TEL/AML1 Overcomes Drug Resistance Through Transcriptional Repression of Multidrug Resistance-1 Gene Expression

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Abstract
The t(12;21)(p12;q22) chromosomal aberration, which is frequently observed in pediatric precursor B-cell acute lymphoblastic leukemia (ALL), generates the TEL/AML1 chimeric gene and protein. TEL/AML1-positive ALL has a favorable prognosis, and one possible reason is that this subtype of ALL rarely shows drug resistance. AML1/ETO, another AML1-containing chimeric protein, has been shown to transcriptionally repress the activity of the multidrug resistance-1 (MDR-1) gene promoter; thus, we examined whether TEL/AML1 also represses MDR-1 gene expression, possibly preventing the emergence of multidrug resistance. In this study, we show that the TEL/AML1 protein binds to the consensus AML1 binding site in the MDR-1 promoter and transcriptionally represses its activity. Following transient transfection of TEL/AML1 protein into Adriamycin-resistant K562/Adr cells, we also demonstrate that TEL/AML1 can down-regulate the expression of P-glycoprotein, a product of the MDR-1 gene, and restore the chemosensitivity to the cells. Furthermore, we report that MDR-1 mRNA levels in leukemic cells obtained from TEL/AML1-positive ALL patients are lower than those from TEL/AML1-negative ALL patients. Thus, TEL/AML1 protein acts as a transcriptional repressor of MDR-1 gene expression, and although TEL/AML1 has been implicated in leukemogenesis, its effects on the MDR-1 gene may contribute to the excellent prognosis of TEL/AML1-positive ALL with current therapy. (Mol Cancer Res 2004;2(6):339–47)

Introduction
Multidrug resistance of leukemic cells is a major obstacle to eradicating acute leukemia by using chemotherapy. One of the major mechanisms of multidrug resistance in malignant cells is overexpression of the multidrug resistance-1 (MDR-1) gene, which encodes P-glycoprotein (P-gp). P-gp functions as an energy-dependent efflux pump of chemotherapeutic drugs such as Vinca alkaloids, anthracyclines, and epipodophyllotoxins (1, 2). The prognostic value of P-gp expression for predicting the clinical course of acute myeloid leukemia and the blast crisis of chronic myelogenous leukemia has been established by several previous reports (5-8). However, the impact of P-gp on drug resistance in acute lymphoblastic leukemia (ALL) remains controversial. Kakihara et al. (9) could not find a positive correlation between expression of the MDR-1 gene and clinically classified risk-related groups, and Gekeler et al. (10) observed no difference in MDR-1 mRNA expression levels between primary and relapsed ALL patient samples. In contrast to these reports, other investigators have described that P-gp expression independently correlates with the probability of induction failure and relapse (11, 12) and that in vitro cellular drug resistance can be related to the clinical response to chemotherapy and leukemic relapse (13, 14). This latter conclusion remains to be confirmed.

Recurrent chromosomal aberrations identify subgroups of acute leukemia patients with distinct clinical features and have a strong impact on the characteristics of the leukemia. The t(12;21)(p12;q22) chromosomal translocation is the most frequently occurring genetic abnormality in childhood ALL. This translocation, which is often cryptic, is found in one-fourth of the patients with pediatric precursor B-cell ALL (15), and it generates a TEL/AML1 chimeric gene and protein. TEL is a member of the Ets family of transcription factors; it is required for the maintenance and development of the yolk sac vascular network and for hematopoiesis of all lineages within the bone marrow (16). TEL contains a NH2-terminal helix-loop-helix (HLH) domain (also known as the pointed domain or the SAM domain), which mediates homotypic oligomerization and is critical for normal TEL function (17, 18). AML1 (also known as Runx1) is a transcription factor, which is essential for the development of definitive hematopoiesis. Homozygous loss of AML1 leads to embryonic lethality due to the complete absence of fetal liver-derived hematopoiesis and to central nervous system hemorrhages (19, 20). The DNA binding domain of AML1 is named the runt homology domain (RHD); it binds to the specific...
DNA sequence “TG(T/C)GGT” (21). TEL/AML1 contains the NH2-terminal portion of the TEL and almost the entire structure of AML1B (22), thereby containing both the HLH domain and the RHD, which allows TEL/AML1 to bind to the AML1 binding sequence (23). Competition for binding between AML1 and TEL/AML1 for the AML1 binding sequence can alter the expression of genes normally regulated by AML1, as shown for the interleukin-3 promoter (24), the macrophage colony-stimulating factor receptor promoter (25), and the T-cell receptor β enhancer (26). Repression of gene expression has been shown for other leukemia-associated transcription factors including AML1/ETO [t(8;21)(q22;q22)], CBFβ/MYH11 [inv(16)], and AML1/EVI1 [t(3;21)], which actively recruit nuclear corepressor molecules and histone deacetylases to the regulatory regions of AML1 target genes (21, 27), and similar mechanisms of repression have been described for TEL/AML1 (23, 28).

