].

Further investigations are needed to understand the significance of the potential SFK activation within HTLV-1-infected T cells, considered their importance in the pathogenesis of diseases such as chronic myeloid leukemia and BCLL[149].

These results appear in contrast with the recent findings that p13, in spite of its mitochondrial localization when expressed alone, in the presence of Tax is partially mono-ubiquitinated, stabilized, and re-routed to the Tax-related nuclear bodies[147]. p13 directly binds Tax, decreases Tax binding to the CBP/p300 transcriptional co-activators and, by reducing Tax transcriptional activity in a dose-dependent manner, thereby suppresses viral gene expression[147].

Interestingly, a truncated form of p13 (and hence a truncated p30), lacking of the C-terminal SH3-binding motif, is encoded by the rabbit HTLV-1-infected cell line RH/K34, a renowned cell line for its ability to induce a lethal leukemic-like disease in animal[150]. In contrast with the cell line RH/K30, that produce a low-grade leukemia with acute rejection, a different pattern of tyrosine phosphorylation is noticeable within RH/K34 cells[151]. Remarkably, Vav, a downstream TCR effector, is constitutively phosphorylated and activated only in the RH/K34 cell line. Clearly, while the SH3-binding motif of p13 is not contributing to the constitutive activation of TCR signaling[151], other viral proteins are involved through unidentified mechanisms.

Further functional studies on infected cells and during viral protein co-expression are required to delineate the complex interplay between the viral proteins and the cell, although the presence of putative SH3-binding motifs in p12, p30 and Tax suggests a focused molecular study on their putative physical interaction with SFKs.

HTLV-1 P30 ALTERS CELL CYCLE AND REPRESSES TAX FUNCTION

p30 is a viral accessory protein that localizes with p300 in the nucleus. p30 competes with Tax for the same p300

N-terminal region of binding, eventually repressing Tax transcriptional activity in a dose-dependent manner[139]. Increased availability of ectopically expressed HAT-active p300 reverses the effect of p30. The authors proposed that the intrinsic HAT activity of p300 is utilized to acetylate and potentially modulates the transcriptional regulatory function of p30, but further studies are needed[139].

p30 delays the entry into S phase, decreasing the phosphorylation levels of Rb, while its homologue, HTLV-2 p28, does not. In order to do so, p30 interacts with Cyclin E and with Cdk2 and prevents their activation[152] (Figure 2). p30 also seems to alter the pattern of phosphorylation of the Cdc25C phosphatase, Chk1 checkpoint kinase and Polo-like kinase-1, thereby activating the cell cycle checkpoint at the G2-M phase transition[153] (Figure 2). These data, taken together, suggest a Tax-counteracting effect of p30 on the cell cycle control (as described above), but do not allow a clear characterization of p30 function.

HBZ UTILIZES UBIQUITIN PATHWAYS

HBZ has been shown to negatively regulate basal and Tax-dependent LTR transcription through its ability to interact with specific basic-leucine zipper proteins and to inhibit CREB binding to the LTR[154]. Furthermore, HBZ, like p30, inhibits Tax transcriptional activity competing with Tax for p300 binding[155].

Interestingly, HBZ acts also indirectly on Tax canonical NF-κB transcription, inducing the overexpression of PDLIM2 ubiquitin ligase and interacting with RelA[112]. The authors found that PDLIM2 targets specifically RelA for degradation and that HBZ binds RelA diminishing its DNA binding capacity[112]. Also Tax stability is affected by PDLIM2 as described above. PDLIM2 down regulation in primary ATLL cells[17] suggest a balanced interplay between HBZ and other viral proteins in which HBZ activity is restricted.

Remarkably, HBZ promotes c-Jun degradation without the requirement for ubiquitination[156], counteracting its stabilization promoted by Pin1 (described above). HBZ appears to function as a tethering factor between the 26S proteasome and c-Jun, thus repressing the AP-1 pathway. Incidentally, HBZ promotes also the cell cycle, activating the E2F1 pathway (reviewed in[157]).

HTLV-1 GAG AND UBIQUITINATION

Cells employ PTMs to organize trafficking activities and, in particular, ubiquitination plays a critical role in non-proteolytic functions such as DNA repair, exocytosis and endocytosis[158]. During the exocytosis, membrane scission requires the endosomal-sorting complex-required for transport (ESCRT) machinery and enveloped viruses exploit this system for virion budding[159]. Recently, evidences that arrestin-related trafficking proteins (ARTs, adaptors between WWP1, WWP2 and Itch ubiquitin ligases and ESCRT machinery) are recruited to the site of

viral budding, suggest the involvement of ART-mediated ubiquitination in viral egress[160]. Early work by Derse and collaborators demonstrated that the HTLV-1 Gag matrix domain is involved in virion budding[161]. Gag matrix domain interacts through a late domain (PPxY motif: proline-proline-any amino acid-tyrosine) with WWP1 ubiquitin ligase (know also as NEDD4.1) and is ubiquitinated on the Lys-74 to function. Late domains are short peptide sequences encoded by enveloped viruses to promote the final separation of the nascent virus from the infected cell. Gag interacts with WWP1 at the plasma membrane where it is ubiquitinated and transferred to the late endosomes. There, it interacts with Tsg101, a component of the ESCRT machinery[162]. An ubiquitination-defective Gag mutant produces a substantial decrease in the virion release[161].

Furthermore, Gag binds also the tumor suppressor hDlg, mediating the cell-to-cell contact[163], one of the common mechanism of spreading for HTLV-1.

CONCLUDING REMARKS

The obligatory parasitic nature of viruses requires them to enter and hijack the cellular machinery with the contribution of viral encoded proteins, and to exploit the cellular resources for viral replication whilst blocking, inhibiting or balancing the host defenses.

