FERM domain mutations induce gain of function in JAK3 in adult T-cell leukemia/lymphoma

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Adult T-cell leukemia/lymphoma (ATLL) is an incurable disease where most patients succumb within the first year of diagnosis. Both standard chemotherapy regimens and mAbs directed against ATLL tumor markers do not alter this aggressive clinical course. Therapeutic development would be facilitated by the discovery of genes and pathways that drive or initiate ATLL, but so far amenable drug targets have not been forthcoming. Because the IL-2 signaling pathway plays a prominent role in ATLL pathogenesis, mutational analysis of pathway components should yield interesting results. In this study, we focused on JAK3, the nonreceptor tyrosine kinase that signals from the IL-2R, where activating mutations have been found in diverse neoplasms. We screened 36 ATLL patients and 24 ethnically matched controls and found 4 patients with mutations in JAK3. These somatic, missense mutations occurred in the N-terminal FERM (founding members: band 4.1, ezrin, radixin, and moesin) domain and induced gain of function in JAK3. Importantly, we show that these mutant JAK3s are inhibited with a specific kinase inhibitor already in human clinical testing. Our findings underscore the importance of this pathway in ATLL development and offer a therapeutic handle for this incurable cancer. (Blood. 2011;118(14):3911-3921)

Introduction

ATLL is an aggressive T-cell neoplasm that is resistant to diverse therapeutic approaches. Because the disease is rare, small case series have been published testing cytotoxic chemotherapy, IFN therapy, nucleoside analogues, stem cell transplantation, and immune therapy, but no specific regimen induces durable remission. Cures have been reported after stem cell transplantation but the treatment-related morbidity and mortality are high. Unfortunately, newly diagnosed patients can only expect a median survival of <12 months. Perhaps through better understanding of the unique biology and pathogenesis of ATLL we could generate novel therapeutic targets. Indeed, ATLL is a remarkable example of retrovirally induced cancer in humans. ATLL is caused by infection of T cells by human T-lymphotropic virus-1 (HTLV-1) which is endemic in southwestern Japan, the Caribbean islands, West Africa, and South America. HTLV-1 is a complex retrovirus that expresses multiple gene products that contribute to T-cell transformation; among these, the retroviral oncogene Tax can induce T-cell immortalization on its own and is thought to be absolutely required for ATLL development. Interestingly, ATLL arises in only 5% of patients infected with HTLV-1 and disease presents up to 3 decades after initial infection. This suggests that there are probably multiple oncogenic mutations from the initiating hit of HTLV-1 infection to the development of ATLL. In fact, host somatic mutations in specific genes and pathways are probably required for ATLL and could explain the low penetrance and long latency.

One major pathway implicated in ATLL development is the activation of IL-2 (and related cytokines) signaling. In fact, the IL2 and IL2RA (α chain or CD25) genes are trans-activated by tax protein. The IL-2Rβ and IL-2Rγ chains are constitutively expressed on T cells but the high-affinity IL-2R requires the IL-2Ra chain. Thus, Tax-expressing T cells are induced to proliferate because of autocrine signaling from IL-2 to the IL-2R, composed of α, β, γ chains; other HTLV-1 gene products may contribute to this same pathway. On ligand binding, the nonreceptor tyrosine kinases, JAK1 and JAK3, initiate phosphorylation of downstream substrates such as the STAT5 transcription factors, A and B, which in turn activate target genes that allow cell-cycle entry (ie, CCND2) and block apoptosis (ie, BCL2L1). JAK3 is constitutively bound to the IL-2Rγ (also γc) chain and is used by the receptors for IL-4, IL-7, IL-9, IL-15, and IL-21. Interestingly, IL-9 is up-regulated in some ATLL cells so cytokines other than IL-2 may also use JAK3/γc.

