**Original Article**

**HTLV-I Tax regulates the cellular proliferation through the down-regulation of PIP3-phosphatase expressions via the NF-κB pathway**

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Received March 8, 2012; accepted March 15, 2012; Epub March 20, 2012; Published March 30, 2012

**Abstract:** An oncogenic retrovirus, human T-cell leukemia virus type I (HTLV-I), encodes an oncoprotein, Tax, which plays critical roles in leukemogenesis of adult T-cell leukemia/lymphoma (ATLL) through the pleiotropic actions such as transcriptional regulation, cell cycle control, and transformation. We have previously reported that PTEN and SHIP-1, PIP3 inositol phosphatases that negatively regulate the PI3-kinase signaling cascade, are disrupted in ATLL neoplasias. Overactivation of PI3-kinase signaling has an essential role in onset of ATLL. We report here that both PTEN and SHIP-1 are downregulated by Tax through the NF-κB signaling pathway. Tax expression upregulated phosphorylated Akt, a downstream serine/threonine kinase in the PI3-kinase signaling cascade. Activation of NF-κB pathway also suppressed these phosphatases. An IκBΔN mutant which inhibits the activation of NF-κB prevented PIP3 phosphatase downregulation by Tax. The underlying mechanism of NF-κB mediated suppression of PIP3 phosphatases involved sequestration of the coactivator p300 by p65. These down-regulations of PIP3 phosphatases were found to be essential for the Tax-induced cell proliferation. Thus, our results suggest that HTLV-I Tax downregulates PIP3 phosphatases through the NF-κB pathway, resulting in increased activation of the PI3-kinase signaling cascade in human T-cells and contributing to leukemogenesis.

**Keywords:** HTLV-I, Tax, PTEN, SHIP-1, NF-κB

**Introduction**

Human T-cell leukemia virus type I (HTLV-I) preferentially infects and subsequently transforms T-cells into acute malignant cells, resulting in adult T-cell leukemia/lymphoma (ATLL) [1]. ATLL generally presents following prolonged incubation periods ranging from 40 to 60 years. The vast majority of infected individuals remain clinically asymptomatic, with only 2-5% developing neoplasia. An accumulation of leukemogenic events within HTLV-I-infected T-cells are required for the development of ATLL [2]. The HTLV-I genome has a unique region at its 3’end that was originally designated as the pX region, which includes Tax, Rex, and certain accessory proteins [2]. Tax plays central roles in tumorigenesis and contributes to its own pathogenesis through its capacity to immortalize primary T-cells, to transform rodent fibroblasts, and to induce leukemia in transgenic mice expressing Tax [3-5]. The majority of the oncogenic properties of Tax are related to its ability to activate the expression of cellular genes that control T-cell proliferation and differentiation through induction of the constitutive activation of NF-κB, a transcriptional regulator of numerous cellular genes [6]. Tax also indirectly activates transcription by recruiting or modifying the activity of cellular transcription factors, including cyclic AMP responsive element binding protein (CREB), serum-responsive factor (SRF) and NF-κB. Tax is also reported to directly activate oncogenes and inactivate tumor suppressor genes.
In T-cells, NF-κB indirectly promotes T-cell activation and proliferation through the induction of immune regulatory cytokines. In addition, NF-κB transcriptional regulation directly modulates T-cell function by regulating cell survival through the induction of Bcl-xL and cIAP [7]. It is thought that over activation of NF-κB leads to abnormal regulation of gene expression and NF-κB is involved in oncogenesis. NF-κB is known to bind to the multifunctional protein CBP/p300, which is a scaffold protein in multicomponent transcriptional regulatory complexes [8]. Several lines of evidence indicate that CBP/p300 proteins are rate-limiting for transcription. Competition for a limited amount of CBP/p300 upon NF-κB activation has been previously proposed as a mechanism for either transcriptional regulation or induction of apoptosis [8]. The sequestration of CBP/p300 by NF-κB also down-regulates the transcriptional activity of several transcription factors, including p53 and c-myb [9, 10]. Abnormal regulation of CBP/p300 by NF-κB is thought to have an important role in the oncogenesis.