HTLV-1 is capable of altering many cellular pathways and this phenomenon has been studied in immortalized cell lines and in *ex vivo* samples. Single or groups of viral proteins have been investigated *in vitro* or in cell culture under ectopic expression conditions. All the results are far from delineating the picture, but have highlighted a plethora of useful information about the regulation of cellular pathway through PTMs. HTLV-1 infection generates deregulation of many cellular proteins. Hsp-90 overexpression seems to favour Akt signaling and β-catenin-dependent cell proliferation. TCR signaling is hampered for the downregulation of CD3 and infected T cells seem to borrow components of the BCR pathway, making possible a conceptual parallelism between ATLL and BCLL. The downstream Ras-Raf-ERK pathway is turned on, suggesting the activation of MAP kinase and JNK as well. Jak/Stat pathway is constitutively activated in ATLL cells but is an unsuitable target for therapeutic treatments being redundant for proliferation.

Cell cycle is strictly controlled by PTMs and after infection is promoted by upregulation of Aurora kinases. It suffers major alterations for counteracting functions of upregulated cell proteins, Tax, HBZ and p30: while a G1 phase progression is promoted by Tax, Pin1 and HBZ mRNA, G2/M transition is blocked by Tax. Upregulated Kip1 and Waf1 inhibit G1/S/G2 transition but are takled by Akt activation. It seems that Tax acts, upstream and downstream, on Akt activation, promoting FoxO-4 and p53 degradation and Bad inactivation, with the ultimate effect of altering cell cycle, response to DNA damage and apoptosis.

Tax function on gene transcription is hampered by p13, p30 and HBZ, but its activities on other cellular pathways, such as TCR, are far from being understood. Most likely, different Tax PTMs redirect Tax localization and function to specific compartments, where Tax is as essential as for transcription. For instance, the indirectly determined Tax phosphorylation on Ser-160 appears to stabilize the protein only during the mitosis, and the negative crosstalk between ubiquitination and SUMOylation on Lys-280 and Lys-284 seems to enable Tax shuttling between the cytoplasm and the nucleus. Centrosomes, ER and Golgi apparatus appears to be active site of recruitment for Tax and its cellular partners. Akt pathway, NF-κB pathway and DNA damage response require physical interaction between cell proteins and Tax. And also TCR signaling is probably affected directly by Tax for its putative SH3-binding motifs, as suggested by unpublished data (Pagano MA, personal communication) that show constitutive tyrosine phosphorylation in Tax-expressing cells. Like Tax-1, Tax2B undergoes to several PTMs, some of which have no discernible effects ascribed to date.

Other accessory proteins are essential to sustain the infection and the viral replication, and are subjected to modifications that could explain differences in localization and in function. Rex-1 and Rex-2 differ for phosphorylation residues and seems to work under phosphorylation. p12 induces NFAT activation while inhibiting the TCR pathway downstream Syk kinases, and its post-translational truncated form p8 localizes in the plasma membrane affecting TCR signaling and forming the virological synapse for viral spreading. p13 binds and constitutively activates SFKs, TCR effectors, suggesting an analogy with other leukemias involving overactivation of SFKs. Furthermore, a C-terminal truncated form of p13 and p30 in HTLV-1-infected cell promotes Vav hyperphosphorylation and a fatal disease in rabbit. At last, HBZ functions and Gag contribution in viral budding involve the recruitment of ubiquitin ligases.

Many HTLV-1 proteins affect and are modulated by cellular pathways implicated in phosphorylation, ubiquitination and other PTMs. One of the problems associated to the study of PTMs is that the proportion of modified residues in a specific protein could account for functional differences associated to the system (*in vivo, ex vivo, in culture* or *in vitro*), or associated to the described model (animal model, cell line). The application of the existing knowledge in several systems might solve partially the problem of recognizing relevant PTMs.

This data collection, not exhaustive of the clinical and molecular work done on HTLV-1, consents to appreciate the extensive interplay of the viral proteins with the host cellular machinery. The molecular mechanisms related to the virus replication are partially controlled by PTMs, that in turn are to some extent controlled by viral proteins. In ATLL, Akt pathway, downstream both Jak/Stat and TCR signaling, has revealed important in cell proliferation, survival and apoptosis abolition, together with Ras pathway. Both represent a possible target for pharmacological

treatments. Jak/Stat instead has proven to be a pharmacological target only in specific leukemic settings. TCR signaling seems promising as a suitable pharmacological target. In ATLL, it is not responsive to external stimuli and is highly altered by the expression of a battery of enzymes proper of B cells, suggesting a parallelism between ATLL and BCLL. Hsp-90 could be involved in the overactivation of TCR signaling and, in particular, should be tested its putative involvement in SFK activation in ATLL. Furthermore, the identification of putative SH3-binding motifs in p12, p30 and Tax, and of an actual SH3-binding motif in p13 suggests the need to study the interplay between HTLV-1 proteins and SFKs, downstream TCR, as a prerequisite for the suitability of such kinases as pharmacological target.

Our knowledge is presently incomplete at least partly for the paucity of time-course studies on infected synchronized cells, which would extend the comprehension of the dynamic nature of PTMs. Further studies are needed that involve co-expression of viral proteins (or, alternatively, siRNA synchronous knockdown of more viral proteins within infected cells) to consider the balance between different and often opposite functions. More sophisticated lymphocyte models, with inducible expression of different HTLV-1 proteins, will provide new tools for the dissection of this fatal oncovirus. More insights and efforts are therefore needed to delineate novel mechanistic insights on viral exploitation of PTM pathways, which should provide a foundation for new treatment strategies to specifically target ATLL.

ACKNOWLEDGMENTS

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