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News and Commentary

Proapoptotic regimes for HTLV-I-transformed cells: targeting Tax and the NF-κB pathway

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Human T-cell lymphotropic virus type I (HTLV-I)-associated adult T-cell leukemia/lymphoma (ATL) is a malignancy of activated T cells resistant to chemotherapy. The viral transactivator protein Tax is responsible for several aspects of the malignant transformation such as proliferation, resistance to apoptosis, genetic instability, tumor dissemination, immune escape and chemotherapy resistance. Through all of these activities, Tax acts as a powerful oncogene and represents the most attractive viral protein for targeted therapy. Among Tax properties, activation of the NF-κB pathway plays a crucial role in the proliferation and transformation of the infected cells. Indeed, most of the activated cellular genes in HTLV-I-infected cells are through this mechanism. In this review, we summarized the molecular mechanisms of Tax-induced NF-κB activation. We then presented the effect, and mechanism of action, of new potential treatment modalities targeting Tax and/or the NF-κB pathway, including arsenic trioxide, interferon alpha and proteasome inhibitors.

Overview on ATL

The HTLV-I is the causative agent of adult ATL[1] and of tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). [2] ATL is an aggressive lymphoproliferative disorder[3] characterized by the proliferation of constitutively activated CD4+ T cells with convoluted nuclei and basophilic cytoplasm. The serum of ATL patients contains antibodies against HTLV-I[1] and the HTLV-I provirus is clonally integrated in the ATL cells. The disease occurs in native individuals from the HTLV-I endemic regions (i.e. the southern Japan, the Carribean, intertropical Africa, Brazil and northern Iran).[4] Although HTLV-I can be transmitted intravenously or through sexual intercourse, vertical transmission through breast-feeding is required for ATL development. Out of the 10–20 million people who are infected with HTLV-I worldwide, only 3–10% develop ATL after a latent period of infection.

ATL remains of very bad prognosis due to severe immunosuppression and to intrinsic resistance of leukemia cells to high doses of chemotherapy. Combination chemotherapy regimen, in particular those designed for treatment of aggressive non-Hodgkin's lymphoma or acute lymphoblastic leukemia, have little effect in the treatment of ATL patients (reviewed in Bazarbachi and Hermine[3] and Bazarbachi *et al*., 2004[5]), with a median survival around 6 months in the acute form. Important advances in the treatment of ATL were reported with the combination of zidovudine and interferon-α (IFN-α) (reviewed in Bazarbachi and Hermine[3] and Bazarbachi *et al*., 2004[5]). This antiviral therapy exhibits a high response rate in ATL patients, especially in previously untreated acute ATL type and prolongs survival. However, most patients eventually relapse, which underlines the need for new therapeutic approaches.

The HTLV-I Oncoprotein Tax

Preceding the development of ATL, there is an initial stage of polyclonal infection due to virus replication followed by an oligoclonal expansion of infected cells. [6] These clonal expansions, at least early after infection, result from the expression of the viral transactivator protein Tax. Tax is a 40 kDa phosphoprotein that is encoded by the pX region of the viral genome. It is predominantly present in the nucleus and can shuttle into the cytoplasm using a nuclear export signal (NES).[7] Tax alters many cellular pathways (Figure 1). It activates several transcription factors, inhibits apoptosis, and interferes with the function of several DNA repair mechanisms and cell cycle regulators.[8]

Transcription factors

Tax interacts with transcription factors to activate several major cellular transcription factor pathways including CREB/ATF, SRE, AP-1 and NF-κB. Tax does not bind to DNA by itself but to transcription factors that bind specific enhancers. Indeed, Tax is a potent trans-activator of the HTLV-I long terminal repeat (LTR), together with the promoters of several important cellular genes such as interleukin-2, interleukin-15 and their cognate receptors, thereby initiating and sustaining an autocrine pathway of T-cell activation.[9]

Cell cycle

Tax deregulates the normal cell cycle control in T cells by targeting different regulators of cell cycle progression. Although Tax does not interact with Rb directly, it forms a complex with $p16^{INK4a}$ and $p15^{INK4b}$, two cell cycle inhibitors