The PI3-kinase signaling cascade is essential for a variety of biological processes, including proliferation, T-cell activation, regulation of apoptosis, and cell movement [11]. PI3-kinase activity produces phosphatidylinositol-3, 4, 5-trisphosphate (PIP3) by the phosphorylation of phosphatidylinositol-4, 5-bisphosphate (PIP2), which subsequently regulates downstream effector molecules. In contrast, PIP3 phosphatases, such as the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) tumor suppressor and Src homology 2 domain containing inositol polyphosphate phosphatase-1 (SHIP-1), modulate various biological phenornenos dephosphorylate PIP3 and act as negative regulators of the PI3-kinase/Akt signaling cascade [11]. The alteration of PI3-kinase/Akt signaling disrupts a signaling equilibrium and can thus lead to cellular transformation [12]. Recently, we demonstrated that alteration of the signaling cascade through PIP3 phosphatase disruption was essential for ATLL-type multilobulated nuclear formation, an important diagnostic marker and indicator for development of ATLL via abnormal T-cell proliferation [13]. Recent studies demonstrated that Tax induces abnormal cell growth through the production of PIP3 and/or the activation of a down-stream effector of PI3-kinase, the kinase Akt [14]. However, how Tax modulates PIP3 production and the mechanism of Akt signaling activation has not been established. Current evidence suggests the possibility that abnormal regulation of the PI3-kinase signaling cascade in ATLL-T cells and Tax expressing cells may be common underlying mechanisms altered PIP3 phosphatase gene expression.

In this study, we show that Tax down-regulates the expression of PIP3 phosphatases through NF-κB activation, leading to the overactivation of PI3-kinase signaling cascade in T-cells. We also report that activated NF-κB directly binds to p300 and influences the expression of not only PTEN but also SHIP-1 as a result of p300 sequestration by activated NF-κB. These results suggest that the mechanism of Tax-regulated alteration of the PI3-kinase signaling cascade involves suppression of PIP3 phosphatase gene expression through NF-κB-mediated inhibition of p300 recruitment. We also demonstrate that Tax-mediated alteration of the PI3-kinase signaling cascade is essential for abnormal cell proliferation by HTLV-I Tax.

Materials and methods

Ethics statement

All the experimental protocol was approved by the Ethics board of Tokyo University of Science ( Permit Number: 0003). Written informed consent was provided by each individual participant before sample collection and study procedures began.

Cell culture and treatment

Highly-purified T-cells (>97%) were isolated from healthy adult volunteers following informed consent. This study was approved by the Ethics Committee of Tokyo University of Science.

We utilized JPX-9 cells, subclones of human acute lymphocytic leukemia T-cell line Jukat cell which have been used elsewhere for human leukemia investigation in this study (generously gifted by Dr. K. Sugamura at Tohoku University) [15]. JPX-9 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. CV-1 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml
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Retroviral gene transfer, plasmid construction and transient transfection

pMX retroviral vectors and Plat-A packaging cells were kindly supplied by Dr T. Kitamura (University of Tokyo) [16]. Plat-A cells were maintained in high glucose DMEM medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were passaged at equivalent cell numbers into a 100mm culture dish 48 h before transfection. After reaching approximately 50% confluence, the cells were transfected with plasmids that had been preincubated for 30 min with 100 µl of serum-free medium containing FuGENE 6 (Roche, Penzberg, Germany).

