Cell signaling modifiers for molecular targeted therapy in ATLL

Cell signaling modifiers for molecular targeted therapy in adult T-cell leukemia/lymphoma

Naoki Mori

Division of Molecular Virology and Oncology, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan

TABLE OF CONTENTS

1. ABSTRACT

Adult T-cell leukemia/lymphoma (ATTL) is a malignancy of peripheral T lymphocytes caused by human T-cell leukemia virus type 1 (HTLV-1) infection. Available therapies for ATLL have minimal efficacy, with few responders and poor survival. New therapies are clearly needed for ATTL patients. Three decades of research in this field has resulted in accumulation of a wealth of knowledge about the molecular pathways underlying the proliferation of HTLV-1-infected T cells. Inappropriate over- and under-activation of various signaling pathways can contribute to pathological processes such as neoplasia. Molecular and pharmacological interventions that target the aberrant state of activation are thus of potential therapeutic benefit. Here we review how signal transduction pathway components including nuclear factor-kappaB (NF-kappaB), activator protein-1 (AP-1), janus kinase-signal transducer and activator of transcription (JAK-STAT), and phosphatidylinositol 3-kinase (PI3K)-Akt contribute to the pathogenesis of ATLL. The targeted inhibition of such molecules to suppress the growth of HTLV-1-infected T cells both *in vitro* and *in vivo* is also discussed. The potential translation of such strategies into effective therapies for patients with ATLL may improve the poor outcome associated with this neoplasia.

2. INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) is a unique malignancy of mature $CD4^+$ T cells caused by a delta-type retrovirus, the human T-cell leukemia virus type 1 (HTLV-1) (1-3). About 10-20 million people worldwide are currently infected with HTLV-1, and the infection is endemic in southwestern Japan including the Okinawa

prefecture, the Caribbean islands, the areas surrounding the Caribbean basin, and Central Africa. After infection, double-stranded DNA from $CD4^+$ T cells is synthesized from virus RNA by reverse transcriptase, followed by integration into the host gene as a provirus. HTLV-1 persists as provirus DNA integrated in the host DNA, and is transmitted in a cell-to-cell manner by three main routes: mother-infant (mainly through breast-feeding), sexual contact, and blood transfusion. Epidemiological studies have indicated a higher incidence of ATLL in male HTLV-1 carriers, implicating vertical infection as a risk factor for ATLL (4). ATLL develops in 2-5% of HTLV-1-infected individuals after a long latent period, suggesting a multistage process of immortalization and transformation of T lymphocytes (Figure 1). Clinically, ATLL is subclassified into four subtypes: acute, lymphoma, chronic, and smoldering. In the relatively indolent smoldering and chronic types, the median survival time is ≥2 years. Unfortunately, no effective curative therapy for ATLL exists and the condition often progresses to death with a median survival time of 13 months in aggressive cases (5). Death is usually due to severe infection or hypercalcemia, often associated with resistance to aggressive combination chemotherapy. Therefore, new therapeutic strategies for ATLL need to be established.

3. CLONAL PROLIFERATION OF HTLV-1-INFECTED T CELLS AND ATLL CELLS

Dysregulation of certain signaling pathways may be oncogenic for ATLL. These include the nuclear factor-kappaB (NF-kappaB), activator protein-1 (AP-1), janus kinase-signal transducer and activator of transcription (JAK-STAT), and

phosphatidylinositol 3-kinase (PI3K)-Akt pathways. These pathways are involved in regulating apoptosis, cell cycle regulation, tumor proliferation, and cell migration (6). The HTLV-1 sequence contains two long terminal repeats (LTRs) in the 3' and 5' ends. HTLV-1-mediated transformation of T lymphocytes depends on the 40-kDa Tax phospho-oncoprotein (7), which potently increases viral gene expression through these LTRs. Tax can be used to immortalize primary human T cells, and when expressed as a transgene, it provokes leukemogenesis and lymphomagenesis in animals (7,8). Tax activates NF-kappaB, AP-1, and PI3K-Akt pathways (6,9,10). In addition, Tax is a major target of cytotoxic T lymphocytes (CTLs) (11) thereby Tax expression induces an immune response. Thus, the expression of Tax in HTLV-1-infected T cells introduces advantages and disadvantages for host cell survival. To escape from CTLs, ATLL cells frequently lose the expression of Tax via several mechanisms (12). In addition, targeted inhibition of the constitutively activated signaling pathways in ATLL (Figure 1) induces apoptosis. Interestingly, treatment of transformed fibroblast cell lines derived from Tax transgenic mice with Tax antisense oligonucleotides caused a 90% reduction in Tax expression, but had no effect on cell growth or ability to form tumors *in vivo* (13). These findings are consistent with Tax being required only in the initiation of transformation, and not during maintenance.

Animal experiments conducted using HTLV-1 infectious clones with mutated accessory genes demonstrated that the accessory *p12*, *p30*, *rex*, *p13*, and HTLV-1 basic leucine zipper factor (*HBZ*) genes help to establish a chronic HTLV-1 infection *in vivo* (14-18). HBZ, encoded by the minus strand of the provirus (19), is expressed continuously and permanently in infected cells. HBZ inhibits the Tax-mediated

transactivation of viral transcription from the 5’ LTR by heterodimerizing with JUN and CREB2 (20). Rex and p30 also negatively regulate viral transcription (21,22). Thus, HBZ, Rex, and p30 seem capable of escaping the host immune surveillance system. Interestingly, HBZ RNA promotes ATLL cellular proliferation (23). Thus, HBZ may contribute to the development and maintenance of the leukemic process. As described above, ATLL cells do not always need Tax expression in the later stage of leukemogenesis. However, ATLL cells frequently show somatic alterations of genomic DNA that controls the cell cycle and hypermethylation of tumor suppressor gene promoter regions (12). Thus, genetic and epigenetic changes imprinted into the cellular genome seem to contribute to multistep leukemogenesis (Figure 1).

4. MOLECULAR TARGETS

4.1. NF-kappaB

NF-kappa B signaling is an important regulatory pathway for cell growth, apoptosis, inflammation, the stress response, and many other physiological processes (24). In mammals, the NF-kappaB family consists of RelA (p65), RelB, c-Rel, NF-kappaB1 (p50 and its precursor p105), and NF-kappaB2 (p52 and its precursor p100); these components can form transcriptionally active complexes in various combinations. NF-kappaB proteins are kept inactive by association with inhibitory proteins in the cytoplasm, including IkappaB alpha, IkappaB beta, IkappaB epsilon, as well as the p105 and p100 precursors of p50 and p52, respectively.