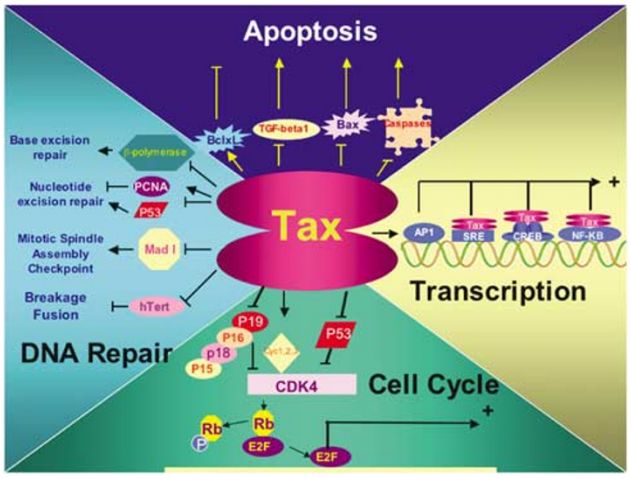

Figure 1 Cellular pathways altered by the HTLV-I oncoprotein Tax. Tax activates the CREB/ATF, AP-1 and NF-κB transcription factors, upregulates antiapoptotic (Bcl_{xL}, caspase inhibitors) and downregulates proapoptotic (Bax) proteins, represses p53 activity, DNA polymerase beta, telomerase (hTert), PCNA and MAD-1 and interferes with the function of several cell cycle regulators including cyclins and cdk inhibitors

that bind to CDK4 rendering it inactive. Tax binding interferes with the ability of $p16^{INK4a}$ and $p15^{INK4b}$ to repress CDK4 activity resulting in Rb phosophorylation.[8,10] Other examples are cycD2, Pro-IL16 and CDK4.[11] Tax can also interfere with the cell cycle progression through the transcriptional activation or repression of $p18^{INK4c}$, cyc A, cyc C, cyc D_2, cyc E, CDK2, $p21^{(CIP1/WAF1)}$ and E2F.[11] Moreover, Tax interferes with the regulation of the cell cycle at the post-translational level by forming a complex with cyclin D3, which induces its phosphorylation.[12]

Genetic instability

Tax interferes with several DNA repair mechanisms. It represses the expression of DNA polymerase-β, an enzyme involved in base excision repair.[13] Tax also suppresses the nucleotide excision repair through the transcriptional upregulation of PCNA and through the inactivation of p53.[11] Moreover, Tax represses the expression of human telomerase hTert, thus interfering with protective mechanisms used to prevent inappropriate breakages fusion.[14] Tax has also been found to bind Mad1, a mitotic spindle checkpoint constituent, and to impair its function.[11]

Apoptosis

Although some reports suggested that Tax expression induces apoptosis, it is generally accepted that Tax protects HTLV-I-infected cells from both spontaneous and chemotherapy-induced apoptosis. Indeed, Tax induces the expression of Bcl-xL, an antiapoptotic protein, through the NF-κB and the CREB pathways.[15] Tax also represses the transcription of the proapoptotic *bax* gene.[16] In addition, Tax inhibits the caspase cascade in an NF-κB-dependent manner through the induction of the caspase inhibitors X-IAP, cIAP-1 and c-IAP-2.[17] Moreover, despite elevated expression of p53 in several HTLV-I-transformed lymphocytic cell lines, p53 is not fully active in these cells. Indeed, in ATL-derived cells, p53 is sometimes mutated but most frequently functionally inactive,[18], and Tax-dependent activation of the NF-κB pathway was reported to play a critical role in the inhibition of p53 transactivation function.[19] Tax alone is capable of suppressing the p53 transcriptional activity and can abrogate G1 arrest and apoptosis induced by p53.[20] Tax mediates p53 inhibition through the phosphorylation of p53 on serines 15 and 392.[19]

Microenvironment

Tax also influences the microenvironment: it induces the synthesis of TGF-β, inhibits TGF-β signal transduction in infected cells,[21] induces angiogenesis, metalloproteinases and gap junction-mediated communication between infected cells and endothelial cells,[22] hence contributing to the extravasation and invasiveness of ATL cells.[23]

Therefore, Tax is at the root of several aspects of the malignant transformation such as proliferation, growth factor independence, inactivation of tumor suppressors, resistance to apoptosis, genetic instability, tumor dissemination, immune escape and chemotherapy resistance. Indeed, Tax expression in human peripheral blood mononuclear cells is sufficient to induce immortalization of CD4+ T cells.[24] Tax also transforms rodent fibroblast cell lines and induces tumors in its transgenic mice.[25,26] Through all of these activities, Tax acts as a powerful oncogene and represents a very attractive viral protein for targeted therapy. However, among the wide-ranging properties of Tax oncoprotein, activation of the NF-κB pathway plays a crucial role in the proliferation and transformation of the infected cells, and hence also represents a critical therapeutic target.