Many clinical studies have revealed that TEL/AML1-positive ALL has a favorable prognosis (17, 29, 30), and one possible reason is that this type of ALL rarely shows drug resistance (31). t(8;21)-positive AML, which contains the MDR-1 promoter contains one potential AML1 binding site (TGTGGT), which is located between −94 and −89 bp upstream of the major transcriptional start site (Fig. 1A). We performed electrophoretic mobility shift assays to examine the specific binding of TEL/AML1 to the AML1 binding site of the MDR-1 promoter. Bacterially expressed TEL/AML1 protein binds to the oligonucleotide probe, which contains AML1 binding sequence (Fig. 2A, lane 2), and this DNA-protein gel shift complex is largely eliminated by adding a 100-fold molar excess of unlabeled wild-type competitor (Fig. 2A, lane 3) and is almost completely eliminated by adding a 300-fold molar excess of the same competitor (Fig. 2A, lane 4). In contrast, similar amounts of mutant competitor cannot compete out the DNA-protein binding (Fig. 2A, lanes 5 and 6), demonstrating that TEL/AML1 binds to the AML1 binding site in a sequence-specific manner.

We also performed chromatin immunoprecipitation (ChIP) assays to confirm the binding of TEL/AML1 to the MDR-1 promoter. K562 cells were transiently transfected with control or TEL/AML1 expression plasmids, and anti-Xpress antibody was used to perform immunoprecipitation. Immunoblotting assays showed that this antibody was able to detect the Xpress epitope fused to TEL/AML1 protein (Fig. 2B). Cells immunoprecipitated without antibody (Fig. 2C, lanes 2 and 4) were used as negative controls. The amplification product was seen only when both the TEL/AML1 expression plasmid and the Xpress antibody were used (Fig. 2C, lane 5). Therefore, ChIP assays also demonstrated the binding of TEL/AML1 to the AML1 binding site of the MDR-1 promoter.

**Results**

**TEL/AML1 Specifically Binds to the TGTGGT Sequence of the Human MDR-1 Promoter**

The human *MDR-1* promoter contains one potential AML1 binding site (TGTGGT), which is located between −94 and −89 bp upstream of the major transcriptional start site (Fig. 1A). To define the transcriptional regulatory activity of TEL/AML1 on the *MDR-1* promoter, we performed transient cotransfection assays in K562 cells using one of the expression

![FIGURE 1. Schematic representation of the human MDR-1 promoter and the TEL/AML1 cDNAs.](Image)
plasmids, which contain the TEL/AML1 cDNA or its deletion mutants (shown in Fig. 1B), and a luciferase-based reporter plasmid, which contains the AML1 binding site in the MDR-1 promoter. Wild-type TEL/AML1 down-regulated the transcriptional activity of MDR-1 promoter 50% compared with the empty expression vector, whereas the mutant TEL/AML1 constructs, which lack either HLH domain or RHD, had no suppressive effect (Fig. 3A). Thus, TEL/AML1 can repress the expression of the MDR-1 gene, and this repression requires both the HLH domain and the RHD. We also demonstrated that TEL/AML1 could repress the MDR-1 promoter activity in a dose-dependent fashion (Fig. 3B). In contrast, TEL/AML1 did not change the activity of the mutant MDR-1 reporter plasmid, which contains a mutated AML1 binding sequence (CAGATT) instead of a wild-type sequence (TGTGGT; Fig. 3C). This result suggests that the intact AML1 binding site is necessary for the repression by TEL/AML1.

In addition, we examined the transcriptional activities of wild-type AML1A or AML1B on the MDR-1 promoter (Fig. 3D). AML1A, which lacks the transactivation domain of AML1B, had no effect on the promoter. Interestingly, AML1B, which retains the transactivation domain, also did not change the promoter activity.