Physiological activation of NF-kappaB occurs mainly through either the canonical or noncanonical pathway (Figure 2). Both pathways involve inducible

phosphorylation of IkappaB proteins by the multiprotein IkappaB kinase (IKK) that contains two catalytic subunits, IKK alpha (IKK1) and IKK beta (IKK2), as well as the regulatory subunit IKK gamma (or NEMO for NF-kappaB essential modifier). The canonical pathway of NF-kappaB activation is stimulated by a range of stimuli, including proinflammatory cytokines (25,26). Activated IKK phosphorylates IkappaB proteins, thereby inducing IkappaB polyubiquitinylation and subsequent proteolytic degradation by the proteasome. NF-kappaB dimers, p50 and RelA, are released following degradation of IkappaB. These molecules then translocate into the nucleus, and activate the transcription of several genes. The noncanonical pathway of NF-kappaB activation represents an additional specialized signaling cascade that is particularly important in mature B cells. This pathway is stimulated by a restricted set of cell-surface receptors that belong to the tumor necrosis factor (TNF) receptor superfamily, including CD40, the lymphotoxin beta receptor, and BAFF receptor (26). This pathway activates NF-kappaB-inducing kinase (NIK) and IKK alpha to directly phosphorylate NF-kappaB2/p100. This process induces partial proteolysis of p100 to p52, which preferentially dimerizes with RelB. Although each NF-kappaB subunit has distinct regulatory functions, many of the target genes are common to several NF-kappaB dimers.

The target genes that are relevant for lymphocyte biology are grouped into several functional classes: positive cell cycle regulators, antiapoptotic factors, inflammatory and immunoregulatory genes, and negative feedback regulators of NF-kappaB. Tax can bind directly to IKK gamma and stimulate both NF-kappaB pathways (9) (Figure 2). However, these signaling cascades may not fully explain

ATLL biology, because cells no longer expressing Tax continue to show constitutively activated NF-kappaB (27) and upregulated NF-kappaB-dependent proteins. Indeed, NIK was recently implicated in constitutive NF-kappaB activation in ATLL cells (28).

Chemical inhibition of NF-kappaB signaling has been investigated in primary ATLL cells and HTLV-1-infected T-cell lines using Bay 11-7082, an irreversible inhibitor of IkappaB alpha phosphorylation (29), dehydroxymethylepoxyquinomicin (DHMEQ), an inhibitor of nuclear translocation of RelA (30), and 2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl nicotinonitrile (ACHP), an inhibitor of IKK (31). Rapid and efficient inhibition of NF-kappaB activity and downregulation of its targets in these cells has also been reported (29-31). Such inhibition induces extensive apoptosis and G_1 cell cycle arrest, confirming the NF-kappaB pathway as a potential therapeutic target in ATLL. Other potential inhibitors of NF-kappaB include the following: proteasome inhibitors, bortezomib (32); a human immunodeficiency virus protease inhibitor, ritonavir (33); retinoids, all-trans retinoic acid (34) and NIK-333 (35); the purine analog fludarabine (36); arsenic trioxide (37); the histone deacetylase inhibitors, depsipeptides (38), MS-275 (39), suberoylanilide hydroxamic acid (39), and LBH589 (39); HSP90 inhibitor, 17-AAG (40); the β-galactoside-binding lectin galectin-9 (41), fucoidan, a natural sulfated polysaccharide extracted from *Cladosiphon okamuranus Tokida* (42); curcumin, a natural compound present in turmeric (43), and oridonin, a natural diterpenoid purified from *Rabdosia rubescens* (44). Although these compounds have limited specificity for the NF-kappaB pathway, they induce apoptosis and G_1 and/or G_2/M cell cycle arrest in ATLL cells and HTLV-1-infected T-cell lines. Based on the

above background, NF-kappaB is an attractive target for therapeutic intervention and NF-kappaB inhibitors are suitable candidates for translating this strategy into clinical medicine.

4.2. AP-1

The AP-1 transcription factor is composed mainly of Jun, Fos, and ATF protein dimers. AP-1 proteins are considered oncogenic, although recent studies have challenged this view by demonstrating that some AP-1 proteins such as JunB and c-Fos have tumor-suppressor activities (45). Unstimulated T cells show low basal level of AP-1 proteins, while T-cell activation rapidly induces the *jun* and *fos* genes (46). AP-1 is the second major survival pathway dysregulated by HTLV-1. ATLL cells and HTLV-1-infected T-cell lines express a number of AP-1 proteins, including c-Fos, Fra-1, c-Jun, JunB, and JunD (47,48); all of these except JunB are activated by Tax at the transcriptional level (47). Tax activates transcription through the AP-1 site and induces AP-1 DNA-binding activity (48,49). However, the AP-1 binding complex in ATLL cells and HTLV-1-infected T-cell lines contains JunD, but not the characterized Fos family members (48). Unmodified and in the absence of other factors, JunD forms unstable complexes with DNA (50). Thus, a factor different from currently known AP-1 transcription factors is likely to be a component of the AP-1 binding complex in ATLL cells and HTLV-1-infected T-cell lines. Recent studies showed high expression levels of Fra-2, JunB, and JunD in ATLL cells, while knockdown of Fra-2 and JunD reduced cell proliferation in HTLV-1-infected T-cell lines, whereas knockdown of JunB did not (51). AP-1 activity can be regulated also at the post-transcriptional level

by the activation of c-Jun N-terminal kinase (JNK) (52). JNK phosphorylates c-Jun within its N-terminal transactivation domain, thereby enhancing its transactivation potential (53). JNK also phosphorylates and potentiates the activity of JunD and ATF-2 (53). Tax could therefore modulate this pathway by constitutively activating JNK (54).

The complementary strand of the HTLV-1 genome encodes HBZ (19), which is expressed in ATLL cells (23). The original two-hybrid screen using CREB-2 as bait identified HBZ as a nuclear basic leucine zipper protein, resembling both AP-1 and CREB/ATF. HBZ has both positive and negative effects on AP-1-dependent transcription and Tax activation of HTLV-1 LTR. HBZ inhibits the transcriptional activation of HTLV-1 LTR via c-Jun, by forming heterocomplexes with c-Jun that are subsequently degraded (20,55). In contrast, HBZ stimulates JunB- or JunD-mediated activation of AP-1 under certain conditions (20,56). Thus, both Tax and HBZ might be important in the constitutive activation of AP-1.

