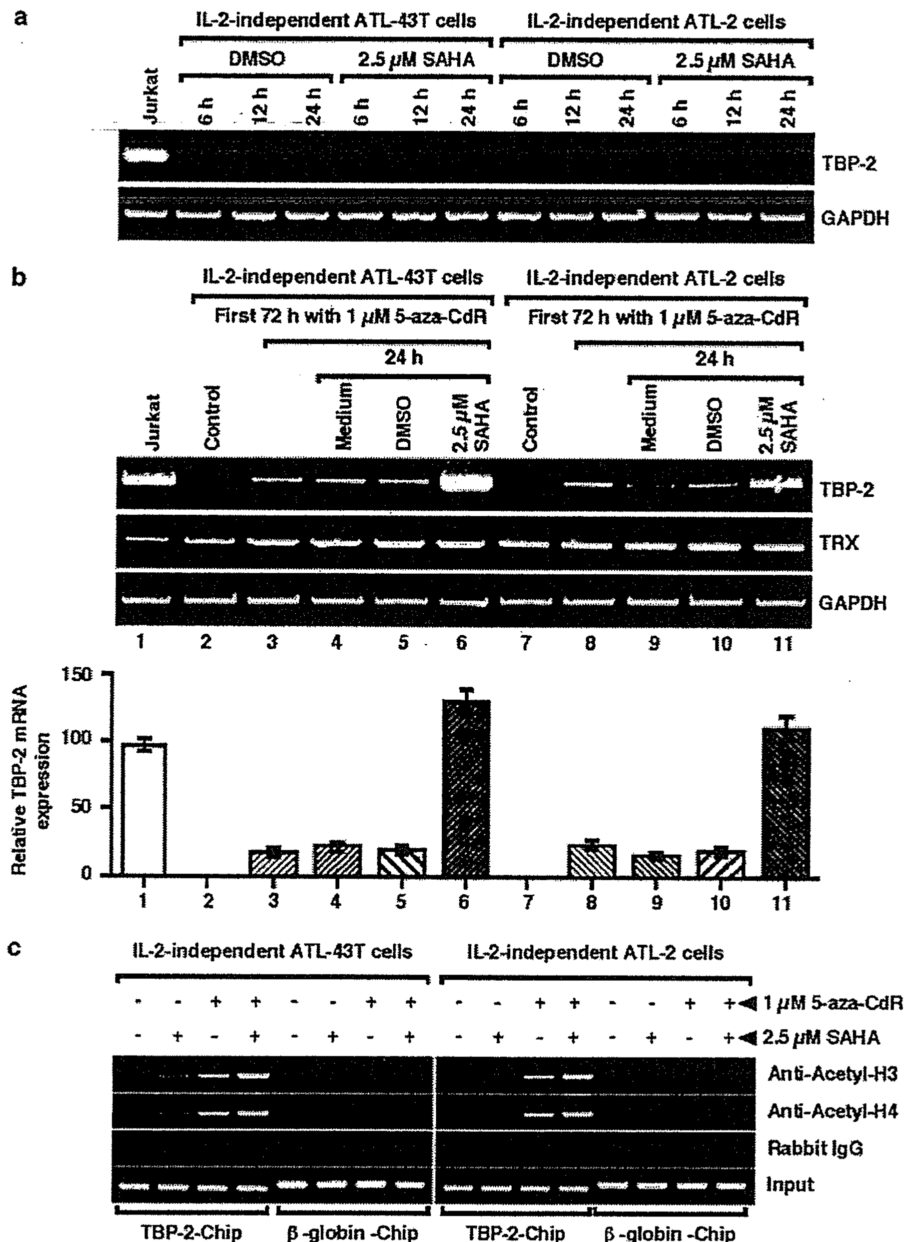


Figure 3 Restoration of thioredoxin-binding protein-2 (*TBP-2*) expression in interleukin-2 (IL-2)-independent HTLV-I-infected T cells by sequential treatment with 5-aza-2′-deoxycytidine (5-aza-CdR) and suberoylanilide hydroxamic acid (SAHA). (**a**) TBP-2 expression in SAHA-treated IL-2-independent ATL-43 T and ATL-2 cells. The cells were treated with 2.5 μM SAHA at the indicated time points and analysed by semiquantitative RT–PCR. (**b**) TBP-2 and TRX expression in the cells sequentially treated with 5-aza-CdR and SAHA. The cells were treated with 1 μM 5-aza-CdR for the first 72 h, then with complete medium, DMSO, or 2.5 μM SAHA for 24 h. The bar graph shows mean values of real-time RT–PCR from three replicates. Similar results were obtained in three separate experiments. Error bars represent s.d. 18S ribosomal mRNA expression was used as a cDNA loading control. (**c**) The histone acetylation status of the *TBP-2* promoter region (TBP-2-Chip) and *β-globin* (β-globin-Chip) determined by chromatin immunoprecipitation (Chip) assay. Assays were performed three times with the indicated antibodies, followed by PCR analysis.

proliferated well regardless of IL-2. In these cells, TBP-2 expression was completely abrogated and the cell growth did not change in response to IL-2. In Figure 4e (ATL-43T) and Figure 5e (ATL-2), statistical analysis of the day-3 data showed a significant ($P<0.05$) difference in growth between the presence and absence of IL-2 in IL-2-dependent cells and sequentially treated IL-2-independent cells, but not in untreated IL-2-independent cells. These results suggested that restored TBP-2 expression is associated with the response to IL-2 and suppression of uncontrolled IL-2-independent growth.