TEL/AML1 Down-Regulates the Expression of P-gp

Having demonstrated that TEL/AML1 binds to the MDR-1 promoter and acts as a transcriptional repressor, we examined whether TEL/AML1 could down-regulate P-gp expression, a product of the MDR-1 gene. K562/Adr cells, which constitutively express P-gp, were transiently transfected with the TEL/AML1 expression plasmids. After incubation for 24 hours, immunoblotting assays using an anti-P-gp antibody showed suppression of P-gp levels when TEL/AML1 was expressed in these cells (Fig. 4A). Using densitometric analysis, we found that TEL/AML1 suppressed P-gp expression by 56% compared with the cells transfected with the empty expression plasmids. In contrast to wild-type TEL/AML1, the two TEL/AML1 deletion mutants, which lack either the HLH domain or the RHD, had no effect on P-gp expression (Fig. 4B). Our results indicate that the expression of TEL/AML1 is sufficient to down-regulate cellular P-gp expression. This likely reflects transcriptional repression of the MDR-1 gene, as both the HLH domain and the RHD are essential for this effect of TEL/AML1.

TEL/AML1 Restores the Chemosensitivity to K562/Adr Cells

The restoration of the chemosensitivity by TEL/AML1 was examined. K562/Adr cells were transiently transfected with
either control or TEL/AML1 expression plasmids and treated with various concentrations of Adriamycin. After incubation for 48 hours, viability of cells was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays. The growth of K562/Adr cells expressing TEL/AML1 was inhibited by as low as 1 to 5 \( \mu \text{mol/L} \) Adriamycin, whereas the growth of mock-transfected K562/Adr cells was not suppressed by up to 10 \( \mu \text{mol/L} \) Adriamycin (Fig. 5). IC\(_{50}\) of K562/Adr cells expressing TEL/AML1 was \( \sim 30 \mu \text{mol/L} \) and IC\(_{50}\) of control K562/Adr cells was \( \sim 50 \mu \text{mol/L} \). These results suggest that TEL/AML1 can restore the sensitivity of K562/Adr cells to chemotherapy.

**MDR-1 Expression Is Suppressed in Leukemic Blasts of ALL With TEL/AML1**

Many previous reports have described that pediatric pre-B-cell ALL, which expresses TEL/AML1, shows high chemosensitivity and a favorable prognosis (17, 29, 30). Therefore, we examined the MDR-1 mRNA levels of leukemic blasts obtained from eight ALL patients using a real-time quantitative reverse transcription-PCR method (Fig. 6). Seven of eight cases were euploid. One TEL/AML1-negative case was hyperdiploid (DNA index 1.22), although chromosomal analysis showed normal karyotype. Leukemic cells from TEL/AML1-positive ALL patients showed lower MDR-1 mRNA expression compared with cells from TEL/AML1-negative patients; this difference was not quite statistically significant likely due to the small number of patient samples examined.

Repression of MDR-1 gene expression in ALL blasts by TEL/AML1 could confer sustained chemosensitivity to the leukemic cells, and this may be one explanation for the favorable clinical outcome seen in ALL with t(12;21).

**Discussion**

The development of intensive chemotherapeutic regimens has markedly improved the treatment outcome of patients with hematologic malignancies. However, drug resistance of leukemic cells that emerges during chemotherapy is a major problem limiting the eradication of leukemia. P-gp, which is encoded by MDR-1 gene, extrudes various kinds of chemotherapeutic drugs outside of the cell and plays an important role in the multidrug-resistant phenotype (1-4). A previous study has reported that