Immunoblotting analyse

Immunoblotting was performed according to methods described previously [13]. Cells were washed in phosphate-buffered saline and lysed in lysis buffer (Tris–HCl 50mM (pH 7.5), 1mM EGTA, 5mM EDTA, 1% Triton X-100, 50mM NaF, 0.2mM Na3VO4) in the presence of protease inhibitors (0.1mM PMSF, 1ug/ml leupeptin, 1ug/ml pepstatin, 1ug/ml aprotinin). Cell extracts were incubated on ice for 15 min, centrifuged (10000xg) at 4°C for 10 min, and then supernatants were collected. Tris-Glycine-gels, 4-20% (Daiichi Chemicals, Tokyo, Japan), were used as recommended by the manufacturer. Proteins were then transferred to PVDF membranes (Daiichi Chemicals, Tokyo, Japan), membrane non-specific binding sites were blocked for 30 min in non fat dry-milk or BSA at room temperature (RT), and membrane-bound proteins analyzed using specific antibodies. Antibodies included anti-GFP polyclonal antibody (pAb), are purified from rabbit serum using ion exchange chromatography (MBA, Nagoya, Japan), anti-PTEN pAb, which are produced by immunizing rabbit with a synthetic peptide (KLH-coupled) derived from the carboxy-terminal sequence of human p65 (Upstate Biotechnology, Lake Placid, NY, USA), anti-p300 monoclonal antibody (mAb) (NM11, BD Bioscience, San Jose, CA, USA), anti-β-actin mAb (SIGMA-Aldrich) according to the manufacturer’s instructions. After washing membranes, bound primary Abs were visualized with a horseradish peroxidase (HRP)-conjugated F(ab’)2 fragment against rabbit IgG (ICN/Cappel, Aurora, OH, USA) using enhanced chemiluminescence (ECL) (Amersham Biosciences, England).

Immunoprecipitation

For the immunoprecipitation of p65 and p300, the cell lysates were incubated with anti-p65 pAb (Upstate Biotechnology) and anti-p300 (BD Bioscience) and incubated with protein-G Sepharose beads (Amersham Biosciences, England). The beads were washed with lysis buffer and the bound proteins were solubilized by boiling for 5 min in SDS sample buffer. Immunoblotting was performed according to methods described. The membranes were then incubated with anti-GFP pAb (MBL), anti-p65 pAb (Upstate Biotechnology) and anti-p300 mAb (BD Bioscience). After washing, the bound primary Abs were detected as described above.

RNA isolation and real-time reverse transcription PCR

The expression of PTEN, SHIP-1, and β-actin were assessed by real-time reverse transcription PCR. Total RNA was prepared from cells under various conditions using TRIZOL Reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNAs were synthesized using random primers and a First-Strand cDNA Synthesis kit (Toyobo, Tokyo, Japan). PCR mixtures were prepared using SYBR Premix Ex Taq (Takara, Tokyo, Japan) containing 0.2 mM of each primer and amplification reactions were performed. The sequences of PTEN, SHIP-1 and Bcl-xl primer pairs were: PTEN (forward, 5’-cca atg ttc agt ggc gga act-3’; reverse, 5’-gaa ctt gtc ttc ccg tcg tgt g-3’); SHIP-1 (forward, 5’-cga caaga gcc tga gtc ctt tt-3’; reverse, 5’-ggt taa gag ccc caa acc aga a-3’); and β-actin (forward, 5’-gcc acg gtc gct ctc a-3’; reverse, 5’-gaa ccc ctg att gcc aat g-3’). Gene expression levels of PTEN and SHIP-1
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were measured using the ABI Prism 7000 Sequence Detection System (Applied Biosystems). PCR product levels were estimated by the measurement of the intensity of fluorescence of SYBR Green. Gene expression levels were normalized to β-actin mRNA expression.

**Short-hairpin RNA constructs**

p300-specific knockdowns in JPX-9 cells were performed by the expression of hairpin siRNA using a pSuper.neo+gfp in pSuper RNAi system (OligoEngine, Seattle, WA, USA). To generate the p300- and control luciferase-RNai plasmid, a unique nucleotide sequence p300 (5'-aac ccc tcc tct tca gca cc-3') and luciferase (5'-cgt acg cgg gaa tac ttc ga-3'), derived from the mRNA transcript of p300 and luciferase, were synthesized and cloned into pSuper.neo+gfp vector according to the manufacturer’s instructions.