Most remarkably, as ATLL progresses, Tax gene expression is lost by methylation or deletion in a majority of patients. Despite this, many ATLL cell lines remain hypersensitive to γc-using cytokines, such as IL-2, and show constitutive activation of downstream substrates of JAK3 such as STAT5a and PI3K/AKT. Mutations in γc-restricted cytokine signal transduction components (eg, JAK3) could explain the activation of the pathway in the absence of Tax transcriptional stimulus. The JAK family kinases are central players in normal and malignant hematopoiesis and are candidates for oncogenic somatic mutations. These nonreceptor tyrosine kinases are recruited for signal transduction by cytokine receptors that do not possess tyrosine kinase activity on their own. Activating mutations in JAK2 are clonal in a large
number of myeloproliferative disorders (MPDs) and recently mutations in JAK1 and JAK3 have been described in acute myeloid and lymphoblastic leukemias.17-20 The JAK proteins are composed of FERM, SH2, pseudokinase and kinase domains (Figure 1A).12 The FERM (named after founding members band 4.1, ezrin, radixin, and moesin) is predominantly responsible for binding the cytoplasmic tails of cytokine receptor chains.21,22 The pseudokinase domain structurally resembles a kinase domain but does not function as one and is thought to have regulatory function.23 This domain is a hotspot for most of the mutations described in JAK1-3 in MPDs and leukemias. In this study, we analyzed the JAK3 gene in ATLL patients and found 4 ATLL patients with missense mutations in the N-terminal FERM domain. We show that these mutations cause gain of function in the kinase. Most importantly, we found the mutant JAK3s were inhibited by a specific JAK3 inhibitor, tofacitinib, which is already in human clinical testing for autoimmune disorders.24,25 Our studies propose JAK3 kinase as a therapeutic target in ATLL.

Methods
Patient samples, sequencing, and mutational analysis
Genomic DNA was isolated from 36 ATLL patients consented and treated at the NIH Clinical Center under an institutional review board (IRB)–approved protocol. Most of these patients were from Jamaica. Likewise, 24 ethnically matched (Jamaica or Haiti; see supplemental Table 5, available on the Blood Web site; see the Supplemental Materials link at the top of the online article) control patients were consented and had their whole blood drawn for genomic DNA preparation under an IRB-approved protocol. The entire JAK3 gene was analyzed by PCR amplification of specific exons (supplemental Table 2) and analyzed for sequence alterations by SpectrumeX’s Reveal Genetic Analysis System on samples where the leukemia cells were purified from the buffy coat. Standard Sanger DNA sequencing (GeneWiz and Vanderbilt Core Sequencing facility) was subsequently used to analyze all potential mutations as determined by Reveal Genetic Analysis System. Multiple independent PCR amplicons cloned into pGEM-TEasy (Promega) were used to analyze samples that did not have pure leukemic samples or in some instances had <10% leukemic cells. Oligonucleotides used for PCR are available in supplemental Table 2. The proposed JAK3 FERM domain structure was determined by ClustalW alignment with the FAK FERM domain and analyzed with PyMol using FAK FERM coordinates. The crystal structure of the JAK3 kinase domain was available from the RCSB Protein Data Bank.26

Viruses and plasmid constructs
MSCV-ires-GFP (MIG) constructs expressing mutant JAK3 cDNAs were made by introducing mutations into the wild-type (WT) JAK3 using the Stratagene QuiikChange kit (supplemental Table 3). The WT JAK3 and A572V JAK3 mutant constructs were provided by Dr Brian Druker (Oregon Health and Science University, Portland, OR) cloned into MIG. Site-directed mutagenesis was verified by full sequencing of the JAK3 cDNA insert. Virus was made by transient transfections of JAK3-MIG constructs into Phoenix cells by the CaPO4 method along with pCMV-IL2Rα and pCMV-STAT5A constructs. Viral titers were determined by flow cytometric analysis of 3T3 cells for GFP expression. pCMV6-IL2Rβ and pCMV6-STAT5A constructs were obtained from the Mammalian Gene Collection. IL2Rα was
cloned into pcDNA 3.1(+; Invitrogen) from pNCY-IL2Rx (Open Biosystems) construct. IL-2Ry was amplified from HuT-102 cDNA and cloned into pcDNA 3.1(+). All inserts were fully sequenced.