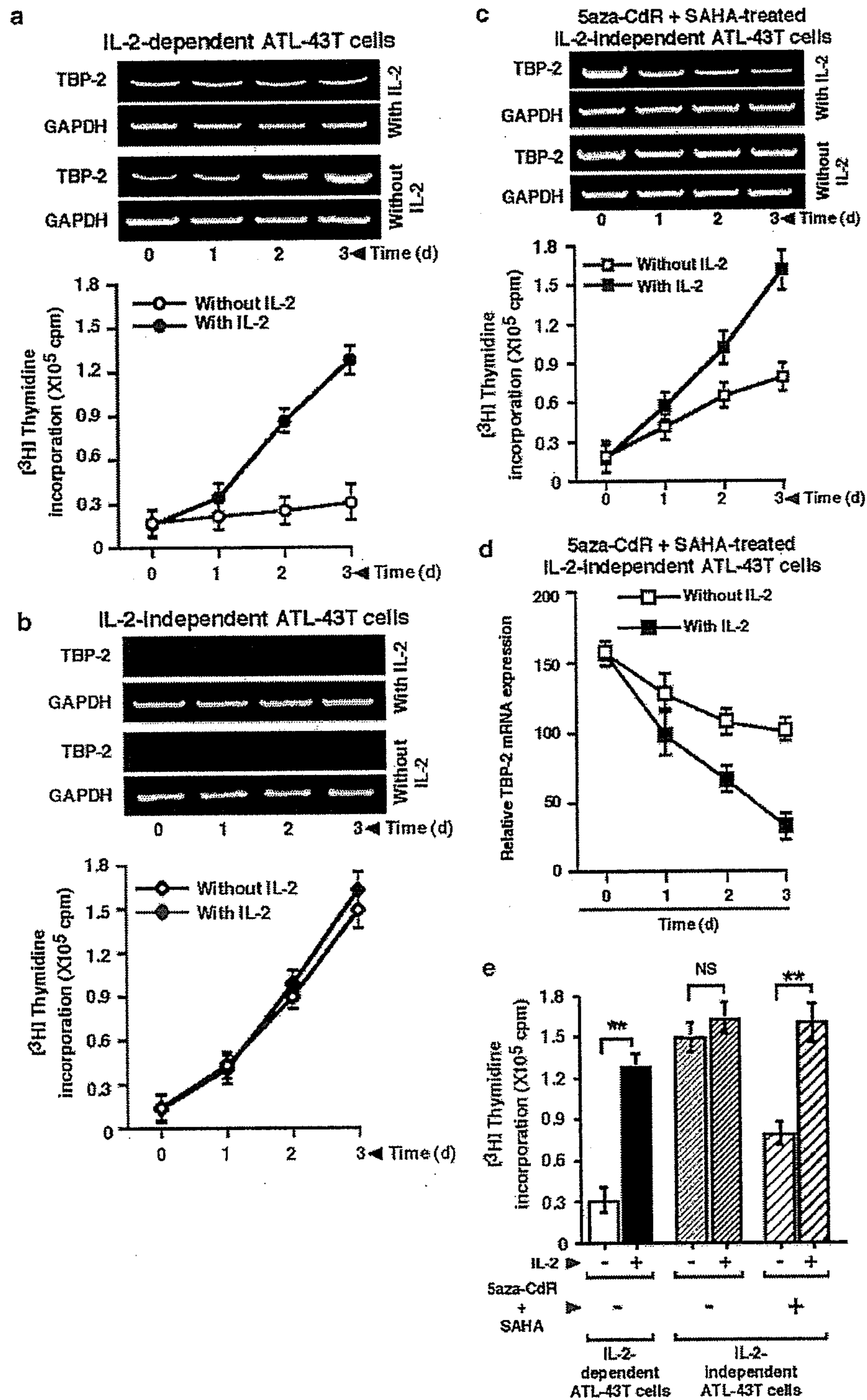
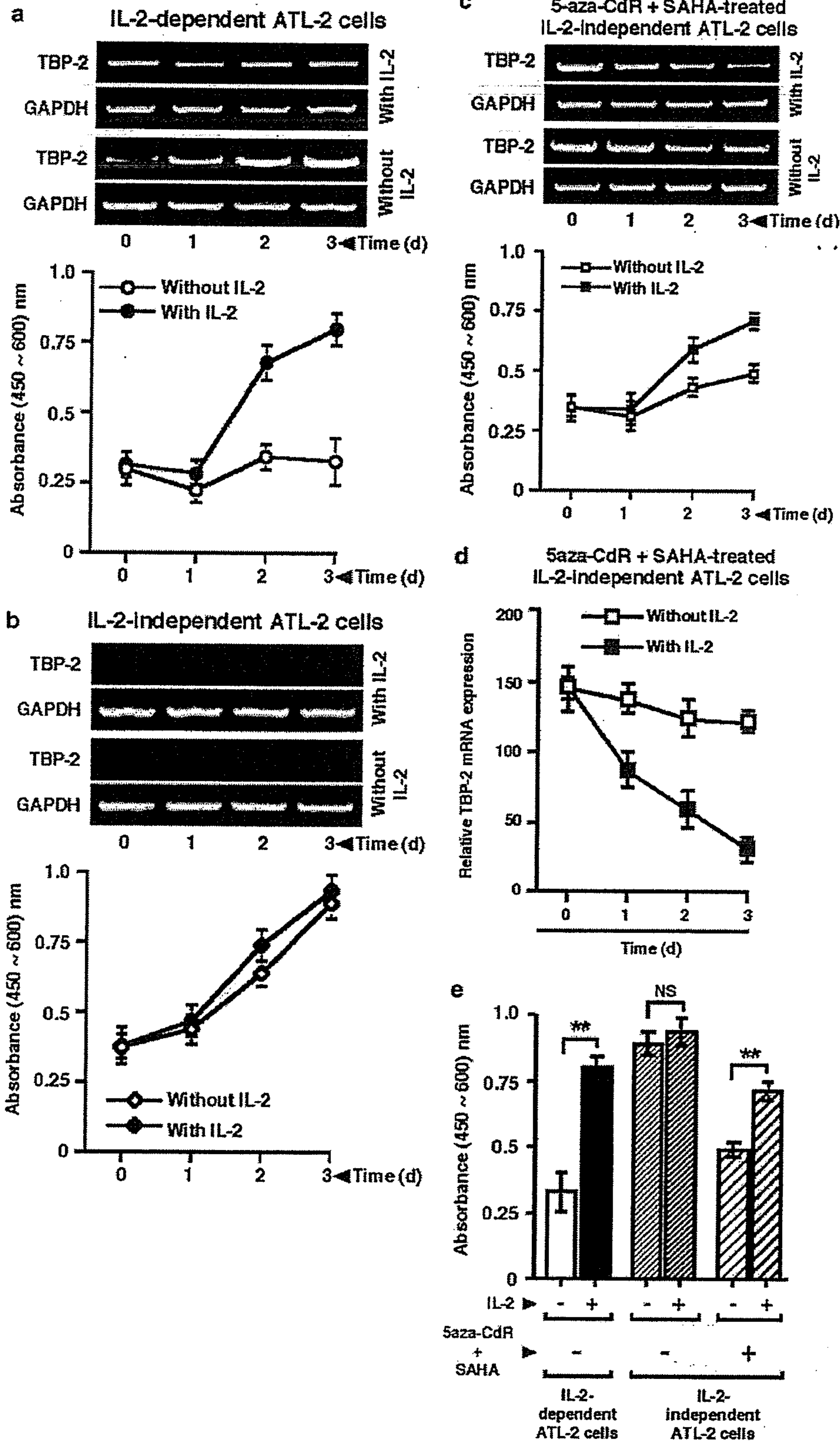