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**FIGURE 3.** Transcriptional repression of MDR-1 promoter activity by TEL/AML1. K562 cells were transiently cotransfected with expression plasmids, luciferase reporter plasmid, and an internal control plasmid to assess transfection efficiency. Mock, empty expression plasmid. Data are representative of three experiments with similar results. **A.** TEL/AML1 (T/A) down-regulates the MDR-1 promoter activity, whereas TEL/AML1 deletion mutants (T/A dHLH and T/A dRHD) have no effect. *, \( P < 0.05 \). **B.** Increasing amount of TEL/AML1 plasmid represses luciferase activity in a dose-dependent manner. *, \( P < 0.05 \). **C.** Compared with the wild-type MDR-1 reporter plasmid (WT), the luciferase activity is not repressed by using the reporter plasmid carrying mutant AML1 binding sequence (mut). *, \( P < 0.05 \). **D.** Wild-type AML1 proteins, AML1A or AML1B, do not affect MDR-1 promoter activity compared with negative control.
the complete remission rate was significantly lower in MDR-1-positive acute nonlymphoblastic leukemia cases (23 of 71, 32%) than in the MDR-1-negative cases (64 of 79, 81%; ref. 6). MDR-1 mRNA expression levels were measured in acute myeloid leukemic patients in another study, and longer overall survival was found in the MDR-1 mRNA-negative patients (8 months for MDR-1 mRNA-positive patients compared with 19 months for MDR-1 negative patients; ref. 7). Furthermore, another group found that the chance of achieving first complete remission differed between MDR-1-positive ALL patients (56%) and MDR-1-negative patients (93%), and they observed a higher relapse rate in MDR-1-positive patients (81%) compared with MDR-1-negative patients (37%; ref. 11). Drug resistance can also be associated with other efflux pumps such as multidrug resistance–related protein 1 (34, 35) and lung resistance protein (36), which are thought to cooperate in vivo with P-gp. However, several investigators have shown that the expression level of multidrug resistance–related protein 1 or lung resistance protein does not correlate with prognosis in patients with childhood ALL (9, 37).

The TEL/AML1 chimeric gene is the most common genetic abnormality in pediatric ALL. TEL/AML1-positive ALL patients have significantly lower rates of relapse compared with TEL/AML1-negative patients (0 of 22 cases of relapse versus 16 of 54 cases; ref. 17). In another study, only 10% of relapsed pediatric ALL cases expressed TEL/AML1 as compared with 20% to 25% of newly diagnosed ALL cases (30). While some investigators have reported that the presence of TEL/AML1 may correlate only with chemosusceptibility and may not contribute to more favorable long-term prognosis, large prospective studies with long-term follow-up must be conducted to settle this issue (38-40).

Although AML1B can activate or repress gene transcription (33, 41), Hiebert et al. (23) have demonstrated that the HLH domain derived from TEL also results in TEL/AML1 functioning as a repressor of transcription. Our current results are also consistent with the idea that TEL/AML1 can act by itself as a transcriptional repressor on various promoters. In contrast, the study of TEL/AML1 on the macrophage colony-stimulating factor receptor promoter showed that TEL/AML1 had no significant effect on the promoter by itself (42). We also observed that neither AML1B nor AML1A influenced the activity of the MDR-1 promoter, which is consistent with the previous finding that AML1A had no activity on the MDR-1 promoter (33). However, these observations contrast with our data that both AML1A and AML1B could transcriptionally activate the interleukin-3 gene promoter (43). Although it contains a transactivation domain, AML1B is unable to function as an activator on the MDR-1 promoter. Accordingly, we speculate that regulation of the MDR-1 gene could be a “gain-of-function” property of the AML1 chimeric proteins.

Transcription factors generally regulate gene expression in conjunction with cofactors such as coactivators or corepressors (27, 44). Fenrick et al. (45) showed that TEL/AML1 can bind the corepressor mSin3A. AML1 can also interact with mSin3A, and TEL can associate not only with mSin3A but also with N-CoR and histone deacetylase-3, which are required for TEL-mediated repression (46, 47). While both TEL and AML1 B associate with mSin3A, TEL/AML1 appears to bind mSin3A much more stably than either wild-type protein. Our results indicate that TEL/AML1 suppressed MDR-1 expression by one half at both transcription and protein levels. On the other hand, AML1/ETO can repress the same gene expression by ∼4- to 6-fold (33). Hiebert et al. (28) reported that AML1/ETO can make contacts with more corepressors than TEL/AML1. This fact might be the reason of the difference between TEL/AML1 and AML1/ETO.

This is the first study to show that MDR-1 gene expression is transcriptionally repressed by the TEL/AML1 chimeric protein, which could help explain the good therapeutic outcome of TEL/AML1-positive ALL. The lower MDR-1 mRNA expression in leukemic cells obtained from TEL/AML1-positive ALL patients supports our hypothesis; however, an analysis of a larger number of patient samples will be necessary to demonstrate the significance of this difference.