**Cell proliferation assay**

Cell proliferation was determined by using either direct cell counting or the Cell Counting Kit-8 (Dojindo Labs, Tokyo, Japan) according to the manufacturer’s protocol. The cell viability in each well was determined by reading the optical density at 450 nm.

**Results**

*HTLV-I Tax induces the activation of PI3-kinase/Akt pathway via the down-regulation of PTEN and SHIP-1*

It has been reported that alteration of PI3-kinase signaling cascade induced abnormal cell proliferation in both ATLL cells and Tax-expressing cells [4, 13, 14]. In order to study the mechanism of this regulation, we analyzed the role of Tax in regulating the expression of PTEN and SHIP-1 in peripheral blood T-cells (PBT) isolated from healthy donors which has been activated by CD3 and CD28 stimulation. We found that transduction of Tax significantly reduced the expression of not only PTEN but also SHIP-1 proteins by Western blot analysis (Figure 1A). The expression of Tax also significantly decreased the expression of those PIP3-phosphatase mRNAs in activated PBT (Figure 1B). JPX-9 cells, which were transfected with a Tax gene regulated by a metallothionein promoter and inducible by CdCl2, were commonly used for Tax-function analysis [15]. When JPX-9 cells were treated with CdCl2, the expressions of the PIP3-phosphatase mRNAs were significantly decreased as described previously (data not shown). We also transfected with Tax cDNA to JPX-9 cells and analyzed the effect of the expressions of those mRNAs whether the reduction of those mRNA expressions induced by CaCl2 could be due to the direct effect by the Tax expression. Not only PTEN mRNA but also SHIP-1 mRNA were also significantly decreased the transduction of Tax in JPX-9 cells (Figure...
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1B). Next, we determined the effects of Tax expression on Akt, a downstream effector of PI3-kinase, in activated PBT. We found that Tax induced the phosphorylation of Akt. Interestingly, phosphorylated Akt (p-Akt) levels were inversely correlated with PTEN and SHIP-1 expression (Figure 1C). These results indicate that Tax expression induced the activation of the PI3-kinase/Akt signaling pathway through the down-regulation of PI3 phosphatases PTEN and SHIP-1.

**Tax-mediated downregulation of PI3-phosphatase mRNA expression is involved in NF-κB activation**

It is well known that Tax modulates gene expression and associated cellular events through the constitutive activation of NF-κB [6]. Tax expression induced the nuclear localization of p65, the transactivation subunit of NF-κB complexes, and upregulated the expression of a downstream target gene, Bcl-xL [2]. We investigated whether Tax could downregulate the mRNA expressions of PI3 phosphatases, PTEN and SHIP-1, through NF-κB pathway. Tax M22 mutant (T130L131-A130S131), which is thought to lack the ability to activate the NF-κB pathway but retains the ability to transactivate the CREB-dependent HTLV-I long terminal repeat (HTLV-I LTR) [2, 17], could have a diminished ability to downregulate PTEN and SHIP-1 mRNA expression as compared to wild-type Tax in PBT (Figure 2A left panel). Although Tax M22 could also significantly downregulate the mRNA expressions of the PI3 phosphatases, Tax M22 had a partially diminished ability to downregulate those mRNA expressions (Figure 2A right panel).