Figure 4 Thioredoxin-binding protein-2 (TBP-2) expression and cell growth in response to interleukin-2 (IL-2) in ATL-43T cells. The cells were cultured for 3 days in the absence or presence of IL-2 and cell growth was analysed using the thymidine incorporation assay. The expression of TBP-2 was analysed by semiquantitative RT–PCR. (a) IL-2-dependent ATL-43T cells, (b) IL-2-independent ATL-43T cells, and (c) IL-2-independent ATL-43T cells after sequential treatment with 5-aza-CdR and SAHA. (d) Quantitative real-time RT–PCR analysis of TBP-2 mRNA expression in the samples of Figure 4c. Data are representative of three separate experiments and the mean±s.d. from three replicates. (e) Statistical analysis of the day-3 data. Data are representative of three separate experiments and the mean±s.d. from three replicates. Nonsignificant and significant ($P<0.05$) differences are indicated by NS and (**), respectively, as determined with Student's *t* test.


a
IL-2-dependent ATL-2 cells
TBP-2
GAPDH
TBP-2
GAPDH
With IL-2
Without IL-2
0 1 2 3◀Time (d)
Without IL-2
With IL-2
Absorbance (450 ~ 600) nm
b
IL-2-independent ATL-2 cells
c
5-aza-CdR + SAHA-treated
IL-2-independent ATL-2 cells
d
5aza-CdR + SAHA-treated
IL-2-independent ATL-2 cells
Relative TBP-2 mRNA expression
Time (d)
e
NS
IL-2
5aza-CdR + SAHA
IL-2-dependent ATL-2 cells
IL-2-independent ATL-2 cells

Ectopic expression of TBP-2 suppressed cell growth and partially restored IL-2 responsiveness
To further analyse the role of TBP-2 in the response to IL-2, we utilized HTLV-I-transformed MT-2 cells ectopically expressing TBP-2 (Nishinaka *et al.*, 2004a). TBP-2 protein expression in these transfectants was confirmed (Figure 6a). While the control clones C_1 and C_4 proliferated well regardless of IL-2, the TBP-2-overexpressing clones T_1 and T_2 proliferated less without IL-2 than with IL-2 ($P<0.05$; Figure 6b). Addition of IL-2 suppressed the expression of TBP-2 in TBP-2-overexpressing clones T_1 and T_2 (Figure 6b; Agarose gel snaps). These results suggested that the TBP-2 expression suppressed cell growth and partially restored responsiveness to IL-2. We further analysed the relationship between TBP-2 expression and IL-2-dependency in IL-2-dependent ATL-43T and ATL-2 cells by knocking down TBP-2 expression using RNA-interference (RNAi). The expression of TBP-2 was downregulated in these cells after the transfection with RNAi of TBP-2 but not with control RNAi (Figure 6c). The cells transfected with nonsilencing control RNAi proliferated in the presence of IL-2 but not in the absence of IL-2, while those cells transfected with TBP-2 RNAi showed partial proliferation in the absence of IL-2 (Figure 6d). These results suggest that loss of TBP-2 expression causes HTLV-I-infected IL-2-dependent cells to acquire a partial capability for growth without IL-2.