Therapeutic approaches that modulate gene transcription have already shown to be effective in diseases such as acute promyelocytic leukemia (48). Sequence-specific DNA binding molecules, which mimic the transcriptional activity of tumor suppressor proteins such as p53 or Rb, could have broad

**FIGURE 4.** Down-regulation of cellular P-gp expression by TEL/AML1. K562/Adr cells, which constitutively express P-gp, were transiently transfected with the TEL/AML1 expression plasmid, and the cellular extracts were prepared for immunoblot analysis to detect P-gp. Relative intensity ratios of the bands measured by densitometric analysis are also shown. The intensity of P-gp to actin in mock-transfected samples was assigned a value of 1. A, TEL/AML1 suppressed P-gp expression in K562/Adr cells compared with the empty expression plasmid (mock) or plasmid expressing β-galactosidase (LacZ). B, Mutant TEL/AML1 constructs (T/A dHLH or T/A RHD) did not down-regulate P-gp expression.
spectrum antitumor activity against various cancers. In addition, several investigators have engineered synthetic proteins, which can bind to the promoter region of specific genes (49). These proteins, called designer transcription factors, consist of artificial zinc finger motifs and can bind and activate or repress genes such as \( \text{MDR-1} \) (50), erythropoietin (51), \( \text{erbB-2} \) (52), \( \text{erbB-3} \) (52), and PPAR\( \gamma \) (53). Our study and others have demonstrated that RHD-containing chimeric proteins act as transcriptional repressors of the \( \text{MDR-1} \) gene. Therefore, introduction of a functional RHD-containing suppressor, which can suppress genes abnormally up-regulated in cancer cells, could serve as a novel therapy.

Materials and Methods

Cell Culture

K562 and K562/Adr (Adriamycin-resistant strain) cell lines were maintained in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA), 100 units/mL penicillin, and 100 \( \mu \)g/mL streptomycin.

Plasmids

\( \text{MDR-1} \) genomic DNA (54) was digested with \( \text{XhoI} \) and \( \text{BglII} \) restriction endonucleases, and the fragment, which contains nucleotides from \(-133 \) to \(+286 \) relative to the transcriptional start site of the \( \text{MDR-1} \) promoter (with the AML1 binding site), was subcloned into pGL3-basic vector (Promega, Madison, WI). The mutant \( \text{MDR-1} \) promoter plasmid was generated by replacing the \( \text{KpnI-CeII} \) fragment of the wild-type reporter plasmid to the same fragment carrying mutated AML1 binding sequence (TGTGGT was substituted to CAGATT). This fragment was prepared by synthetic oligonucleotides.

The pCMV5-vector-based \( \text{TEL/AML1} \), \( \text{AML1A} \), and \( \text{AML1B} \) expression plasmids were described previously (24). The pCMV5-TEL/AML1 contains the entire structure of \( \text{TEL/AML1} \) cDNA, whereas the pCMV5-TEL/AML1 divergent RHD or the pCMV5-TEL/AML1 divergent HLH lack the RHD or the HLH domain of the \( \text{TEL/AML1} \) cDNA, respectively (24).

Preparation of TEL/AML1 Proteins

The \( \text{TEL/AML1} \) cDNA was subcloned into the pET-30b(+) plasmid (Novagen, Madison, WI). Bacterially expressed TEL/AML1 protein was prepared according to the manufacturer’s instructions (Novagen). Briefly, \( \text{Escherichia coli} \) strain BL21 (DE3) cells were transformed with recombinant plasmids and cultured at 37°C overnight. The subcloned cDNAs were expressed following induction with 1 mmol/L isopropyl-\(-\)\( \beta \)-D-\( \text{thiogalactopyranoside} \) (Sigma Chemical) for 4 hours at 37°C. Cells were resuspended in 1 mL of 50 mmol/L Tris-2 mmol/L EDTA (pH 8) and lysed with 50 \( \mu \)g/mL lysosome (Sigma Chemical), sonicated, and centrifuged; the supernatant was then recovered. To avoid degradation of the proteins, 1 mmol/L phenylmethylsulfonyl fluoride (Sigma Chemical), 10 \( \mu \)g/mL aprotinin (Sigma Chemical), and 10 \( \mu \)g/mL leupeptin (Sigma Chemical) were used. The expressed proteins in the supernatant were separated by 7.5% SDS-PAGE, and the S-Tag HRP

FIGURE 5. Restoration of the chemosensitivity by TEL/AML1. K562/Adr cells were transiently transfected with the TEL/AML1 expression plasmid (●) or mock (◆) and treated with various concentrations of Adriamycin for 48 hours. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays demonstrated that the growth of the cells expressing TEL/AML1 was inhibited at a lower concentration of Adriamycin compared with mock-transfected K562/Adr cells.