**Flow cytometry and Western blots**

Flow cytometry experiments were performed in the Vanderbilt Flow Cytometry Shared Resource core. Flow cytometry data acquisition was performed on an LSRII 3 laser (BD Biosciences) and analyzed with FACSDiva software. BaF3 cells transduced with various MIG-JAK3s were sorted for GFP on a FACS Aria (BD Biosciences). These cells were also stained with Vybrant Dyecycle violet (Invitrogen) and the proportion of cells in each cell cycle stage was determined by flow cytometry and analyzed using FlowJo software. Immunoprecipitations were performed in BaF3 cells transduced with MIG-JAK3 using anti-JAK3 (Santa Cruz Biotechnology Inc) and anti-γc Abs (R&D Biosystems). Detection of total and phosphorylated protein targets was performed using the LI-COR Odyssey Infrared Imaging System. Abs used were anti-phospho-STAT5 (Y694; BD Transduction Laboratories/BD Pharmingen), anti–phospho-AKT (S473), anti-ERK1/2, anti–phospho-ERK1/2 (T202/T204; Cell Signalling Technology), anti-STAT5, anti-IL2Rx, anti-IL2Rβ, anti-γc, anti-JAK3, anti-STAT3, anti–phospho-STAT3 (Y705), and anti–phospho-JAK3 (Y980; Santa Cruz Biotechnology Inc) and 680- and 800-nm infrared dye-conjugated secondary Abs (LI-COR).

**Cell lines and inhibitor assays**

BaF3 cells were obtained from Dr Elizabeth Yang (Vanderbilt). OP9-DL1 and OP9-GFP cell lines were provided by Dr Juan Carlos Zúñiga-Pflücker (University of Toronto). The following reagents were obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health): HuT-102 and MT-2 cell lines. We obtained HEK 293T and Phoenix cell lines through ATCC. All cell lines were maintained in recommended media (supplemental Table 4). The T-cell differentiation assay was performed using JAK3−/− fetal liver cells. JAK3−/− mice were acquired from The Jackson Laboratory.23 JAK3−/− fetal liver cells were collected at embryonic day (E) 15.5. Mononuclear cells were isolated using lymphocyte separation medium (MediaTech). Cells were magnetically sorted for c-kit+Lin−Sca-1− (KL5) cells (StemCell Technologies Inc) and verified by flow cytometry. Cells defined as KL5 were then transduced with MIG-JAK3, and cocultured with irradiated OP9-DL1 or OP9-GFP cells in MIG-JAK3 separation medium (MediaTech). Cells were magnetically sorted to enrich newly identified mutations in ATLL. We isolated JAK3 FERM domain mutations in ATLL patients do not show loss of function

**Results**

**JAK3 FERM domain mutations in ATLL patients do not show loss of function**

We analyzed 36 ATLL patients for mutations in the JAK3 gene by heteroduplex analysis, PCR cloning, and direct sequencing. In 4 patients (11%), we found sequence variants that were not present in single nucleotide polymorphism (SNP) databases or in 24 ethnically matched control individuals51 (Figure 1A), supplemental Figure 1, supplemental Tables 1 and 5). Some of our samples were purified leukemic blasts from apheresed patients and some were admixed with normal cells of the buffy coat. The frequency of one of these mutations based on cloned PCR products (10%) correlated with the frequency of leukemic cells in genomic DNA samples as estimated by quantitative PCR for tax (supplemental Figure 2). Missense mutations L156P, R172Q, and E183G occurred in highly conserved residues and were somatically acquired (Figure 1A). The E183G mutation was recurrent because it was found in 2 patients. All mutations clustered within the FERM domain of JAK3 whose major function is to bind the cytoplasmic tail of γc.21,35 In humans, autosomal-recessive SCID is caused by loss of function of JAK3.36 Some of the germline SCID mutations described occur in the FERM domain of JAK3 and abolish binding to γc.35 Therefore, we sought to rule out loss of function of JAK3 because of these newly identified mutations in ATLL. We isolated JAK3−/− fetal liver (Lin−Sca-1+c-Kit+) hematopoietic stem and progenitor cells (HSPCs) and cocultured them with OP9-GFP or OP9-DL1 stromal cells as described.28 JAK3 knockout cells failed to proliferate or differentiate into CD4+CD8+ double-positive (DP) T cells but JAK3−/− HSPCs transduced with WT JAK3 were rescued (Figure 1C). A previously described activating mutant JAK3 A572V and the ATL mutations also rescued proliferative and differentiation defects in the knockouts (Figure 1B-D).19,27,28 Interestingly, T-cell differentiation and proliferation did not occur in the transduced cells if IL-7 was omitted from culture. We evaluated our mutant JAK3s for γc binding by coimmunoprecipitation assays and found that mutant JAK3s bound γc to the same extent as WT JAK3 (supplemental Figure 3). These experiments showed that the ATLL...
JAK3 mutations in the FERM domain did not cause loss of function but also did not show gain of function in this assay.