Discussion

We revealed that in IL-2-independent cells, the expression of TBP-2 is completely silenced due to DNA methylation and histone deacetylation (Figures 1–3). DNA methylation and histone deacetylation with the downregulation of gene expression for tumor suppressors such as CDKN2A, p53, E-cadherin and VHL tumor suppressor have been also reported (Ushijima and Okochi-Takada, 2005). TBP-2 is a regulator of the cell cycle and apoptosis (Butler *et al.*, 2002; Nishinaka *et al.*, 2004a). The *TBP-2* gene is downregulated during oncogenesis (Butler *et al.*, 2002; Han *et al.*, 2003), reported as a suppressor of melanoma metastasis (Goldberg *et al.*, 2003), and implicated in tumor progression in TBP-2 knockout mice (Oka *et al.*, in preparation). Therefore, the *TBP-2* gene seems to be an onco-suppressive gene. An HDAC inhibitor, SAHA, did not augment TBP-2 expression alone but induced the expression only after the reversal of DNA methylation (Figure 3a and b). Demethylation slightly induced histone acetylation (Figure 3c). These results indicate a link between DNA methylation and histone deacetylation, consistent with previous reports (Coombes *et al.*, 2003; Satoh *et al.*, 2003).

In HTLV-I-infected IL-2-dependent cells as well as murine IL-2-dependent CTLL-2 cells (data not shown), TBP-2 mRNA expression was upregulated on the deprivation of IL-2 and cells did not grow, whereas the expression was downregulated to within the normal range on the addition of IL-2 and cells started to grow, as shown by the current study and elsewhere (Nishinaka *et al.*, 2004a). TBP-2 gene expression is also induced by treatment with vitamin D_3 (Chen and DeLuca, 1994), HDAC inhibitors (Butler *et al.*, 2002), and serum deprivation (Han *et al.*, 2003). Therefore, expression of the *TBP-2* gene seems to be transcriptionally induced under conditions associated with cell growth arrest. TBP-2 mRNA expression paralleled the protein level (Nishinaka *et al.*, 2004a). The level of TBP-2 showed an inverse correlation with cell growth in response to IL-2 (Figure 4 and 5). TBP-2 may play a key role in the regulation of IL-2-dependent growth. TBP-2 gene regulation seems to be a sensor of deprivation of IL-2 signal. IL-2 deprivation upregulates TBP-2 expression transcriptionally, leading to cell growth arrest. Addition of IL-2 to the IL-2-deprived cells downregulates the expression of TBP-2 to the basal level, leading to proliferation. Therefore, loss of TBP-2 or loss of TBP-2 gene regulation causes loss of the growth control mechanism.

The ectopic expression of TBP-2 in MT-2 cells (Figure 6a) suppressed cell growth and restored the responsiveness to IL-2, although the response was partial (Figure 6b). Similarly, knockdown of TBP-2 by RNAi in IL-2-dependent cells (Figure 6c) caused a partial loss of IL-2-dependent growth control (Figure 6d). We consider the reason why TBP-2 overexpression only partially restored IL-2 dependence to be as follows. Since the transformation of HTLV-I seems to be a multistep process, TBP-2 alone may not be enough to restore the dependence on IL-2. In addition, the overexpressed *TBP-2* gene is less responsive to exogenous stimuli such as IL-2. The gene regulation of TBP-2 expression may be important for full IL-2-dependence. Overexpression of TBP-2 did not change the expression of the IL-2 receptor, or STAT binding activity (data not shown). Further study is needed to elucidate the mechanism of the regulation of TBP-2 expression in response to IL-2. The mechanism by which TBP-2 regulates growth suppression is under investigation. In our previous report, we observed a similar growth suppressive effect on overexpression of TBP-2 in other HTLV-I-transformed T cells (IL-2-independent

Figure 5 Thioredoxin-binding protein-2 (*TBP-2*) expression and cell growth in response to interleukin-2 (IL-2) in ATL-2 cells. The cells were cultured for 3 days in the absence or presence of IL-2 and cell growth was analysed by the MTT assay as described in 'Materials and methods'. The expression of TBP-2 was analysed by semiquantitative RT–PCR. (a) IL-2-dependent ATL-2 cells, (b) IL-2-independent ATL-2 cells, and (c) IL-2-independent ATL-2 cells after sequential treatment with 5-aza-CdR and SAHA. (d) Quantitative real-time RT–PCR analysis of TBP-2 mRNA expression in the samples of (c). Data are representative of three separate experiments and the mean ± s.d. from three replicates. (e) Statistical analysis of day-3 data. Data are representative of three separate experiments and the mean ± s.d. from three replicates. Nonsignificant and significant ($P<0.05$) differences are indicated by NS and (**), respectively as determined with Student's *t*-test.