ATLL cells that show weak or no expression of Tax have constitutively activated AP-1 (48). In addition, depsipeptide (38), 17-AAG (40), fucoidan (42), and curcumin (57) suppress AP-1 activation. These inhibitors also have remarkable antiproliferative effects on HTLV-1-infected T-cell lines and primary ATLL cells *in vitro*, and depsipeptide inhibits growth of tumors inoculated subcutaneously into severe combined immunodeficiency mice (38). The inhibition of AP-1 activity is associated with inhibition of JunD expression (40,42,57). Together, these results implicate AP-1-targeting agents in promising therapies for ATLL.

4.3. JAK-STAT

The JAK-STAT signal transduction pathway was first identified in studies of transcriptional regulation in response to interferon (58). It was subsequently implicated more generally in signaling responses to cytokines, hormones, and growth factors (59). JAK-STAT signaling is a major cytokine-stimulated regulatory pathway of T lymphocyte function (60). Specifically, the STAT family of transcription factors is essential for cytokine-regulated processes including growth and proliferation via the activation of downstream genes (61). STAT proteins are activated by JAK proteins, a group of receptor-associated enzymes with tyrosine phosphorylation activity. Whereas JAK and STAT proteins are normally unphosphorylated and inactive in quiescent lymphocytes, JAK1, JAK3, STAT3, and STAT5 become activated in normal T lymphocytes in response to the cytokine, interleukin-2 (IL-2), and are constitutively tyrosine phosphorylated in HTLV-1-transformed T cells (62-64) and ATLL cells (65). The mechanism underlying the constitutive activation of JAK-STAT after HTLV-1 infection is still unclear. Nicot and colleagues (66) reported that the $p12^{I}$ protein, encoded by the pX open reading frame I of HTLV-1, binds to the IL-2 receptor (IL-2R) beta chain, thus activating STAT5 through activation of JAK1 and JAK3. Upon IL-2 stimulation, IL-2R beta and associated signaling molecules are phosphorylated. This in turn recruits SH2-homolog-containing protein-tyrosine phosphatase-1 (SHP-1) to the IL-2R complex, dephosphorylating IL-2R beta, JAK1, and JAK3 to elicit the earliest negative regulation of IL-2-mediated JAK-STAT signaling. However, SHP-1 expression is significantly diminished or undetectable in several HTLV-1-infected T-cell lines that possess constitutively activated JAK-STAT proteins (67). This supported the notion that SHP-1 functions as a tumor suppressor by antagonizing the

growth-promoting and oncogenic potentials of tyrosine kinases. HTLV-1-immortalized T cells are initially IL-2-dependent, but can acquire independence from exogenous IL-2 support following prolonged periods in culture (62). The acquisition of constitutively activated JAK-STAT proteins correlates with a loss of IL-2 dependency (62,63), as does the downregulation of SHP-1 expression (67). Constitutive activation of JAK-STAT pathway was also associated with augmented cell cycle progression in ATLL cells (65). These results argue that ATLL development involves a constitutively activated JAK-STAT pathway.

AG490, a JAK-specific inhibitor, blocked the phosphorylation of JAK1, JAK3, STAT3, and STAT5, and the DNA-binding activity of STAT3 and STAT5 in HTLV-1-infected T-cell lines and primary ATLL cells (68). AG490 inhibits the growth of these cells by inducing G_1 cell cycle arrest and apoptosis. This finding was indirectly supported by studies using cyclin-dependent kinase inhibitor, roscovitine, to induce apoptosis in the HTLV-1-transformed T-cell line MT-2 (69). Roscovitine inhibited STAT5 activation by blocking its tyrosine phosphorylation. In addition, ectopic expression of a dominant-negative form of STAT5 induced apoptosis in MT-2 cells (69). Roscovitine and dominant-negative STAT5 also reduced the expression of antiapoptotic protein XIAP, and STAT5 was associated physically with the XIAP promoter (69). Curcumin also suppresses JAK3, STAT3, and STAT5 activity in HTLV-1-infected T-cell lines (70). Thus, the JAK-STAT pathway is also a suitable target for treatment of ATLL.

4.4. PI3K-Akt

The serine/threonine kinase Akt is a central downstream signaling component for growth factors, cytokines, and other cellular stimuli. Activated receptor tyrosine kinases activate PI3K through either direct binding or tyrosine phosphorylation of scaffolding adaptors, such as IRS1, which then bind and activate PI3K. PI3K is a heterodimer composed of a catalytic subunit (p110) and an adaptor/regulatory subunit (p85). PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3) in a reaction reversible by PTEN, a PIP3 phosphatase. Akt and PDK1 bind to PIP3 at the plasma membrane, where PDK1 phosphorylates the activation loop of Akt on Thr308. Activated receptor tyrosine kinase signaling also activates the mammalian target of rapamycin (mTOR) complex 2 (mTORC2) via an unknown mechanism, and mTORC2 phosphorylates Akt on the hydrophobic Ser473 (71) (Figure 3).

Akt activation mediates multiple biological activities including increased survival, proliferation, and growth of tumor cells. The effect of Akt on the survival of cancer cells is related to its antiapoptotic properties. Akt directly phosphorylates several components of the cell death machinery. For example, phosphorylation of Bad (a proapoptotic member of the Bcl-2 family) prevents its binding and consequent inactivation of the survival factor Bcl-xL. Similarly, Akt phosphorylates and inhibits the catalytic activity of proapoptotic protease caspase-9. The antiapoptotic effect of Akt is also mediated by phosphorylation of the forkhead box O (FOXO) transcription factor, which inhibits the nuclear translocation and activation of proapoptotic FOXO gene target proteins, such as Bim and Fas ligands. In addition to its direct antiapoptotic effect, Akt also acts indirectly on NF-kappaB, a central regulator of cell death. Akt activates

NF-kappaB via direct phosphorylation and activation of IKK, thereby augmenting the transcriptional activity of NF-kappaB family member, RelA. Akt also negatively affects the proapoptotic tumor suppressor p53 via the phosphorylation and activation of p53-binding protein murine double minute-2 (Mdm2), a ubiquitin ligase-containing proteasome that induces the degradation of p53 (72).