**ATLL mutations show increased phosphorylation of STAT5A**

Next, we reconstituted IL-2 signaling in HEK 293T cells, which lack IL-2R proteins, by cotransfecting plasmids expressing the heterotrimeric IL-2R, β, γc, WT or mutant JAK3, and the JAK3 substrate STAT5A. Equivalent amounts of protein were expressed in each transfection as quantified by Western blotting with infrared dye-labeled secondary Abs. We quantified phosphorylated STAT5A using a primary Ab specific to phospho-Y694 of STAT5A (Figure 2), a residue uniquely phosphorylated by JAK3. Cotransfection of the IL-2R components with STAT5A showed no phospho-STAT5A in the absence of JAK3 (lane 3 of Figure 2A). Cotransfection of IL-2R, β, γc, STAT5A, and WT JAK3 induced phospho-STAT5A only in the presence of exogenous IL-2 (100 U/mL; Figure 2A lane 4). We consistently found increased phospho-STAT5A with mutant JAK3 cotransfection in comparison to WT JAK3 cotransfection (quantification in Figure 2B). Equivalent expression of JAK3 was seen in each transfection and phospho-STAT5A levels were normalized to JAK3 protein expression (Figure 2B). The L156P JAK3 mutant did not show statistically significant increase in phospho-STAT5A levels in this assay but was biologically active in BaF3 cells (Figure 3A).

To verify that the observed phospho-STAT5A was caused by JAK3 kinase activity, we introduced a K855A mutation that creates a catalytically inactive kinase (Figure 2B bottom panel bar 5) into a JAK3 construct with the E183G FERM domain mutation. This compound mutant expressed stable protein to the same extent as WT JAK3 (Figure 2A) but showed no phosphorylation of STAT5A when cotransfected with IL-2R, β, γc, and STAT5A (Figure 2A lane 10 and C bottom panel bar 5). As expected, the P132T SNP introduced into the JAK3 construct showed the same level of phospho-STAT5A as WT JAK3 (Figure 2B bottom panel bar 4). Interestingly, the Y100C SCID mutant showed less phospho-STAT5A than WT JAK3 but did not completely abolish phospho-STAT5A. Because we only observed phosphorylation of STAT5A in the presence of IL-2, the Y100C mutant may retain some binding to γc when overexpressed. Thus, reconstituted IL-2 signaling in 293T cells shows that the ATLL mutations induce increased phosphorylation of STAT5A compared with WT JAK3 protein.

Increased levels of phospho-STAT5A were observed in experiments where JAK1 was cotransfected but with the same overall pattern (data not shown).

**Mutant JAK3s confer cytokine independent growth in BaF3 cells**

We transduced BaF3 cells using recombinant MIG retroviruses expressing mutant or WT JAK3s and sorted for GFP+ cells. BaF3 cells require phosphorylation of STAT5 through IL-3 signaling but become IL-3 independent when gain of function tyrosine kinases are expressed that signal through the same pathway. As expected, untransduced BaF3 cells and those transduced with WT JAK3 died after 3 days in culture without IL-3 (Figure 3A). These cells transduced with WT JAK3 were maintained in culture with the addition of IL-3. Remarkably, BaF3 cells transduced with mutant JAK3s grew without IL-3 and were continually passaged as stable cell lines (Figure 3A).