Overview on NF-κB

The NF-κB family

NF-κB is a transcription factor that regulates the expression of numerous cellular genes involved in the immune response, inflammation and apoptosis. NF-κB also plays an important role in oncogenesis.[27] Although the members of this family are ubiquitously expressed, they are inactive because they are sequestered in the cytoplasm by the inhibitory proteins of the IκB family. Their transcriptional activity depends on their nuclear translocation where they can control the expression of target genes coding for cytokines, growth factors and adhesion molecules.[28]

NF-κB was first described in 1986 as a nuclear factor capable of binding to the enhancer of the immunoglobulin kappa light chain gene in B lymphocytes. The NF-κB family of transcription factors was then defined on the basis of their homology in their large N-terminal domain known as Rel homology domain (RHD). RHD is a conserved domain of 300 amino acids that contains a DNA-binding domain, a dimerization domain, a region for interaction with the inhibitory proteins IκB and a nuclear localization signal (NLS).[28,29]

Five members of the NF-κB family have been identified in mammalian cells: p65/Rel A, c-Rel, RelB, p50/p105 and p52/p100. These members are divided into two groups based on their structure, function and mode of synthesis. The first group is composed of p50 and p52, which are synthesized as inactive cytosolic precursors p105 (NF-κB1) and p100 (NF-κB2), respectively.[29] These precursor proteins share in their C-terminal domain, ankyrin motifs that permit protein–protein interaction. The maturation of these proteins, through their proteolytic cleavage, gives rise to smaller forms p50 and p52 deprived of the ankyrin motifs. The second group is composed of p65 (RelA), c-Rel and RelB, produced as transcriptionally active proteins that possess in addition to their conserved RHD, a C-terminal transactivation domain. These proteins are capable of homo or heterodimerization within the members of this group, with all the possible combinations, except for RelB, which dimerizes only with p50 or p52.[30] Only the hetero or homodimers, containing a transactivating region, are capable of directly activating the transcription. Hetero or homodimers containing only p50 and/or p52, inhibit transcription but may become activators when they are bound to Bcl3, an atypical protein of the IκB family. The most common NF-κB complexes are heterodimers composed of a RelA member and p52 or p50.[27]

The proteins of the Rel/NF-κB family are maintained in the cytoplasm in an inactive form, bound to an IκB family member of which eight are identified: IκB-α, IκB-β, IκB-δ, IκB-ε, IκB-γ, Bcl3 and the precursors p100 and p105.[31–33] IκB-α, IκB-β and Bcl3 possess at their C-terminal region a PEST destabilizing sequence.[34] The protein IκB-γ is identical to the C-terminal domain of p105 and IκB-δ corresponds to the C-terminal domain of p100. All these proteins share a five to seven repeated ankyrin motifs.[28] These repeated ankyrin motifs, are sequences of around 30 amino acids permitting the interaction of IκB proteins with the NF-κB family members. This interaction masks the nuclear localization domain of NF-κB proteins, which therefore, remain in the cytoplasm.[35] The IκB proteins have distinct affinities for the different NF-κB dimers. For example, IκB-α and IκB-β bind preferentially to c-Rel or RelA, whereas Bcl3 binds only to dimers composed of p50 and p52.[36]