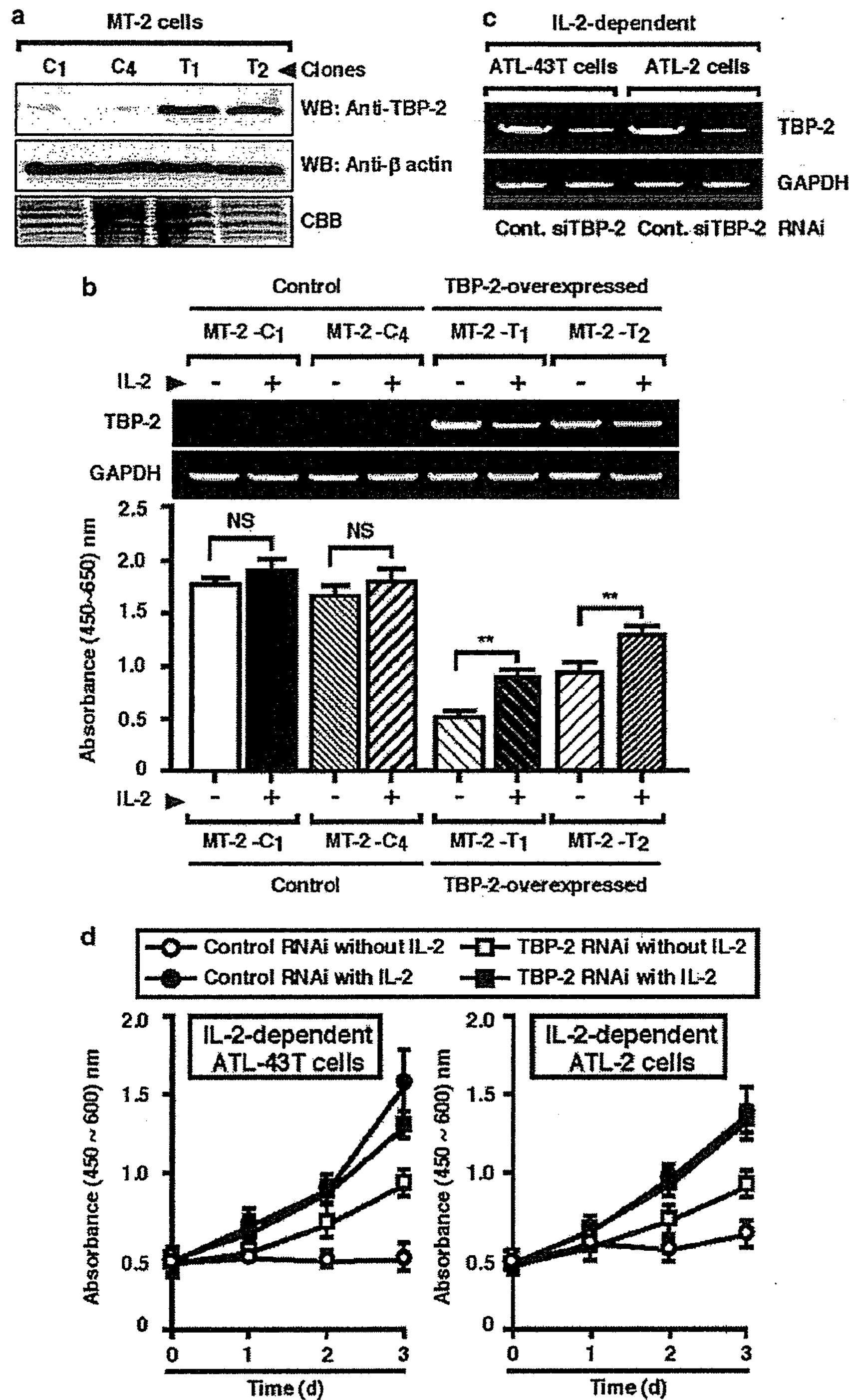


Figure 6 Relationship between TBP-2 expression and interleukin-2 (IL-2)-dependency in HTLV-I-infected T cells. (**a**) The MT-2 transfectants were stably transfected with *TBP-2* (T_1 and T_2) or control vector (C_1 and C_4) and the TBP-2 expression level was confirmed by Western blotting with monoclonal anti-TBP-2 antibody. Western blotting with monoclonal anti-β-actin antibody and staining with Coomassie Brilliant Blue R-250 (*CBB*) were used to monitor the amount of sample loaded. (**b**) Partial recovery of IL-2 dependency by overexpression of TBP-2. The TBP-2-overexpressing T_1 and T_2 or control C_1 and C_4 clones of MT-2 cells were cultured for 3 days in the absence or presence of IL-2 and cell proliferation was analysed by the MTT assay using WST-1 reagent. The upper panel shows the TBP-2 expression level analysed by semiquantitative RT–PCR. The cell proliferation data for day 3 in the absence or presence of IL-2 represent the mean±s.d. from three replicates. Similar results were obtained in three separate experiments. Nonsignificant and significant ($P<0.05$) differences are indicated by NS and (**), respectively, as determined with Student's *t*-test. (**c**) The expression of TBP-2 in cells transfected with RNAi of TBP-2. The level of TBP-2 was analysed by semiquantitative RT–PCR after 48 h of transfection. (**d**) IL-2-independent growth by knockdown of TBP-2 in HTLV-I-infected IL-2-dependent cells. After transfection with RNAi of TBP-2 or control, the cells were cultured for 3 days in the absence or presence of IL-2 and cell growth was analysed by MTT assay as described in 'Materials and methods'. The same results were obtained from three separate experiments.