Based on the importance of the Tax NF-κB activation motif, we next examined whether the activation of NF-κB affected the PTEN and SHIP-1 mRNA expression in PBT and JPX-9 cells. The expression of the p65 subunit of NF-κB, which mimics activated NF-κB, significantly increased Bcl-xL expression and NF-κB-Luc activity as described previously [18, 19]. We also found that p65 drastically decreased both PTEN and SHIP-1 mRNA expression in both activated PBT and JPX-9 cells (Figure 2B). We also examined the effects of a dominant-negative form of IkB (IkBΔN) [20], which is resistant to phosphorylation and degradation and inhibits the activation of NF-κB, on the down-regulation of PTEN and SHIP-1 by Tax. IkBΔN blocked Tax-induced downregulation of phosphatase mRNA expression (Figure 2C). These results suggested that the NF-κB pathway would play central roles for the downregulation of PI3 phosphatase mRNA expression by Tax, although it is possible that other active domain and/or functions of Tax may also partially relate to PTEN/SHIP-1 mRNA down regulation.

**NF-κB reduces PI3 phosphatase mRNA expression by sequestering p300**

We further investigated the possibility that the underlying mechanism for Tax-mediated downregulation of PTEN and SHIP-1 mRNA expression involved the direct interaction between p65 and p300. Using highly p300-expressing cells isolated from p300 wild-type-transfected cells (p300wt) by cell sorting, we found that p300 expression resulted in increased endogenous PTEN and SHIP-1 mRNA expression (Figure 3A). In contrast, p300-specific knockdown using shRNA led to decreased expression of these PI3 phosphatase mRNAs (Figure 3B). It was possible that p300 directly increased the expression of the phosphatase mRNAs and/or that these mRNAs were induced by interaction between transduced p300 and endogenous activated NF-κB. The NF-κB p65 subunit is known to interact with the C/H1 and KIX domains in the N-terminal region of p300 and to require the sufficient transcriptional activity of p300 [10]. We confirmed that an N-terminal deleted form of p300 (p300ΔN), which is unable to bind with p65, could not induce the transcriptions of p300-dependent genes such as cIAP2 and XIAP [21] (data not shown). Transfection of p300ΔN significantly reduced phosphatase mRNA induction as compared to p300wt (Figure 3A). We also examined the effect of these p300wt, p300ΔN and N-terminal region of p300 (p300N), which could bind with p65, on p65-induced downregulation of PTEN and SHIP-1 mRNA expression. The expressions of p300wt and p300N inhibited p65-induced mRNA downregulation (Figure 3C). In contrast, p300ΔN had a significantly reduced block mRNA downregulation as compared to p300wt and p300N (Figure 3C).

We next examined the direct interaction between NF-κB p65 subunit and p300 using immunoprecipitation experiments in JPX-9 cells. When JPX-9 cells were transfected with p65 and p300ΔN, we found that p300ΔN could not interact with NF-κB p65 (Figure 3D, left panel). In contrast, p300N could bind with p65 and interfere to the binding between p65 and endoge-
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Nous p300 both experiments by the immunoprecipitation using antibodies against p65 and endogenous p300 (Figure 3D, center or right panel). These results indicate that NF-kB p65 directly associated with p300 and reduced PTEN and SHIP-1 mRNA expression through the sequestration of p300.

Downregulations of PIP3 phosphatases expressions play an essential role in Tax-mediated cellular proliferation

The promotion of cellular proliferation by Tax is involved in oncogenic property of Tax [3]. Finally, we investigated the effects of the expressions of PIP3 phosphatases on Tax-mediated cellular proliferation. Previous report had showed that the cellular proliferation of CV-1 cells was promoted by the transduction of Tax gene [14]. The transduction of Tax downregulated both expressions of mRNAs and proteins of PTEN and SHIP-1 in CV-1 cells (Figure 4A and 4B). The transduction of PTEN and/or SHIP-1 genes rescued both expressions of mRNAs and proteins of those PIP3 phosphatases (Figure 4A and 4B). The proliferation of CV-1 cells found to be significantly increased by the expression of Tax as described previously [14] (Figure 4C). This Tax-mediated CV-1 cell proliferation was significantly reduced by the transductions of PTEN and SHIP-1 genes (Figure 4C). These results suggest that the downregulations of PIP3 phosphatase expressions are essential for the Tax-mediated cellular proliferation.