IκB-α, the best studied family member, is a 37 kDa protein, which becomes phosphorylated at serines 32 and 36 upon cellular activation (see below). IκB-β, a 45 kDa protein, contains six ankyrin motifs like IκB-α and binds to the same NF-κB dimers. IκB-ε was cloned and identified as a protein interacting specifically with p52. IκB-ε contains six ankyrin motifs and associates uniquely with c-Rel or RelA dimers.[32] Bcl3 contains seven ankyrin motifs, but differs in its nuclear localization, and is considered to be an activator of the transcriptional activity of NF-κB.[37] The inhibitory proteins IκB regulate the activity of NF-κB through both the cytoplasmic retention of NF-κB dimers and the inhibition of the NF-κB binding to the DNA. Two types of complexes are retained in the cytoplasm: the NF-κB dimers bound to the inhibitory proteins like IκB-α, and the heterodimers formed of p105 or p100 associated to another protein of the NF-κB family.[34] Free IκB-α may also be imported to the nucleus where it can interact with NF-κB dimers, destabilizing their interaction with DNA.[28,34]

Activation of the NF-κB pathway

One of the remarkable features of NF-κB is the diversity of signals that induce its activation. Indeed, these include stress signals (ionizing radiation, ultraviolet light and free radicals), infections (bacterial lipopolysaccharide, viral proteins such as Tax) or numerous proinflammatory cytokines (TNF-α, IL-1).[38] All these signals lead to the activation of the IKK complex (IκB kinase). In 1996, the phosphorylation of IκB-α by a high molecular weight complex (700 kDa) was described[39] and two groups identified the active kinases of this complex, IKK-α and IKK-β. These kinases phosphorylate directly IκB-α and IκB-β. A third element of this complex was later identified and named NF-κB essential modulator (NEMO), IKKAP-1 (IKK-associated protein-1) or IKK-γ (IκB kinase gamma).[40,41] The proteins IKK-α and IKK-β interact with each other through their interaction domain 'helix-loop-helix' and interact with NEMO through their leucine zipper domain. The activation of the IKK complex is equally regulated by other kinases capable of interacting with this complex and of phosphorylating IKK such as NF-κB activating kinase (NAK), mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase 1 (MEKK1) and NF-κB-inducing kinase (NIK).[42,43]

Once activated, the IKK complex phosphorylates IκB-α on serine residues 32 and 36. IκB-α then becomes conjugated to ubiquitin and is degraded. This ubiquitylation takes place specifically at lysines 21 and 22, and IκB-α is then degraded by the 26S proteasome complex. IκB-α degradation exposes the NLS of NF-κB dimers, inducing their rapid translocation to the nucleus. NF-κB then bind consensus sequences present in the promoters of several cellular genes and activate their transcription.[28] One of the genes activated by NF-κB is *IκB-α,* which is implicated in a negative feedback loop. Indeed, *de novo* synthesized IκB-α can enter the nucleus through its NLS, releases the NF-κB complex from DNA and exports these dimers to the cytoplasm through its NES.[44] This mechanism, contributes to transient NF-κB activation. The events leading to the degradation of IκB-β and IκB-ε are similar to those of IκB-α. In the case of the precursor p105, its cleavage to p50 involves similar events of phosphorylation, ubiquitylation and degradation. However, in contrast to IκB-α, the proteasome only degrades the C-terminal part of p105, leaving the N-terminal intact.[45,46]

Mechanisms of Tax-Induced Activation of the NF-κB Pathway

Both HTLV-I-infected and Tax-expressing cells exhibit a constitutive nuclear expression of NF-κB.[47] Indeed, Tax is a powerful activator of the NF-κB pathway and acts at multiple levels to initiate and maintain NF-κB activation (Figure 2). First Tax is capable of inducing a constitutive activation of the IKK complex, leading to the phosphorylation of IκB-α and IκB-β proteins, allowing the constitutive presence of the NF-κB transcription factor in the nucleus.[48,49] Tax activates IKK by interacting with the noncatalytic subunit IKK-γ, facilitating the recruitment of Tax to the catalytic subunits IKK-α and IKK-β.[50,51] Tax–IKK-γ interaction requires two leucine zipper domains, present in the N and C-terminal part of IKK-γ, and the leucine repeat region (LRR) of Tax.[52] In fact, LRR, which