In addition to promoting cell survival, Akt also enhances proliferation and growth of tumor cells via its effects on cell cycle regulation by modulating cyclin D1. Akt directly inhibits glycogen synthesis kinase-3 (GSK3) to block the phosphorylation and subsequent degradation of cytoplasmic signaling molecule beta-catenin, allowing it to be translocated to the nucleus. Nuclear beta-catenin combines with different transcription factors such as TCF/LEF-1 to upregulate cyclin D1 expression. This induces cell cycle progression via regulation of RB hyperphosphorylation and inactivation. Phosphorylation of cyclin D1 by GSK3 positively regulates its proteasomal degradation. In a similar way, reduced phosphorylation of cyclin D1 by GSK3 promotes the stabilization of this protein. Akt also phosphorylates p21 and p27, thus inhibiting their antiproliferative effects by retaining them within the cytoplasm and blocking their role in cell cycle inhibition (73).

Akt kinase also mediates phosphorylation and activation of mTOR, which regulates biogenesis by activating p70 ribosomal S6 kinase (S6K) and enhancing translation. mTOR also inhibits 4E-binding protein 1 (4E-BP1), a translational repressor. The subsequent enhanced mRNA translation upregulates multiple proteins involved in cell cycle progression from G_1 to S phase (72). mTOR is also involved in the upregulation of hypoxia-inducible factor-1alpha (HIF-1alpha). HIF-1

transcriptionally activates a variety of genes that promote survival and proliferation of tumor cells by mediating angiogenesis, oxygen transport, glycolysis, glucose uptake, growth factor signaling, invasion, and metastasis, thereby promoting an aggressive tumor phenotype (74).

The PI3K signaling pathway is disrupted in HTLV-1-infected T-cell lines and ATLL cells (10,75-80). Tax promotes PI3K-Akt activation by directly binding the regulatory subunit (p85) of PI3K. Activated Akt then signals through downstream factors to activate AP-1 (10). Tax activation of Akt is also linked to NF-kappaB activation and p53 inhibition (76). Furthermore, activation of Akt by Tax prevents beta-catenin degradation and leads to its accumulation in the cell (81), and increases HIF-1alpha protein synthesis (82). Fukuda et al. (75) reported high levels of phosphorylated Akt in ATLL cells and downregulated expression of inositol phosphatases PTEN and SH2-containing inositol phosphatase-1 (SHIP-1), which antagonize the PI3K-Akt pathway. Application of LY294002, a PI3K inhibitor, to cultured HTLV-1-infected T cells prevented Akt phosphorylation and induced both apoptosis and G_1 cell cycle arrest, suggesting the importance of PI3K-Akt activation in the overall survival of HTLV-1-infected T cells (10,75,76,78,79,83). Rapamycin, mTOR inhibitor, also inhibited growth and induced G_1 cell cycle arrest in HTLV-1-infected T-cell lines (79). Recent studies demonstrated that curcumin (78), 17-AAG (40), and the cyclooxygenase-2 inhibitor, celecoxib (84) suppress Akt activation in HTLV-1-infected T-cell lines and ATLL cells. Thus, there is strong evidence for targeting the PI3K-Akt pathway as a promising treatment strategy for individuals with ATLL.

5. SUMMARY AND PERSPECTIVE

One of the major challenges in ATLL treatment is the inherent resistance or acquisition of resistance to several cytotoxic therapies currently used or under investigation. Studies of the molecular mechanism underlying resistance to apoptosis identified several survival pathways. Such signaling pathways present the potential for more precise targeting of antiapoptotic gene products involved in these survival pathways through the use of pharmacological inhibitors to reverse the resistance. The major survival pathways that regulate resistance to apoptosis are the NF-kappaB, AP-1, JAK-STAT, and PI3K-Akt pathways. All of these are hyperactivated in ATLL cells, but not in normal lymphocytes, and all contribute to the transcriptional and translational regulation of several antiapoptotic and survival gene products. Targeting these pathways is therefore a promising approach to dysregulate ATLL cell resistance to apoptosis. In this review, we focused on the deregulation of cellular signaling processes in ATLL. The multistep and complex nature of leukemogenesis clearly makes the constitutive activation of more than one molecule necessary to induce transformation. Thus, clinical trials will also need to validate each potential molecular target-based drug used alone and in combination.

6. REFERENCES

1. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, and Gallo RC: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 77, 7415-7419 (1980)
2. Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita KI, Shirakawa S, and Miyoshi I: Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl Acad Sci USA* 78, 6476-6480 (1981)
3. Yoshida M, Miyoshi I, and Hinuma Y: Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA* 79, 2031-2035 (1982)
4. Tajima, K: The 4th nation-wide study of adult T-cell leukemia/lymphoma (ATL) in Japan: estimates of risk of ATL and its geographical and clinical features. The T- and B-cell Malignancy Study Group. *Int J Cancer* 45, 237-243 (1990)
5. Yamada Y, Tomonaga M, Fukuda H, Hanada S, Utsunomiya A, Tara M, Sano M, Ikeda S, Takatsuki K, Kozuru M, Araki K, Kawano F, Niimi M, Tobinai K, Hotta T, and Shimoyama M: A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. *Br J Haematol* 113, 375-382 (2001)
6. Hall WW and Fujii M: Deregulation of cell-signaling pathways in HTLV-1 infection. *Oncogene* 24, 5965-5975 (2005)

7. Grassmann R, Aboud M, and Jeang K-T: Molecular mechanisms of cellular transformation by HTLV-1 Tax. *Oncogene* 24, 5976-5985 (2005)
8. Hasegawa H, Sawa H, Lewis MJ, Orba Y, Sheehy N, Yamamoto Y, Ichinohe T, Tsunetsugu-Yokota Y, Katano H, Takahashi H, Matsuda J, Sata T, Kurata T, Nagashima K, and Hall WW: Thymus-derived leukemia-lymphoma in mice transgenic for the Tax gene of human T-lymphotropic virus type I. *Nat Med* 12, 466-472 (2006)
9. Sun S-C and Yamaoka S: Activation of NF-κB by HTLV-I and implications for cell transformation. *Oncogene* 24, 5952-5964 (2005)
10. Peloponese J-M Jr and Jeang K-T: Role for Akt/protein kinase B and activator protein-1 in cellular proliferation induced by the human T-cell leukemia virus type 1 tax oncoprotein. *J Biol Chem* 281, 8927-8938 (2006)
11. Kannagi M, Ohashi T, Harashima N, Hanabuchi S, and Hasegawa A: Immunological risks of adult T-cell leukemia at primary HTLV-I infection. *Trends Microbiol* 12: 346-352 (2004)
12. Taylor GP and Matsuoka M: Natural history of adult T-cell leukemia/lymphoma and approaches to therapy. *Oncogene* 24, 6047-6057 (2005)
13. Kitajima I, Shinohara T, Bilakovics J, Brown DA, Xu X, and Nerenberg M. Ablation of transplanted HTLV-I Tax-transformed tumors in mice by antisense inhibition of NF-κB. *Science* 258, 1792-1795 (1992)
14. Collins ND, Newbound GC, Albrecht B, Beard JL, Ratner L, and Lairmore MD: Selective ablation of human T-cell lymphotropic virus type 1 $p12^{I}$ reduces viral infectivity in vivo. *Blood* 91, 4701-4707 (1998)