We analyzed phosphorylation of STAT5A (Y694), STAT3 (Y705), ERK1/2 (T202 and Y204), and AKT (S473) after acute withdrawal of IL-3 to identify those events that correlated with the cytokine-independent growth observed in Figure 3A. We divided the IL-3 withdrawal cultures in 2 and added IL-3 to one group before preparing whole-cell lysates or immunoprecipitates. As shown in Figure 3B, phosphorylation of Y980, a residue in the activation loop of JAK3 that is autophosphorylated, was minimal in WT JAK3 (lanes 3-4) but constitutive in all the mutant JAK3s (lanes 6-13). We did not observe phospho-Y980 in BaF3 cells expressing the E/K compound mutant JAK3 (data not shown), confirming that Y980 is autophosphorylated; and, IL-3 did not consistently affect this phosphorylation. Phospho-STAT5A was detected in all the BaF3 cells expressing mutant JAK3s (Figure 3B lanes 6-13) but not in untransduced BaF3 cells or BaF3 cells transduced with WT JAK3. Phospho-STAT5A was increased after the addition of IL-3 which induces this substrate phosphorylation through JAK2. AKT phosphorylation at S473 was increased in all the mutant JAK3-expressing BaF3 cells (Figure 3B lanes 5-12) compared with untransduced and WT JAK3-expressing BaF3s (Figure 3B lanes 1-4). ERK1/2 and STAT3 phosphorylations were present in a different pattern than JAK3, STAT5, and AKT. Phospho-ERK1/2 was not detectable in the mutant JAK3-expressing cells without the presence of IL-3 and STAT3 was minimally phosphorylated without IL-3. This basal level of phospho-STAT3 was the same in untransduced BaF3 and in cells expressing WT or mutant JAK3 and could be stimulated with IL-3 addition. In summary, the phosphorylations of JAK3, STAT5, and AKT were correlated with cytokine-independent

![Figure 2. JAK3 FERM mutations cause increased phosphorylation of STAT5A in reconstituted IL-2 signaling in 293T cells.](image-url)
growth of mutant JAK3-expressing cell lines whereas ERK1/2 and STAT3 phosphorylations were not correlated.

BaF3 cells that expressed mutant JAK3s grew without IL-3 and were not impaired in their proliferation as they showed cell-cycle profiles comparable with BaF3 cells maintained in IL-3 (Figure 3C). Retroviral integration site analysis showed that the stable BaF3 lines expressing mutant JAK3s were polyclonal; and, no integrations were found near genes that could account for cytokine-independent growth (data not shown).

Mutant JAK3 proteins are more stable in BaF3 cells

The JAK proteins have been shown to undergo proteasome-mediated degradation after activation.\(^ {38,39}\) We sought to analyze the stability of WT and mutant JAK3 proteins in the BaF3 cells. We applied cycloheximide to BaF3 cells and performed Western blots for JAK3 protein. We quantified JAK3 protein using fluorescent secondary Abs and plotted the mean of 5 independent experiments (Figure 3D). Linear regression analysis of the curves showed that the mutant JAK3s clustered together with similar decay times (A572V, \(t_{1/2} = 4.3\) hours; L156P, \(t_{1/2} = 3.2\) hours; R172Q, \(t_{1/2} = 4.1\) hour; E183G, \(t_{1/2} = 4.5\) hours) whereas WT JAK3 decayed more rapidly (\(t_{1/2} = 1.96\) hours). In fact, the mutant JAK3 proteins had half-lives 2-fold greater than WT JAK3 protein.