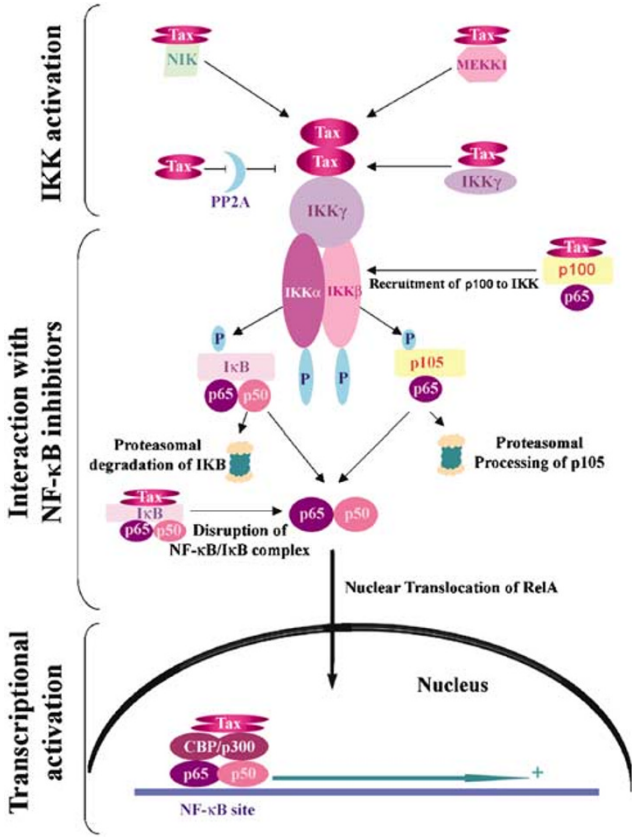

Figure 2 Molecular mechanisms of Tax-induced activation of the NF-κB pathway. Tax acts at multiple levels to initiate and maintain NF-κB activation: (1) Tax dimers activate IKK by interacting with the noncatalytic subunit IKK-γ, facilitating the recruitment of Tax to the catalytic subunits IKK-α and IKK-β. Tax can also activate IKK by interacting with upstream kinases such as MEKK1 and NIK and by inhibiting PP2A, the cellular inhibitor of IKK. This leads to activation of IKK resulting in IκBs phosphorylation, ubiquitylation and proteasomal degradation. IκB-α degradation exposes the NLS of NF-κB dimers, inducing their rapid translocation to the nucleus. (2) Tax also directly interacts with NF-κB inhibitors: it binds to the ankyrin domain of IκB-α, which prevents its interaction with NF-κB factors, recruits p100 to the IKK complex and enhances the proteasome-dependent processing of p105 into p50. (3) At the transcriptional level, Tax physically interacts with the RelA subunit of NF-κB, and recruits the transcriptional coactivators CBP/p300 to RelA. This favors NF-κB binding to consensus sequences present in the promoters of several cellular genes and activates their transcription

contains three leucine residues repeated after each seven residues, does not bind directly with IKK-γ, but it favors the homodimerization of Tax, which in turn induces IKK-γ oligomerization and consequently, the catalytic activity of IKK.[52,53] In addition, Tax can constitutively activate IKK, by interacting with some upstream kinases such as MEKK1.[54] NIK also seems to play an important role in the IKK activation by Tax, since negative-dominant mutants of NIK inhibit NF-κB activation by Tax.[55] Other studies demonstrated that Tax favors the phosphorylation of IKK-γ by IKK-β.[56] Moreover, Tax can affect the serine/threonine protein phosphatase 2A (PP2A), the cellular inhibitor of IKK. Tax mutants incapable of binding PP2A, whether they are capable or not of binding to IKK, have no effect on the NF-κB pathway.[57] Finally, Tax cooperates with IKK by physically recruiting IKK-α to p100, triggering phosphorylation-dependent ubiquitylation and processing of p100 to p52.[58]

Second, Tax is capable of interacting with the inhibitory proteins IκB, through their ankyrin motifs. This interaction activates NF-κB by disrupting NF-κB/IκB complexes (dissociation model) or by recruiting IκB members directly to proteasome (proteasome targeting model). One of Tax's targets is the precursor p105.[59] Tax targets p105 to the proteasome, to accelerate its cleavage to the active form p50. Indeed, the weak interaction between the HC9 subunit of the proteasome and the precursor p105 is enforced by the formation of a tertiary complex with Tax.[60] The precursor p100 is another target of Tax, as well as IκB-α and IκB-β,[61,62] In addition to phosphorylation-dependent ubiquitylation, Tax also induces IκB-α degradation by directly favoring its interaction with the proteasome.[63] Moreover, Tax interaction with these inhibitory proteins can influence Tax localization within the cell,[64] as Tax is essentially cytoplasmic when bound to p100, while it is nuclear when bound to p52.