15. Silverman LR, Phipps AJ, Montgomery A, Ratner L, and Lairmore MD: Human T-cell lymphotropic virus type 1 open reading frame II-encoded $p30^{II}$ is required for in vivo replication: evidence of in vivo reversion. *J Virol* 78, 3837-3845 (2004)

16. Younis I, Yamamoto B, Phipps A, and Green PL: Human T-cell leukemia virus type 1 expressing nonoverlapping tax and rex genes replicates and immortalizes primary human T lymphocytes but fails to replicate and persist in vivo. *J Virol* 79, 14473-14481 (2005)

17. Hiraragi H, Kim S-J, Phipps AJ, Silic-Benussi M, Ciminale V, Ratner L, Green PL, and Lairmore MD: Human T-lymphotropic virus type 1 mitochondrion-localizing protein $p13^{II}$ is required for viral infectivity in vivo. *J Virol* 80, 3469-3476 (2006)

18. Arnold J, Yamamoto B, Li M, Phipps AJ, Younis I, Lairmore MD, and Green PL: Enhancement of infectivity and persistence in vivo by HBZ, a natural antisense coded protein of HTLV-1. *Blood* 107, 3976-3982 (2006)

19. Gaudray G, Gachon F, Basbous J, Biard-Piechaczyk M, Devaux C, and Mesnard J-M: The complementary strand of the human T-cell leukemia virus type 1 RNA genome encodes a bZIP transcription factor that down-regulates viral transcription. *J Virol* 76, 12813-12822 (2002)

20. Basbous J, Arpin C, Gaudray G, Piechaczyk M, Devaux C, and Mesnard J-M: The HBZ factor of human T-cell leukemia virus type I dimerizes with transcription factors JunB and c-Jun and modulates their transcriptional activity. *J Biol Chem* 278, 43620-43627 (2003)

21. Hidaka M, Inoue J, Yoshida M, and Seiki M: Post-transcriptional regulator (rex) of HTLV-1 initiates expression of viral structural proteins but suppresses expression of regulatory proteins. *EMBO J* 7, 519-523 (1988)

22. Zhang W, Nisbet JW, Albrecht B, Ding W, Kashanchi F, Bartoe JT, and Lairmore MD: Human T-lymphotropic virus type 1 $p30^{II}$ regulates gene transcription by binding CREB binding protein/p300. *J Virol* 75, 9885-9895 (2001)

23. Satou Y, Yasunaga J, Yoshida M, and Matsuoka M: HTLV-I basic leucine zipper factor gene mRNA supports proliferation of adult T cell leukemia cells. *Proc Natl Acad Sci USA* 103, 720-725 (2006)

24. Lin A and Karin M: NF-κB in cancer: a marked target. *Semin Cancer Biol* 13, 107-114 (2003)

25. Hayden MS and Ghosh S: Signaling to NF-κB. *Genes Dev* 18, 2195-2224 (2004)

26. Bonizzi G and Karin M: The two NF-κB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 25, 280-288 (2004)

27. Mori N, Fujii M, Ikeda S, Yamada Y, Tomonaga M, Ballard DW, and Yamamoto N: Constitutive activation of NF-κB in primary adult T-cell leukemia cells. *Blood* 93, 2360-2368 (1999)

28. Saitoh Y, Yamamoto N, Dewan MZ, Sugimoto H, Martinez B VJ, Iwasaki Y, Matsubara K, Qi X, Saitoh T, Imoto I, Inazawa J, Utsunomiya A, Watanabe T, Masuda T, Yamamoto N, and Yamaoka S. Overexpressed NF-κB inducing kinase contributes to the tumorigenesis of adult T-cell leukemia and Hodgkin Reed-Sternberg cells. Blood [Epub ahead of print] (2008 Feb 27)

29. Mori N, Yamada Y, Ikeda S, Yamasaki Y, Tsukasaki K, Tanaka Y, Tomonaga M, Yamamoto N, and Fujii M: Bay 11-7082 inhibits transcription factor NF-κB and induces apoptosis of HTLV-I-infected T-cell lines and primary adult T-cell leukemia cells. *Blood* 100, 1828-1834 (2002)

30. Watanabe M, Ohsugi T, Shoda M, Ishida T, Aizawa S, Maruyama-Nagai M, Utsunomiya A, Koga S, Yamada Y, Kamihira S, Okayama A, Kikuchi H, Uozumi K, Yamaguchi K, Higashihara M, Umezawa K, Watanabe T, and Horie R: Dual targeting of transformed and untransformed HTLV-1-infected T cells by DHMEQ, a potent and selective inhibitor of NF-κB, as a strategy for chemoprevention and therapy of adult T-cell leukemia. *Blood* 106, 2462-2471 (2005)

31. Sanda T, Asamitsu K, Ogura H, Iida S, Utsunomiya A, Ueda R, and Okamoto T: Induction of cell death in adult T-cell leukemia cells by a novel IκB kinase inhibitor. *Leukemia* 20, 590-598 (2006)

32. Satou Y, Nosaka K, Koya Y, Yasunaga J-I, Toyokuni S, and Matsuoka M. Proteasome inhibitor, bortezomib, potently inhibits the growth of adult T-cell leukemia cells both *in vivo* and *in vitro*. *Leukemia* 18, 1357-1363 (2004)