Discussion

This study demonstrates that the HTLV-I oncoprotein Tax activated the PI3-kinase signaling cascade by downregulating the PIP3 inositol
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phosphatases PTEN and SHIP-1. It also shows that the PIP3 phosphatases PTEN and SHIP-1 have a common transcriptional regulatory mechanism in T-cells mediated by activated NF-κB. The downregulation of PIP3 phosphatase mRNA expression is mediated by the NF-κB pathway as a result of the sequestration of p300. Furthermore, the downregulations of PIP3 phosphatases are involved in Tax-mediated cellular proliferation. These results suggest that Tax is essential for abnormal T-cell proliferation, resistance to apoptosis, and transformation caused by increased activation of PI3-kinase signaling in HTLV-I-infected cells.

The PI3-kinase signaling cascade is well known to be involved in carcinogenesis, through specific alterations in this pathway [22]. HTLV-I Tax is reported to transactivate cellular gene promoters in T-cells, including those for cytokines, cytokine receptors, and costimulatory molecules, leading to upregulation of many signaling cascades [2]. Tax is also reported to cause cellular transformation by triggering the overactivation of the PI3-kinase signaling cascade [4]. We recently reported that increased activity of the PI3-kinase signaling cascade caused by the suppression of PTEN and/or SHIP-1 was involved in tumorigenesis in ATLL patients [13]. Another paper reported that Tax activation of the PI3-kinase/Akt signaling pathway was essential for the proliferation of HTLV-I-transformed cells [14]. Collectively, these reports suggest that Tax plays an essential role in ATLL pathogenesis via

Figure 3. NF-κB reduces PIP3 phosphatase mRNA expression by the sequestering p300. (A) Expressions of PTEN and SHIP-1 mRNAs in GFP-p300 or GFP-p300ΔN-transfected JPX-9 cells were analyzed using real-time PCR. (B) Expressions of PTEN and SHIP-1 mRNAs in control or p300 short hairpin RNA-transfected JPX-9 cells were analyzed. (C) JPX-9 cells transfected with the combination of GFP-p65, GFP-p300, GFP-p300ΔN or GFP-p300N cDNAs were also analyzed the expressions of PTEN and SHIP-1 mRNAs. Values are presented as the mean ± SEM. *P < 0.05 compared with control; **P < 0.01 compared with control. (D) Interaction of p65 and p300 was analyzed by co-immunoprecipitation. JPX-9 cells were transfected with GFP-p65 and either GFP-p300ΔN or GFP-p300N and immunoprecipitation (IP) was performed using an anti-p65 or an anti-p300 antibodies.
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The transcription factor NF-κB is known to regulate many biological processes, including immunological reactions, cell proliferation, inflammation, and apoptosis [7]. Activation of the NF-κB pathway directly leads to increased transcription of genes from several functional classes, including inflammation, cell-survival, and innate immunity [7]. NF-κB is also known to regulate p53 and c-Myb expression by sequestration of the coactivator CBP/p300 [9, 10]. HTLV-I Tax functions as an intracellular stimulator of IκB kinase, a cellular kinase mediating NF-κB activation by diverse stimuli. Tax also induces the transactivation of NF-κB target genes involved in cell proliferation, survival and migration. Furthermore, it is also known that Tax directly binds to p300 [23]. Tax interaction increases the transcriptional activities of several transcription factors, including CREB, NF-κB and SRF, by increasing the binding affinity between those factors and p300 [2]. In the current study, the TaxM22 mutant, which lacks the ability to activate the NF-κB pathway but retains the ability to transactivate CREB-dependent HTLV-I LTR [2, 17], could be significantly reduced the diminished ability of Tax to downregulate of PIP3 phosphatase mRNA expressions. Therefore, the activation of the NF-κB cascade, but not the direct binding of Tax with transcription factors, would be central roles for the downregulation of PIP3