Third, Tax can interact with p50, p52, p65 and c-Rel.[61,65] Tax can bind to their homology domain, and favors dimer formation, resulting in an increase in their DNA binding and transcriptional activity. Moreover, Tax, RelA, p50 RNA polymerase II and CBP/p300 colocalize in transcriptionally active small discrete nuclear foci,[66,67] and Tax recruits the transcriptional coactivators CBP/p300 to p65/RelA, thereby significantly increasing NF-κB transcriptional activity.

In conclusion, Tax targets the NF-κB pathway at multiple levels, inducing its constitutive activation. Importantly, activation of the NF-κB pathway plays a crucial role in Tax-induced cellular transformation and proliferation and hence represents an interesting therapeutic target. Indeed, a Tax mutant that selectively abrogates the ability of Tax to activate transcription through the NF-κB pathway, loses the ability to immortalize infected cells.[68] Also, in transgenic mice models, Tax-mediated induction of NF-κB activity significantly contributes to *in vivo* tumorigenesis.[69] Understanding the way Tax activates the NF-κB is therefore crucial for designing new therapeutic approaches targeting the viral oncoprotein Tax and/or Tax-mediated induction of NF-κB.

Targeting Tax and the NF-κB Pathway

Clinical studies clearly demonstrated that untargeted conventional chemotherapy is of little benefit in ATL therapy, especially in the acute form. New alternative therapeutics, which act by different mechanisms (targeting the HTLV-I virus, the viral oncoprotein Tax and the secondary genetic events) whether Tax dependent or independent, are therefore needed. Importantly, among the wide-ranging properties of Tax oncoprotein, activation of the NF-κB pathway plays a crucial role in the proliferation and transformation of the infected cells. Indeed, most of the activated cellular genes in HTLV-I-infected cells are through this mechanism. This is the case of the genes coding for IL-2R, TNF-α, the proto-oncogene *c-myc,* antiapoptotic proteins and caspase inhibitors as well as for p53 inactivation.[17,19,70] Overall, Tax-induced activation of the NF-κB pathway is critical for ATL

proliferation, protection from apoptosis, and drug resistance, and hence also represents a critical therapeutic target.

Arsenic trioxide

Arsenic trioxide (As) is a very effective treatment for acute promyelocytic leukemia (APL).[71] Indeed, APL patients resistant to all-*trans* retinoic acid (ATRA) and conventional chemotherapy can still respond to As. *In vitro* studies showed that As triggers relatively APL cell apoptosis at micromolar concentration. Lower doses of arsenic were shown to induce differentiation of the leukemic cells[71] and recent evidence suggests that differentiation is indeed the predominant mechanism of response *in vivo*. At the molecular level, As specifically leads to the degradation of the PML/RARα oncogene generated by the t(15,17) translocation specific for APL,[72] by targeting its PML moiety.

We have shown that IFN-α and As have dramatic synergistic effects to induce cell cycle arrest and apoptosis, specifically in HTLV-I-infected cells and ATL-derived cells.[73] Interestingly, As specifically induced G1 arrest, while IFN-α alone had little effects on these cells. Combining even very low doses of IFN-α to As dramatically diminished cellular proliferation. At the molecular level, combined As and IFN-α induced the degradation of Tax by a proteasome-dependent mechanism. Tax degradation was associated with an upregulation of IκB-α, resulting in a sharp decrease in RelA DNA binding NF-κB complexes due to the cytoplasmic retention of RelA.[74] We then investigated the effects of As alone and As/IFN-α combination on gene networks in HTLV-I-infected leukemic cells and demonstrated that two distinct gene networks were specifically modulated: one is characterized by fast As-induced, extinction of NF-κB target genes, and the other by delayed As/IFN-α triggered cell cycle arrest.[75] The former depends on IκB-α and IκB-ε stabilization by an unknown mechanism, whereas the latter likely reflects Tax degradation. These serial events likely account for the potent and specific effects of the As/IFN-α combination in ATL and provide a mechanistic explanation for the specificity of the As/IFN-a combination in ATL (Figure 3). Reversal by As of NF-κB activation resulted from dramatic stabilization of IκB-α and IκB-ε independently of IKK activity modulation or Tax degradation. Since NF-κB constitutes a well-known survival pathway in ATL cells, its inactivation likely contributes to the induction of apoptosis. However, only the As/IFN-α combination induced a late and massive downregulation of cell cycle regulated genes, concomitantly with Tax degradation by the proteasome and cell death induction. Therefore NF-κB shut-off alone is insufficient for apoptosis induction. Addition of IFN-α to As is required to precipitate cell death, most likely by inducing the degradation of Tax, presumably reversing its oncogenic effects on multiple cellular targets.