33. Dewan MZ, Uchihara J-N, Terashima K, Honda M, Sata T, Ito M, Fujii N, Uozumi K, Tsukasaki K, Tomonaga M, Kubuki Y, Okayama A, Toi M, Mori N, and Yamamoto N. Efficient intervention of growth and infiltration of primary adult T-cell leukemia cells by an HIV protease inhibitor, ritonavir. *Blood* 107, 716-724 (2006)

34. Nawata H, Maeda Y, Sumimoto Y, Miyatake J, and Kanamaru A. A mechanism of apoptosis induced by all-trans retinoic acid on adult T-cell leukemia cells: a

possible involvement of the Tax/NF-κB signaling pathway. *Leuk Res* 25, 323-331 (2001)

35. Okudaira T, Tomita M, Uchihara J-N, Matsuda T, Ishikawa C, Kawakami H, Masuda M, Tanaka Y, Ohshiro K, Takasu N, and Mori N. NIK-333 inhibits growth of human T-cell leukemia virus type I-infected T-cell lines and adult T-cell leukemia cells in association with blockade of nuclear factor-κB signal pathway. *Mol Cancer Ther* 5, 704-712 (2006)
36. Nishioka C, Ikezoe T, Yang J, Koeffler HP, and Taguchi H. Fludarabine induces apoptosis of human T-cell leukemia virus type 1-infected T cells via inhibition of the nuclear factor-κB signal pathway. *Leukemia* 21, 1044-1049 (2007)
37. Nasr R, Rosenwald A, El-Sabban ME, Arnulf B, Zalloua P, Lepelletier Y, Bex F, Hermine O, Staudt L, de The H, and Bazarbachi A. Arsenic/interferon specifically reverses 2 distinct gene networks critical for the survival of HTLV-1-infected leukemic cells. *Blood* 101, 4576-4582 (2003)
38. Mori N, Matsuda T, Tadano M, Kinjo T, Yamada Y, Tsukasaki K, Ikeda S, Yamasaki Y, Tanaka Y, Ohta T, Iwamasa T, Tomonaga M, and Yamamoto N. Apoptosis induced by the histone deacetylase inhibitor FR901228 in human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells. *J Virol* 78, 4582-4590 (2004)
39. Nishioka C, Ikezoe T, Yang J, Komatsu N, Bandobashi K, Taniguchi A, Kuwayama Y, Togitani K, Koeffler HP, and Taguchi H. Histone deacetylase inhibitors induce growth arrest and apoptosis of HTLV-1-infected T-cells via blockade of signaling by nuclear factor κB. *Leuk Res* 32, 287-296 (2008)

40. Kawakami H, Tomita M, Okudaira T, Ishikawa C, Matsuda T, Tanaka Y, Nakazato T, Taira N, Ohshiro K, and Mori N. Inhibition of heat shock protein-90 modulates multiple functions required for survival of human T-cell leukemia virus type I-infected T-cell lines and adult T-cell leukemia cells. *Int J Cancer* 120, 1811-1820 (2007)

41. Okudaira T, Hirashima M, Ishikawa C, Makishi S, Tomita M, Matsuda T, Kawakami H, Taira N, Ohshiro K, Masuda M, Takasu N, and Mori N. A modified version of galectin-9 suppresses cell growth and induces apoptosis of human T-cell leukemia virus type I-infected T-cell lines. *Int J Cancer* 120, 2251-2261 (2007)

42. Haneji K, Matsuda T, Tomita M, Kawakami H, Ohshiro K, Uchihara J-N, Masuda M, Takasu N, Tanaka Y, Ohta T, and Mori N. Fucoidan extracted from *Cladosiphon okamuranus Tokida* induces apoptosis of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells. *Nutr Cancer* 52, 189-201 (2005)

43. Tomita M, Kawakami H, Uchihara J-N, Okudaira T, Masuda M, Takasu N, Matsuda T, Ohta T, Tanaka Y, Ohshiro K, and Mori N. Curcumin (diferuloylmethane) inhibits constitutive active NF-κB, leading to suppression of cell growth of human T-cell leukemia virus type I-infected T-cell lines and primary adult T-cell leukemia cells. *Int J Cancer* 118, 765-772 (2006)

44. Ikezoe T, Yang Y, Bandobashi K, Saito T, Takemoto S, Machida H, Togitani K, Koeffler HP, and Taguchi H. Oridonin, a diterpenoid purified from *Rabdosia rubescens*, inhibits the proliferation of cells from lymphoid malignancies in

association with blockade of the NF-κB signal pathways. *Mol Cancer Ther* 4, 578-586 (2005)

45. Eferl R and Wagner EF: AP-1: a double-edged sword in tumorigenesis. *Nat Rev Cancer* 3, 859-868 (2003)
46. Behrens A, Sabapathy K, Graef I, Cleary M, Crabtree GR, and Wagner EF: Jun N-terminal kinase 2 modulates thymocyte apoptosis and T cell activation through c-Jun and nuclear factor of activated T cell (NF-AT). *Proc Natl Acad Sci USA* 98, 1769-1774 (2001)
47. Fujii M, Niki T, Mori T, Matsuda T, Matsui M, Nomura N, and Seiki M: HTLV-1 Tax induces expression of various immediate early serum responsive genes. *Oncogene* 6, 1023-1029 (1991)
48. Mori N, Fujii M, Iwai K, Ikeda S, Yamasaki Y, Hata T, Yamada Y, Tanaka Y, Tomonaga M, and Yamamoto N: Constitutive activation of transcription factor AP-1 in primary adult T-cell leukemia cells. *Blood* 95, 3915-3921 (2000)
49. Iwai K, Mori N, Oie M, Yamamoto N, and Fujii M: Human T-cell leukemia virus type 1 tax protein activates transcription through AP-1 site by inducing DNA binding activity in T cells. *Virology* 279, 38-46 (2001)
50. Ryder K, Lanahan A, Perez-Albuerne E, and Nathans D: jun-D: a third member of the jun gene family. *Proc Natl Acad Sci USA* 86, 1500-1503 (1989)
51. Nakayama T, Hieshima K, Arao T, Jin Z, Nagakubo D, Shirakawa A-K, Yamada Y, Fujii M, Oiso N, Kawada A, Nishio K, and Yoshie O: Aberrant expression of Fra-2 promotes CCR4 expression and cell proliferation in adult T-cell leukemia. *Oncogene* [Epub ahead of print] (2007 Dec 10)

52. Davis RJ: Signal transduction by the JNK group of MAP kinases. *Cell* 103, 239-252 (2000)

53. Hess J, Angel P, and Schorpp-Kistner M: AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci* 117 (Pt 25), 5965-5973 (2004)