FIGURE 6. Quantitative analysis of \( \text{MDR-1} \) mRNA in human ALL cells with or without TEL/AML1 chimeric gene. Total RNA was extracted from human ALL cells, and the \( \text{MDR-1} \) mRNA was quantified by real-time quantitative reverse transcription-PCR method. The values of each sample are indicated (●). The mean amount of mRNA (−) in TEL/AML1-positive ALL (\( n = 4 \)) was lower than that in TEL/AML1-negative ALL (\( n = 4 \)), yet statistical significance was not confirmed.
LumiBlot kit (Novagen) was used to confirm the integrity of the expressed proteins. The protein structures of TEL/AML1 and its deletion mutants and of wild-type TEL and AML1 are shown in Fig. 1B.

**Electrophoretic Mobility Shift Assays**

The following complementary single-strand oligonucleotides were synthesized and used in electrophoretic mobility shift assays: MDR-1 promoter region (wild-type), 5'-GATCCAGT-CATCTGTGTTAGGGCTGAG-3', containing the AML1 consensus binding sequence (TGTTGG), located from −94 to −89 bp upstream of the transcriptional start site; MDR-1 promoter region (mutant), 5'-GATCCAGGTATCCAGGCTGTAGG-3', containing five nucleotide substitutions (CAGATT) of the TGTTGG sequence.

Complementary strands of these oligonucleotides were annealed at 100°C for 3 minutes and then labeled using T4 polynucleotide kinase (Roche Diagnostics GmbH, Mannheim, Germany) and [γ-32P]ATP. One nanogram of probe was incubated with 1 μL of bacterial culture supernatant containing expressed protein for 25 minutes at room temperature in a 20 μL reaction containing 20 mmol/L HEPES (pH 7.9), 0.5 mmol/L EDTA, 2 mmol/L DTT, 10% glycerol, 50 mmol/L KCl, and 1 μg poly(deoxyinosinic-deoxycytidyllic acid) (Pharmacia, Piscataway, NJ), with or without unlabelled competitors. The molar excess of competitor oligonucleotide is indicated in the figure legends. Following incubation, the samples were loaded onto a 5% nondenatured polyacrylamide gel and run in 0.5× Tris-borate EDTA buffer at room temperature. Gels were dried and exposed to BioMax MS film (Eastman Kodak, Rochester, NY) overnight using intensifying screens at −80°C.

**Transfection and Luciferase Assays**

K562 cells were transiently transfected by electroporation method (55) using a Gene Pulser (Bio-Rad, Hercules, CA). Cells (5 × 10^6) were transfected with 5 μg wild-type or mutant MDR-1 promoter-luciferase reporter plasmids, 5 μg pRL-TK (control plasmid used as an internal control of the transfection; Promega), and 5 or 10 μg pCMV5 expression plasmids (Invitrogen) containing the AML1 consensus sequence (TGTTGG) or its deletion mutants and of wild-type TEL and AML1 are shown in Fig. 1B.

**ChIP Assays**

K562 cells were transiently transfected with pcDNA3.1/HisB or pcDNA3.1/HisB-TEL/AML1 expression plasmid (Invitrogen) by electroporation and incubated for 24 hours. Transfected cells were subjected to Lymphoprep (Axis-Shield PoC AS, Oslo, Norway) density gradient centrifugation, and viable cells were collected. ChIP assay kit (Upstate Biotechnology, Inc., Lake Placid, NY) was used to perform ChIP assays according to the manufacturer’s instructions. Briefly, sonicated cell lysates were incubated with anti-Xpress antibody (Invitrogen), which detects the Xpress tag that is expressed by the pcDNA3.1/His plasmid. The immunoprecipitates were washed, and immunocomplexes were eluted with 500 μL elution buffer. DNA was extracted with phenol/chloroform extraction method and precipitated with ethanol and 20 μg glycogen. Precipitated DNA was amplified by PCR: ChIP assay samples (5 μL) were used in a 50 μL amplification reaction solution containing 5 μL of 10× PCR buffer minus Mg (supplied with Taq DNA polymerase recombinant; Invitrogen), 1.5 μL of 50 mmol/L MgCl2, 1 μL of 10 mmol/L deoxy-nucleotide triphosphate mix, 1 μL each of 10 μmol/L sense and antisense primers, and 0.4 μL of Taq DNA polymerase. The primers of the sequences were as follows: sense primer 5’-GTATTCTGAGGACATGCATT-3’ and antisense primer 5’-CAGGCTTCCCTGTGCCAACAGAG-3’. The thermal cycling conditions included 5 minutes at 94°C followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, and extension at 72°C for 1 minute.