Tax protein was previously shown to bind two subunits of the 19S proteasome.[60,76,77] This binding may either participate in the postactivation degradation of Tax-bound transcription factors, or play a direct role in transcriptional activation *per se*.[78] How the As/IFN-α association triggers Tax degradation by the proteasome is not yet understood. That IFN-α greatly enhances As-induced Tax degradation may relate to the fact that IFN-α modulates the subunit composition of both the catalytic core of the 20S proteasome and its 11S regulatory cap.[79,80] PML, another IFN-α-induced gene, was shown to recruit the 11S proteasome and to be degraded in response to As.[72,81] That Tax is continuously degraded by the proteasome may account for the subsequent presentation of Tax peptides on MHC class I molecules[82] and for the very high frequency of circulating Tax-specific cytotoxic T lymphocytes found in most HTLV-I-infected individuals (reviewed in Bangham[83]). Since Tax is an essential activator of the viral gene expression, which is absolutely indispensable to replication, the As/IFN-α combination may be clinically useful not only in ATL, but also in chronic carriers with high viral loads or during primo-infection.

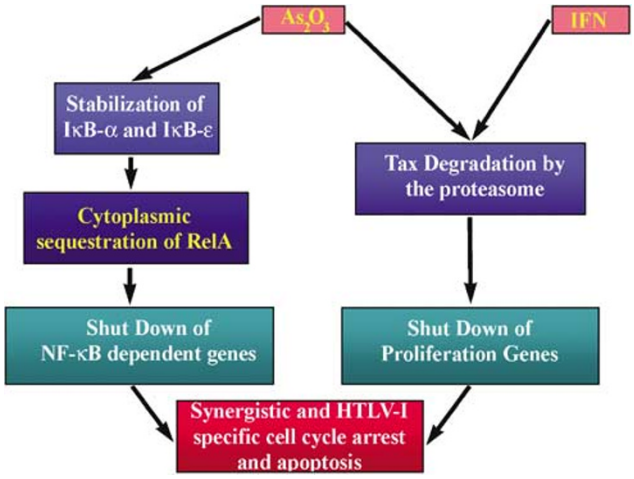

Figure 3 Mechanism of action of arsenic trioxide and IFN-α in HTLV-I-transformed cells. As stabilizes IκB-α and IκB-ε resulting in extinction of NF-κB target genes due to the cytoplasmic retention of RelA. As/IFN combination induces a late and massive downregulation of cell-cycle-regulated genes, due to Tax degradation by the proteasome. These serial events account for the dramatic and synergistic effects of the As/IFN combination, which induces cell cycle arrest and apoptosis, specifically in HTLV-I-infected cells

These findings prompted us to initiate a phase II clinical trial of As/IFN-α combination in seven ATL patients (four acute and three lymphoma) with relapsed/refractory disease after treatment with AZT, IFN-α and chemotherapy.[84] Four patients exhibited a clear initial response (one complete remission and three partial remissions) and one patient is still alive and disease free at 40 months. These preliminary results demonstrate that treatment of ATL with As and IFN-α is feasible and exhibits a clear antileukemic effect, even in refractory patients, although patients reach therapeutic level of As (1 μM) very slowly. Future studies should evaluate the use of intermittent administration of higher doses of As to achieve efficient levels more rapidly without increasing toxicity. We suggest that the best timing for As therapy might be either frontline, combined with AZT/IFN-α, or later as maintenance, also in combination with AZT/IFN-α.