ATL-43 T, ATL-2, and MT-2 cells). We also reported the disappearance of TBP-2 expression in Hut-102, ATL-2, MT-1 and MT-2 cells (Nishinaka *et al.*, 2004a), showing the generality of the finding in HTLV-I-infected cells. Loss of TBP-2 expression may augment the reducing activity of TRX (Nishiyama *et al.*, 1999), enhancing the growth of HTLV-I-infected T cells. In MT-2 cells ectopically expressing TBP-2, the expression of p16 was augmented (Nishinaka *et al.*, 2004a). Overexpression of TBP-2 also suppressed cell growth in MCF-7 cells, in which the p16 gene is deleted (Nishinaka *et al.*, 2004b), suggesting that there are other mechanisms to suppress cell growth. Analyses of gene expression in *TBP-2* knockout cells using Gene chips are underway. TBP-2 is localized to the nuclear compartment (Nishinaka *et al.*, 2004b). The interaction of TBP-2 with transcriptional repressors such as Fanconi anemia zinc-finger (FAZF), promyelocytic leukemia zinc-finger (PLZF) and HDAC1 has been suggested (Han *et al.*, 2003), and we have isolated several nuclear proteins that are important for the regulation of transcriptional events as partners of TBP-2 (Masutani *et al.*, in preparation). Therefore, it could be speculated that TBP-2, augmented by various stimuli, interacts with growth controlling molecules such as an HDAC complex to induce cell growth arrest.

In summary, we showed that the *TBP-2* gene is silenced due to DNA-methylation and histone deacetylation in IL-2-independent cell lines and that TBP-2 expression is closely associated with responsiveness to IL-2-dependent growth. The complete silencing of the *TBP-2* gene is seen in HTLV-I-infected IL-2-independent cells but not in other T-cell lines (Nishinaka *et al.*, 2004a), EBV-transformed cell lines, and other cell lines (unpublished observation). Therefore, the silencing may be specifically linked to the HTLV-I infection. Therefore, the role of the loss of TBP-2 expression seems more important in HTLV-I-infected leukemic cells. Although TBP-2 expression does not seem to be associated with Tax expression in the HTLV-I infected cell lines (data not shown), the role of viral proteins including Tax should be examined further. Clastogenetic changes (point mutations, deletions, substitutions, and translocations) are frequently found in HTLV-I-transformed cells. Disruption of base excision repair, nucleotide excision repair, DNA end stability, telomerase and cell cycle progression leads to an increase in the frequency of genomic mutation. ATL cells are convoluted and known to be aneuploid, suggesting an abnormality of the mitosis checkpoint in HTLV-I transformation (Jeang *et al.*, 2004). These aspects of HTLV-I transformation and ATL leukemogenesis are important. The role of loss of TBP-2 in these aspects of HTLV-I transformation should be also investigated further.

Materials and methods

Cell culture and transfections

Human lymphocytic leukemia T cells (Jurkat) and HTLV-I-infected T cells (ATL-2, MT-2, ED-40515, ATL-35T and ATL-43T) were cultured in RPMI-1640 medium containing 10% heat inactivated fetal calf serum (FCS) and antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml) at 37°C with 5% CO_2 in air. For the maintenance of IL-2-dependent HTLV-I-infected T cells, IL-2 (7.5 ng/ml; Peprotech EC) was added to the culture medium. HTLV-I-infected IL-2-dependent T cells, such as ATL-2, ED-40515, ATL-35T and ATL-43T), were established from ATL patients by culture in the presence of IL-2, whereas IL-2-independent cells were established by the long-term culture of IL-2-dependent cells (Maeda, 1992). Each set of IL-2-dependent and IL-2-independent cells has the same clonal origin as confirmed by the T-cell receptor-β gene rearrangement and HTLV-I proviral integration sites (Maeda, 1992). Stable MT-2 transfectants overexpressing TBP-2 were generated as described previously (Nishinaka *et al.*, 2004a) and maintained in medium containing 2 mg/ml of G418 (Nacalai Tesque).

Treatment of cells with 5-aza-CdR or SAHA

For the treatment with a demethylating reagent, IL-2-independent ATL-43T and ATL-2 cells were cultured in medium supplemented with 1 μM 5-aza-CdR (Sigma) for 3 or 5 days. For the treatment with HDAC inhibitors, cells were cultured in medium supplemented with 2.5 μM SAHA (Alexis Biochemicals). For the sequential treatment with the demethylating reagent and HDAC inhibitors, the cells were cultured in medium supplemented with 1 μM 5-aza-CdR for the first 3 days, then with 2.5 μM SAHA for the next 24 h. After the sequential treatment, the cells were cultured in the presence or absence of IL-2 (1U) for an additional 3 days. SAHA was dissolved in dimethyl sulfoxide (DMSO). After each treatment, cells were placed in fresh medium.