JAK3 mutations confer increased in vitro tyrosine kinase activity

We immunoprecipitated WT or mutant JAK3s from BaF3 cells and incubated the immune complexes with a peptide substrate conjugated to fluorescent beads. Phosphorylation was detected by Förster (or fluorescence) resonance energy transfer (FRET) between the fluorophore-conjugated phospho-specific Ab to the acceptor dye on the substrate. The assay parameters were established and optimized using recombinant kinase domain of JAK3. Incubation of immune complexes with the peptide substrate without ATP resulted in background counts (Figure 4A light gray bars). With the addition of 20μM ATP, counts were detected in recombinant purified JAK3, a truncated protein only expressing the kinase domain, WT JAK3, and all the mutant JAK3s. No kinase activity was detected in immunoprecipitations with isotype control IgG from WT JAK3-transduced BaF3 cells, untransduced BaF3, or the compound mutant, E183G/K855A (E/K). Most remarkably, mutant JAK3 proteins showed increased enzyme activity compared...
with WT JAK3 (P < .001 by Student t test). WT JAK3 activity was less than that of the truncated JAK3 protein but was increased compared with IgG control (P = .008).

A representative immunoprecipitation of JAK3 is shown in Figure 4B after the kinase assay. This was also probed using 4G10, the phosphotyrosine-specific Ab, and an Ab against phospho-Y980 of JAK3. Autophosphorylation of JAK3 (ie, phospho-Y980) was detected for WT and mutant JAK3s (Figure 4B lanes 1-5) but the compound mutant, E/K, did not show autophosphorylation or kinase activity (Figure 4B lane 6). Interestingly, immune complexes that were resuspended in Laemmli buffer for SDS-PAGE immediately after the immunoprecipitation procedure showed phospho-Y980 only for mutant JAK3 proteins and not WT (see Figures 3B lanes 3-12 and 6A lanes 6-21). In contrast, immune complexes that were equilibrated in ATP and kinase buffer followed by SDS-PAGE showed phospho-Y980 in both WT and mutant JAK3 proteins. Thus, the Y980 autophosphorylation assay immediately after immunoprecipitation or in whole-cell lysates was more predictive of enzyme activity than the SDS-PAGE after the kinase assay. This also suggested that autophosphorylation was taking place during the kinase reaction for WT JAK3 proteins. Previous studies have shown that Y980 autophosphorylation can be detected in JAK3 proteins with gain-of-function or loss-of-function mutations and may not be entirely predictive of substrate phosphorylation. In fact, immunoprecipitated JAK3 proteins from transfected 293T cells did not show consistent phospho-Y980.

Molecular modeling suggests autoregulatory function for the JAK3 FERM domain

The cell-based assays and the in vitro kinase results support a gain of function activity for the mutant JAK3s. Interestingly, the JAK3 FERM domain’s potentiation of kinase activity was suspected by O’Shea and colleagues. In their studies, FERM domain mutations have been described in this region as well, D169E, which causes decreased yc association and loss of kinase activity. This SCID mutation is only 3 residues away from a mutation (R172Q) that shows gain of function in our assays. These data suggest that the secondary structure of the JAK3 FERM domain is very sensitive to amino acid substitutions perhaps because of intramolecular contact with the kinase domain, analogous to FAK.