**Figure 4.** The down-regulations of PIP3 phosphatase expressions are essential for Tax-induced cellular proliferation. (A) Protein expressions of PTEN and SHIP-1 in CV-1 cells, which were transfected with GFP-Tax, PTEN and/or SHIP-1 cDNAs, were analyzed by Western blot analysis. (B) Expressions of PTEN and SHIP-1 mRNAs in CV-1 cells, which were transfected with GFP-Tax, PTEN and/or SHIP-1 cDNAs, were analyzed using real-time PCR. (C) Cell proliferation was measured with WST-8 reagent by using a Cell Counting Kit-8 (Dojindo Labs) The cells were transfected with those cDNAs and cultured for 2 days. WST-8 reagent was added for the last 2 h of the culture. The values are presented as the mean ± SEM. *P < 0.05 compared with control or Tax-expressing samples.
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phosphatase transcription. However, it is still possible that other pathways and/or functions of Tax may also partially relate to PTEN/SHIP-1 mRNA down regulation, because Tax plays multiple functions and has various activities through the wide varieties of signaling pathways [2-6]. Although it has been reported that the NF-κB activation suppressed PTEN expression through the p300 pathway [24], the molecular mechanism of transcriptional regulation of SHIP-1, which is modulated by TGF-β [25], is not clarified in detail. Our results present novel evidence that, in addition to PTEN, SHIP-1 expression is regulated by the NF-κB/p300 pathway through sequestration of p300. Furthermore, Tax regulation of PIP3 phosphatase transcription mediated by the same mechanisms via NF-κB/p300 pathway. In our study, the expression of wild-type p300 increased the transcription of PIP3 phosphatases and shRNA-mediated knockdown of endogenous p300 decreased these transcriptions. We considered two possibilities for this effect of p300 on PIP3 phosphatases. First, NF-κB is known to play a role in cell proliferation and cell activation in T-cells, and would likely regulate the activity of p300 at a constant rate. Therefore, ectopic expression of p300 wild type would interfere with NF-κB sequestration of endogenous p300 and increase PIP3 phosphatase gene expression, which is regulated by the balance of NF-κB and p300 activity. We also showed that p300ΔN, a mutant of p300 lacking the C/H1 and KIX domains which is unable to interact with p65, increased PIP3 phosphatase expression. Therefore, the second possible mechanism was that p300 increased PIP3 phosphatase expression through its direct coactivator activity. These observations suggest that NF-κB and p300 play a significant role in Tax-mediated alteration of PI3-kinase signaling through transcriptional regulation of both PIP3 phosphatases, PTEN and SHIP-1.

In this paper, we provide evidence that increased activation of the PI3-kinase signaling cascade, which is induced by Tax, is caused by the down-regulation of PTEN and SHIP-1 PIP3 phosphatases by the NF-κB/p300 pathway through sequestration of p300 and Tax regulation of PIP3 phosphatase transcription. This alteration of the PI3-kinase signaling cascade leads to abnormal cell growth and tumorigenesis in HTLV-I transformed cells. Several reports have stated that Tax expression was not detected in a portion of malignant T-cells from ATL patients [2]. Therefore, the involvement of Tax in ATLL pathogenesis is still unclear. Further studies of the relationship between the regulation of Tax expression and PI3-kinase signaling cascades during the extended period between initial infection and development of ATLL will provide valuable information regarding ATLL pathogenesis in HTLV-I transformed cells.

Acknowledgements

The authors thank K. Sugamura (Tohoku University) for providing JPX-9 cells, T. Kitamura (University of Tokyo) for providing pMX retroviral vectors and PLAT-A cells. This work was partially supported by an “Academic Frontier” Project for Private Universities to T.T. (2003-2010), by a Grant-in Aid for JSPS Fellows (19-4715) to R.F. and by a Grant-in Aid for Scientific Research in Priority Areas (18012044) to T.T. from MEXT Japan.

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References

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