Measurement of TBP-2 mRNA expression by RT–PCR

For the TBP-2 mRNA expression analyses, total RNA was isolated from the cells using Trizol reagent (Invitrogen). cDNA was synthesized using a SuperScript First-Strand Synthesis System (Invitrogen) with oligo dT_{12-18}. The cDNA was amplified by PCR using a KlenTaq-LA DNA polymerase mix (Sigma). The primers used for the amplification were as follows: *TBP-2*, 5′-CCATGGTGATGTTCAAGAAGAT CAAG-3′ (forward) and 5′-CTCAGGGGCATACATAAA GA-3′ (reverse); *TRX*, 5′-ATGGTGAAGCAGATCGAG-3′ (forward) and 5′-TTAGACTAATTCATTAATGGT-3′ (reverse); *CDKN2A*, 5′-TTCGGCTGACTGGCTGGCCA-3′ (forward; exon 1) and 5′-AGCTCCTCAGCCAGGTCCAC-3′ (reverse; exon 2); and *glyceraldehydes-3-phosphate dehydrogenase (GAPDH)*, 5′-ATGGGGAAGGTGAAGGTCGGAG TC-3 (forward) and 5′-CCATGCCAGTGAGCTTCCCGTT C-3′ (reverse). PCR was performed under the following conditions: 32 cycles for *TBP-2* (denaturing at 94°C for 30 s, annealing at 53°C for 1 min, and extension at 72°C for 2 min), 20 cycles for *TRX* (denaturing at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min), 30 cycles for *CDKN2A* (denaturing at 94°C for 30 s, annealing at 61°C for 45 s, and extension at 72°C for 1 min), and 22 cycles for *GAPDH* (denaturing at 94°C for 30 s, annealing at 61°C for 1 min, and extension at 72°C for 90 s). The PCR products were visualized by electrophoresis in 3% NuSieve GTG agarose (Cambrex Bio Science) gel.

Quantitative real-time RT–PCR

For measurement of the quantity of *TBP-2* mRNA, real-time RT–PCR was conducted with a TaqMan Universal Master Mix (Applied Biosystems) using reverse transcribed cDNA as a template, in triplicate. The amplification was performed

using an ABI Prism7000 under the following conditions: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles (95°C for 15 s and 60°C for 1 min). The oligos and TaqMan probe for *TBP-2* were purchased from Applied Biosystems. 18S ribosomal RNA was used as an internal control.

Sodium bisulfite sequencing of genomic DNA
The genomic DNA was prepared from HTLV-I-infected IL-2-dependent or -independent cells using a Puregene DNA isolation kit (Gentra). Sodium bisulfite treatment was performed as reported previously (Nosaka *et al.*, 2000; Yasunaga *et al.*, 2004). For the sequencing of the *TBP-2* promoter region, 100 ng of genomic DNA was amplified by PCR using as primers, 5′-GGAGAAGACATCGGTCCT-3′ (forward) and 5′-CATGATGGAACTGAGTTGGT-3′ (reverse). For bisulfite-sequencing analyses, 100 ng of sodium bisulfite-treated genomic DNA was amplified by PCR using two pairs of methylation-specific primers. For the TATA-box region, a first round of amplification was performed with 5′-GGTTTTAGGGTTAGTGGGA-3′ (forward) and 5′-AAAAACCTTCTTTCCCCCAA-3′ (reverse), followed by a second round of nested PCR with 5′-TTTATTGGATTTGG GAGAA-3′ (forward) and 5′-ATCCAATCTCCACAAACA CTCC-3′ (reverse) primers. For the exon-1 region, a first round of amplification was performed with 5′-GGAAAGAA GGTTTTTTTTTTGA-3′ (forward) and 5′-CCACTTACCT ATTAACTAATCT-3′ (reverse), followed by a second round of nested PCR with 5′-TGATTTTGTTTAGTGTAATTAG-3′ (forward) and 5′-CCTATTAACTAATCTTCCAA-3′ (reverse). Amplification was carried out under the following conditions: 30 cycles (denaturing at 94°C for 30 s, annealing at 53°C for 1 min and extension at 72°C for 2 min), followed by a final extension at 72°C for 8 min. The PCR products were purified using a QIAEX II Gel extraction kit (Qiagen) and ligated into the vector pCR2.1 TOPO (Invitrogen). Inserts from five positive clones of each product were sequenced using M13 primers. Sequencing was performed using Big Dye Terminator reagent (Applied Biosystems) with an ABI PRISM310 Genetic Analyzer.

Chromatin immunoprecipitation (Chip) assay
For analyzing the histone acetylation status in TBP-2-restored cells, a Chip assay was performed as described previously (Spencer *et al.*, 2003). In brief, a chromatin solution was immunoprecipitated overnight at 4°C, using antiacetylated H3 and H4 antibodies (Upstate Biotechnology). Precipitated DNA was analysed by PCR amplification, using a KlenTaq LA DNA Polymerase Mix (Sigma). The following promoter-specific primers were used: *TBP-2* promoter region (207 bp); 5′-TCCAGAGCGCAACAACCAT-3′ (forward) and 5′-AAG CAGGAGGCGGAAACGT-3′ (reverse) or, *β-globin* promoter region (237 bp); 5′-GGCAAGGTGAACGTGGATG AAGTTGGTG-3′ (forward) and 5′-GGAGTGGACAGA TCCCCAAAGGACTCAAAG-3′ (reverse). PCR was carried out under the following conditions: 30 cycles for *TBP-2* (denaturing at 95°C for 30 s, annealing at 53°C for 1 min, and extension at 72°C for 2 min) or, 28 cycles for *β-globin* (denaturing at 95°C for 30 s, annealing at 66°C for 1 min, and extension at 72°C for 2 min). The PCR products were visualized by electrophoresis in 3% NuSieve GTG agarose gel.