54. Jin D-Y, Teramoto H, Giam C-Z, Chun RF, Gutkind JS, and Jeang K-T: A human suppressor of c-Jun N-terminal kinase 1 activation by tumor necrosis factor α. *J Biol Chem* 272, 25816-25823 (1997)

55. Matsumoto J, Ohshima T, Isono O, and Shimotohno K: HTLV-1 HBZ suppresses AP-1 activity by impairing both the DNA-binding ability and the stability of c-Jun protein. *Oncogene* 24, 1001-1010 (2005)

56. Thebault S, Basbous J, Hivin P, Devaux C, and Mesnard J-M: HBZ interacts with JunD and stimulates its transcriptional activity. *FEBS Lett* 562, 165-170 (2004)

57. Tomita M, Kawakami H, Uchihara J-N, Okudaira T, Masuda M, Takasu N, Matsuda T, Ohta T, Tanaka Y, and Mori N: Curcumin suppresses constitutive activation of AP-1 by downregulation of JunD protein in HTLV-1-infected T-cell lines. *Leuk Res* 30, 313-321 (2006)

58. Velazquez L, Fellous M, Stark GR, and Pellegrini S: A protein tyrosine kinase in the interferon α/β signaling pathway. *Cell* 70, 313-322 (1992)

59. Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, and Silvennoinen O: Signaling through the hematopoietic cytokine receptors. *Annu Rev Immunol* 13, 369-398 (1995)

60. Lin J-X and Leonard WJ. The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. *Oncogene* 19, 2566-2576 (2000)

61. Bromberg J: Stat proteins and oncogenesis. *J Clin Invest* 109, 1139-1142 (2002)

62. Migone T-S, Lin J-X, Cereseto A, Mulloy JC, O'Shea JJ, Franchini G, and Leonard WJ: Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-I. *Science* 269, 79-81 (1995)

63. Xu X, Kang S-H, Heidenreich O, Okerholm M, O'Shea JJ, and Nerenberg MI: Constitutive activation of different Jak tyrosine kinases in human T cell leukemia virus type 1 (HTLV-1) tax protein or virus-transformed cells. *J Clin Invest* 96, 1548-1555 (1995)

64. Mulloy JC, Migone T-S, Ross TM, Ton N, Green PL, Leonard WJ, and Franchini G: Human and simian T-cell leukemia viruses type 2 (HTLV-2 and STLV-2_{pan-p}) transform T cells independently of Jak/STAT activation. *J Virol* 72, 4408-4412 (1998)

65. Takemoto S, Mulloy JC, Cereseto A, Migone TS, Patel BK, Matsuoka M, Yamaguchi K, Takatsuki K, Kamihira S, White JD, Leonard WJ, Waldmann T, and Franchini G: Proliferation of adult T cell leukemia/lymphoma cells is associated with the constitutive activation of JAK/STAT proteins. *Proc Natl Acad Sci USA* 94, 13897-13902 (1997)

66. Nicot C, Mulloy JC, Ferrari MG, Johnson JM, Fu K, Fukumoto R, Trovato R, Fullen J, Leonard WJ, and Franchini G: HTLV-1 $p12^{I}$ protein enhances STAT5 activation and decreases the interleukin-2 requirement for proliferation of primary human peripheral blood mononuclear cells. *Blood* 98, 823-829 (2001)

67. Migone T-S, Cacalano NA, Taylor N, Yi T, Waldmann TA, and Johnston JA. Recruitment of SH2-containing protein tyrosine phosphatase SHP-1 to the

interleukin 2 receptor; loss of SHP-1 expression in human T-lymphotropic virus type I-transformed T cells. *Proc Natl Acad Sci USA* 95, 3845-3850 (1998)

68. Tomita M, Kawakami H, Uchihara J-N, Okudaira T, Masuda M, Matsuda T, Tanaka Y, Ohshiro K, and Mori N: Inhibition of constitutively active Jak-Stat pathway suppresses cell growth of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells. *Retrovirology* 3, 22 (2006)

69. Mohapatra S, Chu B, Wei S, Djeu J, Epling-Burnette PK, Loughran T, Jove R, and Pledger WJ: Roscovitine inhibits STAT5 activity and induces apoptosis in the human leukemia virus type 1-transformed cell line MT-2. *Cancer Res* 63, 8523-8530 (2003)

70. Rajasingh J, Raikwar HP, Muthian G, Johnson C, and Bright JJ: Curcumin induces growth-arrest and apoptosis in association with the inhibition of constitutively active JAK-STAT pathway in T cell leukemia. *Biochem Biophys Res Commun* 340, 359-368 (2006)

71. Manning BD and Cantley LC: AKT/PKB signaling: navigating downstream. *Cell* 129, 1261-1274 (2007)

72. Younes H, Leleu X, Hatjiharissi E, Moreau A-S, Hideshima T, Richardson P, Anderson KC, and Ghobrial IM: Targeting the phosphatidylinositol 3-kinase pathway in multiple myeloma. *Clin Cancer Res* 13, 3771-3775 (2007)

73. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, and Gonzalez-Baron M: PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* 30, 193-204 (2004)

74. Bardos JI and Ashcroft M: Hypoxia-inducible factor-1 and oncogenic signalling. *BioEssays* 26, 262-269 (2004)

75. Fukuda R, Hayashi A, Utsunomiya A, Nukada Y, Fukui R, Itoh K, Tezuka K, Ohashi K, Mizuno K, Sakamoto M, Hamanoue M, and Tsuji T: Alteration of phosphatidylinositol 3-kinase cascade in the multilobulated nuclear formation of adult T cell leukemia/lymphoma (ATLL). *Proc Natl Acad Sci USA* 102, 15213-15218 (2005)

76. Jeong S-J, Pise-Masison CA, Radonovich MF, Park HU, and Brady JN: Activated AKT regulates NF-κB activation, p53 inhibition and cell survival in HTLV-1-transformed cells. *Oncogene* 24, 6719-6728 (2005)

77. Liu Y, Wang Y, Yamakuchi M, Masuda S, Tokioka T, Yamaoka S, Maruyama I, and Kitajima I: Phosphoinositide-3 kinase-PKB/Akt pathway activation is involved in fibroblast Rat-1 transformation by human T-cell leukemia virus type I tax. *Oncogene* 20, 2514-2526 (2001)