Mutant JAK3s are sensitive to specific inhibitors

Next, we tested a JAK3-specific inhibitor, tofacitinib, on the BaF3 cells stably expressing mutant JAK3s. We cultured BaF3 cells expressing WT JAK3 or mutant JAK3s with 0.5, 2, or 4μM of the inhibitor or with solvent (DMSO) alone and probed the levels of JAK3 protein, phospho-Y980 of JAK3, and phospho-STAT5A at 4 and 24 hours by quantitative Western blot analysis (Figure 6A). The JAK3 and phospho-STAT5A protein levels were expressed as fold difference with BaF3 cells treated with solvent alone. The BaF3 lines stably expressing mutant JAK3s showed marked dose-dependent decrease in phospho-STAT5A (quantified in Figure 6A bottom panel). At 2μM, phospho-STAT5A levels were decreased by 80% for all BaF3 cells stably expressing mutant JAK3s. Untransduced BaF3 cells showed much lower phospho-STAT5A at steady state (Figure 6A lane 2) and this quantity only
decreased 30% at much higher concentrations (4µM) of tofacitinib; phospho-STAT5A in BaF3 cells transduced with WT JAK3 was similarly resistant to the effects of inhibitor. JAK3 and tubulin were quantified as well. BaF3 cells have STAT5 phosphorylation secondary to the IL-3/JAK2 signaling which may have variable sensitivity to tofacitinib in cell-based assays even though JAK2 kinase activity is inhibited at concentrations comparable with JAK3 inhibition in cell-free enzyme assays. Tofacitinib did not inhibit Y980 phosphorylation to the same extent as STAT5A phosphorylation. Phospho-Y980 in the A572V mutant JAK3 was most sensitive but only decreased to 75% of basal levels at 4µM.
The inhibitor-induced decrease in phospho-STAT5A correlated with decreased growth of these cells (Figure 6B). The IC50 curves demonstrate marked difference between BaF3 cells expressing mutant JAK3s versus those expressing WT JAK3 and untransduced BaF3. Untransduced BaF3 cells and those expressing WT JAK3 were not as sensitive to tofacitinib and curve-fitting did not generate models with high correlation (ie, R^2 > 0.90, see Table 1). Mutant JAK3-expressing BaF3s showed consistent sensitivity to tofacitinib with well correlated curves (Table 1). The decreased cell numbers were because of increased apoptosis and G1 cell-cycle arrest.
Discussion

Data accumulated over many years suggest a central role for JAK3/γc-associated cytokines in ATLL pathogenesis. In this study, we analyzed the JAK3 gene and found 3 missense mutations in the N-terminal FERM domain in 11% of ATLL patients. These mutations cause gain of function in JAK3 kinase activity in cell-based assays and in vitro kinase assays and may contribute to the development of ATLL. The mutant JAK3s caused increased phosphorylation of STAT5A in reconstituted IL-2 signaling in 293T cells and induced cytokine-independent growth in BaF3 cells. One important question is whether the mutant JAK3s require receptor and ligand for signal transduction. The phosphorylation of STAT5A in the 293T cell assay required IL-2 and hematopoietic receptor and ligand for signal transduction. The phosphorylation of the activation loop and the FERM domain at Y119; activation induced by the erythropoietin receptor induces autophosphorylation of the activation loop and the FERM domain at Y119; this phosphorylation causes dissociation of the FERM from the erythropoietin receptor thus exerting negative feedback on the signal. A Y119F mutation introduced into JAK2 creates an enzyme that is more active and more stable. Alanine substitution at the comparable residue in JAK3, Y105, did not abolish JAK3 enzyme that is more active and more stable. Alanine substitution at the comparable residue in JAK3, Y105, did not abolish JAK3 enzyme that is more active and more stable. Alanine substitution at the comparable residue in JAK3, Y105, did not abolish JAK3 enzyme that is more active and more stable. Alanine substitution at the comparable residue in JAK3, Y105, did not abolish JAK3 enzyme that is more active and more stable. 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ATLL but are not as prevalent as they are in T-lymphoblastic leukemias.52

Our results implicate the JAK3/yc pathway as an important therapeutic target in ATLL. Thus far, we have not been able to establish a human ATLL cell line with a gain-of-function JAK3 mutation to formally test oncogene dependence on the pathway. Nevertheless, tofacitinib, a specific JAK3 inhibitor, induced growth of 2 HTLV-1–expressing cell lines that had no mutations in JAK3 and primary ATLL cells whose JAK3 status was unknown.53 This result suggests that there could be oncogene dependence on JAK3 even without a gain of function mutation. For example, the HuT-102 line requires exogenous IL-2 for optimal growth. The MT-2 cells also have deregulation of the JAK3/yc axis in the form of HTLV-I integration 5′ of the IL9R gene. This retroviral insertion induces overexpression of the receptor such that high levels of IL-9R may signal through JAK3 without ligand.54,55 Other components of the JAK3/yc pathway may be mutated in ATLL or other mechanisms may deregulate the pathway because not all patients in our cohort had JAK3 mutations.51 Finally, JAK3-specific inhibitors have been clinically applied in phase 1 and phase 2 studies of autoimmune disease and may be worth testing in ATLL.53

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