**Immunoblotting**

K562/Adr cells were transiently transfected with pcDNA3.1/His-based TEL/AML1 expression plasmids (Invitrogen). Electroporation was performed using the same method described above, except that the cell number (2 × 10^6) and total amount of plasmid DNA (50 μg) were different. After incubation for 24 hours, cells were lysed with radioimmunoprecipitation assay buffer [0.05 mol/L Tris-HCl (pH 8), 0.15 mol/L NaCl, 0.1% NP40, 0.1% SDS, 0.02% sodium deoxycholate, and 1 mmol/L phenylmethylsulfonyl fluoride], and the supernatants were collected. Protein (30 μg) was fractionated by 7.5% SDS-PAGE and transferred to nitrocellulose membrane. Blots were incubated with an anti-P-gp antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and subjected to enhanced chemiluminescence (Amersham Biosciences, Buckinghamshire, United Kingdom) to visualize P-gp expression. Transfection efficiency was confirmed using an anti-Xpress HRP antibody (Invitrogen).

**Cell Proliferation Assay**

K562/Adr cells were transiently transfected with pcDNA3.1/His-based TEL/AML1 expression plasmids (Invitrogen) and incubated for 24 hours. Viable cells were collected by Lymphoprep (Axis-Shield PoC) density gradient centrifugation and resuspended at a final concentration of 1 × 10^6 cells per milliliter, and 100 μL of cell suspension were plated to each well on 96-well cell culture plates in triplicate. Adriamycin (Wako Pure Chemical Industries, Osaka, Japan) was added to each well at various concentrations and incubated for 48 hours at 37°C. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays were performed according to the manufacturer’s instructions (Roche Diagnostics).

**Real-Time Quantitative Reverse Transcription-PCR (TaqMan PCR)**

Bone marrow cells were obtained from human ALL patients under written informed consent. Total cellular RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA). Four cases of ALL with TEL/AML1 and four without TEL/AML1
were analyzed. cDNA was synthesized with 2 μg total cellular RNA by SuperScript First-Strand Synthesis System for reverse transcription-PCR (Invitrogen). The amounts of MDR-1 and β-actin transcripts were measured by real-time quantitative reverse transcription-PCR (TaqMan PCR) method: cDNA sample (1 μL) was used for TaqMan PCR in a 50 μL amplification reaction solution containing 25 μL of 2× TaqMan Universal PCR Master Mix (Perkin-Elmer Applied Biosystems, Foster City, CA), 300 nmol/L each of sense and antisense primers, and 200 nmol/L of TaqMan probe. The primers and TaqMan probe to detect MDR-1 gene were designed using the Primer Express software program (Perkin-Elmer Applied Biosystems). The sequences of the primers and probe are as follows: forward primer 5′-CTCAGACAGGTAGTGTTTGGTT3′, reverse primer 5′-TAGCCCTTTAACCTTGGACA-3′, and probe 5′-AACACCTGAGAGTATTCAAGG-3′. The TaqMan probe was labeled with fluorescent FAM (reporter) at the 5′ end and fluorescent TAMRA (quencher) at the 3′ end. The thermal cycling conditions included 2 minutes at 50°C and 10 minutes at 95°C followed by 40 cycles of 95°C for 15 seconds (for denaturation) and 60°C for 1 minute (for annealing and extension). All reactions were performed in the ABI PRISM 7700 Sequence Detection Systems (Perkin-Elmer Applied Biosystems). The TaqMan β-actin control reagents (Perkin-Elmer Applied Biosystems) served as the control gene. The absolute copy numbers of MDR-1 and β-actin transcripts in the samples were calculated using the standard curve, and results were standardized by the ratio of copy numbers of MDR-1 to those of β-actin in each sample.

Statistical Analysis
Data were compared using paired t test. Results were considered statistically significant when P < 0.05.

References


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