78. Tomita M, Matsuda T, Kawakami H, Uchihara J-N, Okudaira T, Masuda M, Ohshiro K, and Mori N: Curcumin targets Akt cell survival signaling pathway in HTLV-I-infected T-cell lines. *Cancer Sci* 97, 322-327 (2006)

79. Ikezoe T, Nishioka C, Bandobashi K, Yang Y, Kuwayama Y, Adachi Y, Takeuchi T, Koeffler HP, and Taguchi H: Longitudinal inhibition of PI3K/Akt/mTOR signaling by LY294002 and rapamycin induces growth arrest of adult T-cell leukemia cells. *Leuk Res* 31, 673-682 (2007)

80. Akl H, Badran BM, Zein NE, Bex F, Sotiriou C, Willard-Gallo KE, Burny A, and Martiat P: HTLV-I infection of WE17/10 $CD4^+$ cell line leads to progressive

alteration of Ca^{2+} influx that eventually results in loss of CD7 expression and activation of an antiapoptotic pathway involving AKT and BAD which paves the way for malignant transformation. *Leukemia* 21, 788-796 (2007)

81. Tomita M, Kikuchi A, Akiyama T, Tanaka Y, and Mori N: Human T-cell leukemia virus type 1 tax dysregulates β-catenin signaling. *J Virol* 80, 10497-10505 (2006)
82. Tomita M, Semenza GL, Michiels C, Matsuda T, Uchihara J-N, Okudaira T, Tanaka Y, Taira N, Ohshiro K, and Mori N: Activation of hypoxia-inducible factor 1 in human T-cell leukaemia virus type 1-infected cell lines and primary adult T-cell leukaemia cells. *Biochem J* 406, 317-323 (2007)
83. Jeong S-J, Dasgupta A, Jung K-J, Um J-H, Burke A, Park HU, and Brady JN: PI3K/AKT inhibition induces caspase-dependent apoptosis in HTLV-1-transformed cells. *Virology* 370, 264-272 (2008)
84. Sinha-Datta U, Taylor JM, Brown M, and Nicot C: Celecoxib disrupts the canonical apoptotic network in HTLV-I cells through activation of Bax and inhibition of PKB/Akt. *Apoptosis* 13, 33 (2008)

Abbreviations:

AP-1: activator protein-1;

ATLL: Adult T-cell leukemia/lymphoma;

CTL: cytotoxic T lymphocytes;

FOXO: forkhead box O;

GSK: Glycogen synthesis kinase;

HBZ: HTLV-1 basic leucine zipper factor;

HIF: hypoxia-inducible factor;

HTLV-1: human T-cell leukemia virus type 1;

IKK: IkappaB kinase;

IL: interleukin;

IL-2R: IL-2 receptor;

JAK: janus kinase;

JNK: c-Jun N-terminal kinase;

LTR, long terminal repeat;

Mdm2: murine double minute-2;

mTOR: mammalian target of rapamycin;

mTORC: mTOR complex;

NF-kappaB: nuclear factor-kappaB;

NIK, NF-kappaB inducing kinase;

PI3K: phosphatidylinositol 3-kinase;

PIP2: phosphatidylinositol-4,5-bisphosphate;

PIP3: phosphatidylinositol-3,4,5-trisphosphate;

S6K: S6 kinase; 4E-BP: 4E-binding protein;

SHIP: SH2-containing inositol phosphatase

SHP-1: SH2-homology-containing protein-tyrosine phosphatase-1;

STAT: signal transducer and activator of transcription;

TNF: tumor necrosis factor.

Key Words: Adult T-cell leukemia/lymphoma, Human T-cell leukemia virus type 1, Signal transduction, Apoptosis

Send correspondence to: Prof. Naoki Mori, Division of Molecular Virology and Oncology, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan, Tel: 81-98-895-1130, Fax: 81-98-895-1410, E-mail: n-mori@med.u-ryukyu.ac.jp

Figure legends

Figure 1. Schematic model of ATLL leukemogenesis. HTLV-1 is transmitted in a cell-to-cell fashion through breast milk. HTLV-1-infected T cells proliferate clonally requiring Tax and other viral proteins. However, proliferation of HTLV-1-infected T cells is also controlled by the host immune system. The balance between proliferation of HTLV-1-infected T cells and elimination by the host immune system is established in the carrier phase. After a long latency period, ATLL develops in about 5% of asymptomatic carriers. Tax expression is suppressed by several mechanisms, suggesting that Tax is not necessary at this stage. Instead, genetic and epigenetic alterations accumulate progressively in the host genome during the latency period, culminating in the onset of ATLL. CTL: cytotoxic T lymphocytes; ATL: adult T-cell leukemia/lymphoma; HBZ: HTLV-1 basic leucine zipper factor.

Figure 2. Activation of two NF-kappaB signaling pathways by Tax. The noncanonical pathway consists of NIK- and IKK alpha-mediated p100 processing and nuclear mobilization of RelB/p52 heterodimers. Tax triggers activation of this pathway downstream of NIK by activating IKK alpha via IKK gamma and recruiting IKK alpha to p100 to stimulate phosphorylation, ubiquitination, and processing to p52. The canonical pathway consists of IKK-mediated phosphorylation, ubiquitination, and degradation of IkappaB proteins, leading to degradation by the proteasome, and the nuclear translocation of RelA and c-Rel-containing heterodimers. Tax activates the canonical pathway by interacting with IKK gamma and stimulating the catalytic

activity of IKK alpha and IKK beta. Tax may recruit upstream kinases to trigger IKK activation. See text for abbreviations.

Figure 3. Upstream activation of Akt by growth factors. Activation of growth factor receptor protein tyrosine kinases results in autophosphorylation of tyrosine residues. PI3K is recruited to the membrane through direct binding to phosphotyrosine consensus residues of growth factor receptors or through tyrosine phosphorylation of scaffolding adaptors, such as IRS1. This leads to allosteric activation of the catalytic subunit. Activation leads to production of the second messenger PIP3, which in turn recruits a subset of PH domain-containing signaling proteins to the membrane, including PDK1 and Akt. PTEN is a PIP3 phosphatase that negatively regulates the PI3K-Akt pathway. Once activated, Akt mediates the activation and inhibition of several targets, resulting in cell survival, growth, and proliferation through various mechanisms. See text for abbreviations.

Running title: Cell signaling modifiers in ATLL