Such specific As-induced Tax degradation is highly reminiscent of the As-induced PML-RAR degradation observed in acute promyelocytic leukemia,[72] and could represent another oncogene-targeted cancer therapy. Conversely,

at the physiopathologic level, the clinical efficacy of a Tax-targeted treatment strongly favors the concept that continuous Tax expression has a role in maintaining the ATL leukemic phenotype.

Bay 11-7082

A recent report indicated that Bay11-7082, an irreversible inhibitor of IκB-α phosphorylation, induced apoptosis of HTLV-I-infected T-cell lines, but only negligible apoptosis of HTLV-I-negative cells.[85] At the molecular level, it reduced the DNA binding of NF-κB in HTLV-I infected T cell lines and downregulated the expression of the antiapototic gene BclxL.[85] Another report indicated that Bay 11-7082 inhibits ATL growth in immunodeficient mice.[86]

Proteasome inhibitors

Proteasome inhibitors represent a new interesting class in cancer therapy. The 26S proteasome is a critical nuclear and cytoplasmic proteolytic system that regulates cell proliferation, differentiation and apoptosis.[87] This proteolytic pathway normally eliminates intracellular damaged, mutant and misfolded proteins and a variety of short-lived functional proteins such as cyclins and cyclin-dependent kinase inhibitors, NF-κB inhibitors and p53.[87,88] One of the signals targeting proteins toward the proteasome is the conjugation to ubiquitin (reviewed in Hershko and Ciechanover[89]). Proteins can be modified by covalent attachment of ubiquitin molecules to either lysine residues or their N-terminus.[90] Conjugation to a chain containing at least four ubiquitin monomers is required to target a protein to the proteasome for destruction.[91]

It has been shown that Tax physically interacts *in vitro* with the proteasome core[60,76,77,82] and recent studies further showed that Tax binds to assembled proteasomes and enhances their proteolytic activity.[77,82] A recent report indicated that Tax is ubiquitylated in both transfected cells and T-lymphocytes, and that Tax ubiquitylation is needed for proteasome binding.[92]

The proteasome inhibitor PS-341 has demonstrated clinical effect in both multiple myeloma and non-Hodgkin's lymphoma. Several reports investigated its effects on ATL-derived cells in both *in vitro* and *in vivo* models. In mice models of ATL, PS-341 either alone or combined with humanized anti-IL-2Rα, consistently inhibited tumor growth and prolonged survival.[93–95] We and others showed that PS-341 blocked the degradation of IκBα in ATL cells and weakened NF-κB DNA binding,[93–96] resulting in growth arrest and apoptosis of ATL-derived cells, while normal T lymphocytes were not affected.[96] Combination of PS-341 and the chemotherapeutic agents adriamycine or etoposide resulted in an additive inhibition of cell growth.[96] PS-341 treatment of ATL cells stabilized IκBα, IκBβ, IκBε, p21, p27 and p53 proteins, selectively inhibited Rel-A DNA-binding NF-κB complexes and induced ceramide accumulation. Overall, PS-341 affected multiple pathways critical for the survival of HTLV-I-positive cells supporting a potential therapeutic role for PS-341 in ATL patients, whether alone or in combination with chemotherapy.

Conclusion

Conventional chemotherapy has limited benefit in ATL patients given that HTLV-I cells carry an intrinsic resistance to chemotherapy. [97,98] In addition, the constitutive activation of NF-κB, the inhibition of p53 function and the down-regulation of Fas-ligand expression in HTLV-I-positive cells[99] likely protect these cells from chemotherapy-induced apoptosis. Hence, the reversal of NF-κB activation should enhance the chemosensitivity of HTLV-I-positive cells. Although Tax is undetectable in circulating ATL cells, our demonstration that Tax is continuously degraded by the proteasome may account for the subsequent presentation of Tax peptides on MHC class I molecules[82] and for the high frequency of circulating Tax-specific cytotoxic T lymphocytes found in most HTLV-I-infected individuals (reviewed in Bangham[83]). Thus, ATL cells may display a latent, transient and/or low level of Tax expression in preferential sites involved by tumor cells such as skin, gastrointestinal tract and/or other lymphoid organs. Hence, Tax and NF-κB-targeted treatments may be clinically useful not only in ATL, but also in patients with tropical spastic paraparesis/HTLV-I-associated myelopathy, in chronic HTLV-I carriers with high viral loads or during primo-infection.

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