Western blot
Cell lysates were prepared with lysis buffer (150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 20 mM Tris-HCl (pH 7.5), 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM NaF and protease inhibitor cocktail (Roche)). Western blotting was performed as described previously (Nishinaka *et al.*, 2004a). The membrane was blocked with 10% (w/v) skim milk in Tris-buffered saline containing 0.05% Tween-20, then incubated with monoclonal anti-TBP-2 or anti-β-actin (Sigma, St MO) antibodies, followed by peroxidase-conjugated anti-mouse IgG (Amersham Biosciences, Tokyo, Japan). An ECL Western blot detection kit (Amersham, Tokyo, Japan) was used to visualize the epitopes.

Knockdown of TBP-2 expression by RNA interference (RNAi)
Double stranded oligonucleotides (rACAGACUUCGGA GUACCUGdTT) for selective silencing of *TBP-2* or control oligonucleotides (rUUCUCCGAACGUGUCACGUdTT) (Qiagen) were transfected into cells using a human T cell nucleofector kit (Amaxa biosystems, Tokyo, Japan). Eight hours after transfection, IL-2 was added to the culture.

Cell proliferation assay
To analyse the cellular growth, cell proliferation was assayed based on [^{3}H]-thymidine incorporation or with the MTT assay using WST-1 reagent (TaKaRa). Cells (2–5 × 10^3/well) were seeded in 96-well flat-bottomed microtiter culture plates. Cells were cultured for 3 days in the presence or absence of IL-2 (7.5–15 ng/ml) and cell proliferation was assessed at different time points of the culture (day 0–3). For the [^{3}H]thymidine incorporation assay, cells were labeled with 1 μCi of [^{3}H]methyl-thymidine (Amersham) for the last 6 h and radioactivity was measured. For the MTT assay, cells were incubated with WST-1 reagent for the last 2 h and analysed with a Thermo-Max microplate reader.

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References

Butler LM, Zhou X, Xu WS, Scher HI, Rifkind RA, Marks PA *et al.* (2002). *Proc Natl Acad Sci USA* **99**: 11700–11705.
Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB. (1999). *Nat Genet* **21**: 103–107.
Chen KS, DeLuca HF. (1994). *Biochim Biophys Acta* **1219**: 26–32.
Coombes MM, Briggs KL, Bone JR, Clayman GL, El-Naggar AK, Dent SY. (2003). *Oncogene* **22**: 8902–8911.
Gardiner-Garden M, Frommer M. (1987). *J Mol Biol* **196**: 261–282.
Goldberg SF, Miele ME, Hatta N, Takata M, Paquette-Straub C, Freedman LP *et al.* (2003). *Cancer Res* **63**: 432–440.
Han SH, Jeon JH, Ju HR, Jung U, Kim KY, Yoo HS *et al.* (2003). *Oncogene* **22**: 4035–4046.
Holmgren A. (1985). *Annu Rev Biochem* **54**: 237–271.
Jeang KT, Giam C, Majone F, Aboud M. (2004). *J Biol Chem* **279**: 31991–31994.

Maeda M, Shimizu A, Ikuta K, Okamoto H, Kashihara M, Uchiyama T *et al.* (1985). *J Exp Med* **162**: 2169–2174.
Maeda M. (1992). *Hum Cell* **5**: 70–78.
Makino S, Masutani H, Maekawa N, Konishi I, Fujii S, Yamamoto R *et al.* (1992). *Immunology* **76**: 578–583.
Masutani H, Yodoi J. (2002). *Methods Enzymol* **347**: 279–286.
Matsuoka M. (2003). *Oncogene* **22**: 5131–5140.
Migone TS, Lin JX, Cereseto A, Mulloy JC, O'Shea JJ, Franchini G *et al.* (1995). *Science* **269**: 79–81.
Morgan DA, Ruscetti FW, Gallo R. (1976). *Science* **193**: 1007–1008.
Nishinaka Y, Nishiyama A, Masutani H, Oka S, Ahsan MK, Nakayama Y *et al.* (2004a). *Cancer Res* **64**: 1287–1292.
Nishinaka Y, Masutani H, Oka S, Matsuo Y, Yamaguchi Y, Nishio K *et al.* (2004b). *J Biol Chem* **279**: 37559–37565.
Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, Nakamura H *et al.* (1999). *J Biol Chem* **274**: 21645–21650.
Nosaka K, Maeda M, Tamiya S, Sakai T, Mitsuya H, Matsuoka M. (2000). *Cancer Res* **60**: 1043–1048.
Okamoto T, Ohno Y, Tsugane S, Watanabe S, Shimoyama M, Tajima K *et al* (1989). *Jpn J Cancer Res* **80**: 191–195.
Satoh A, Toyota M, Itoh F, Sasaki Y, Suzuki H, Ogi K *et al.* (2003). *Cancer Res* **63**: 8606–8613.
Spencer VA, Sun JM, Li L, Davie JR. (2003). *Methods* **31**: 67–75.
Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J *et al.* (1989). *EMBO J* **13**: 2244.
Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. (1977). *Blood* **50**: 481–492.
Ushijima T, Okochi-Takada E. (2005). *Cancer Sci* **96**: 206–211.
Yasunaga J, Taniguchi Y, Nosaka K, Yoshida M, Satou Y, Sakai T *et al.* (2004). *Cancer Res* **64**: 6002–6009.
Yodoi J, Takatsuki K, Masuda T. (1974). *New Engl J Med* **290**: 572–573.
Yoshida M, Miyoshi I, Hinuma Y. (1982). *Proc Natl Acad Sci USA* **79**: 2031–2035.