[CANCER RESEARCH 62, 3904–3908, July 15, 2002]

Advances in Brief

TEL-AML1, Expressed from t(12;21) in Human Acute Lymphocytic Leukemia, Induces Acute Leukemia in Mice[1]

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Abstract

TEL-AML1 is expressed from the t(12;21) chromosomal translocation in B-precursor acute lymphocytic leukemia (ALL). Creation of the TEL-AML1 fusion disrupts one copy of the *TEL* and *AML1* genes, and loss of TEL or AML1 is also associated with cases of acute leukemia without TEL-AML1. To determine whether TEL-AML1 can contribute to leukemogenesis, we transduced marrow from C57BL/6 mice with a retroviral vector expressing TEL-AML1 or with a control vector. Transduced cells were introduced into irradiated syngeneic recipients. Two of 9 TEL-AML1 mice developed ALL (one T-lineage ALL and one B-precursor ALL), whereas 0 of 20 control mice developed leukemia. The B-precursor ALL was retransplantable and expressed TEL-AML1. We similarly transduced marrow from C57BL/6 mice lacking the overlapping $p16^{INK4a}p19^{ARF}$ genes and transplanted the cells into wild-type recipients. No control mice died, but six of eight TEL-AML1/p16p19 mice died with leukemia. Overall, these findings indicate that TEL-AML1 contributes to leukemogenesis and may cooperate with loss of $p16^{INK4a}p14^{ARF}$ to transform lymphoid progenitors.

Introduction

The TEL-AML1 fusion protein is expressed from the t(12;21)(p13;q22) chromosome in 25% of pediatric and 3% of adult B-ALL[4] cases (1, 2). TEL is an Ets family transcription factor with a COOH-terminal DNA-binding domain. In TEL-AML1, the majority of TEL, with the exception of its DNA-binding domain, is positioned NH_2-terminal to AML1. TEL-AML1 binds AML1 *cis* DNA elements and represses linked reporter genes (3, 4). The AML1 gene itself harbors point mutations in the AML1 DNA-binding domain in 5% of AMLs, and the genes encoding AML1 or its dimerization partner, CBFβ, are involved in several other chromosomal abnormalities in an additional 20% of AML cases. Mutation of the *AML1* gene and expression of the AML1-ETO, CBFβ-SMMHC, or TEL-AML1 fusion proteins apparently have in common the ability to inhibit the activity of endogenous AML1. In this regard, mice lacking either AML1 or CBFβ and knockin mice expressing AML1-ETO or CBFβ-SMMHC each fail to develop definitive hematopoiesis (5). Whereas TEL-AML1 might contribute to leukemogenesis directly, by repressing AML1 target genes or by binding other Ets proteins via its pointed domain, it is possible that disruption of one copy of the *AML1* gene by the t(12;21) translocation is the more critical abnormality. Similarly, disruption of one *TEL* locus might facilitate transformation because the second copy of *TEL* and expression of TEL protein are lost in the majority of ALLs harboring TEL-AML1 and because a small number of B-ALLs without TEL-AML1 lose both *TEL* alleles (6–8). We therefore sought to determine whether TEL-AML1 induces acute leukemia in mice, expressed either alone or together with loss of the overlapping $p16^{INK4a}p19^{ARF}$ genes.

Materials and Methods

DNA Constructs. The MING retroviral vector encodes NGFR-GFP, residues 1–277 of the human low-affinity NGFR (p75), containing its extracellular and transmembrane domain, linked to the NH_2 terminus of full-length enhanced GFP, residues 1–238. The TEL-AML1 cDNA was subcloned upstream of the IRES to generate MING-TEL-AML1.

Preparation of Packaging Lines. NIH 3T3 and ecotropic CRE packaging cells (9) were cultured in DMEM with 10% heat-inactivated calf serum. CRE cells were transfected by calcium phosphate precipitation with 20 μg of MING or MING-TEL-AML1 and 2 μg of pSV40-puro. Pooled transfectants were selected using 2 μg/ml puromycin. Cells (10^6) were then immunoaffinity purified with biotin-conjugated anti-NGFR monoclonal antibody (HB8737; American Type Culture Collection) and streptavidin-coated magnetic beads (Stem Cell Technologies, Vancouver, British Columbia, Canada). After expansion in culture, selected cells were reselected one to three additional times to obtain populations uniformly expressing NGFR. The viral titers generated by the two packaging lines were assessed using a viral RNA slot blot analysis. In brief, viral particles were precipitated using 10% polyethylene glycol 8000 in 0.5 M NaCl. RNA was then extracted using phenol and loaded onto a nylon filter using a slot blot apparatus. A 1.7-kb *Sph*I/*Eco*RI fragment containing the 5′ LTR and adjacent retroviral sequences was then used as probe using a Northern blot procedure (10). As a second approach, NIH 3T3 cells were transduced for 48 h using filtered supernatants from the CRE cell lines and 4 μg/ml Polybrene. NGFR expression in the NIH 3T3 cells was assessed 48 h later by fluorescence-activated cell sorting. Assessment of TEL-AML1 expression by Western blotting was carried out as described previously (10). AML1 antiserum was kindly provided by H. Drabkin, and TEL antiserum was kindly provided by J. R. Downing.

Transduction and Transplantation of Murine Bone Marrow. Donor C57BL/6 or C57BL/6 $p16^{INK4a}/p19^{ARF}(-/-)$ mice (11) were treated with 150 mg/kg 5-fluorouracil i.v., and 3 days later, marrow cells were flushed from the femurs and tibias using QBSF-58 medium (generously provided by Quality Biological Inc.). After red cell lysis with ammonium chloride, the cells were resuspended in QBSF-58 supplemented with 2 mM glutamine, 100 ng/ml murine interleukin 6, 100 ng/ml murine interleukin 3, and 50 ng/ml murine stem cell factor (PeproTech) and cultured for 48 h at 37°C in 5% CO_2. Confluent CRE packaging lines were irradiated with 40 Gy before coculture with 8×10^6 marrow cells/100-mm dish with 4 μg/ml Polybrene for an additional 2 days. Transduced marrow cells (2×10^6) were then injected into the tail veins of recipient wild-type C57BL/6 mice that had received 6.0 Gy of γ-irradiation the day before the injection and 3.5 Gy of γ-irradiation on the day

Received 3/22/02; accepted 5/23/02.

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[1] Suported by NIH Grant HL51388 and a grant from the Children's Cancer Foundation (to A. D. F.), a grant from the National Foundation for Cancer Research (to C. I. C. and A. D. F.), grants from the Foundation of France and the Leukemia Research Foundation (to F. B.), and NIH Training Grant T32 CA60441 (to Y. Y.). A. D. F. is a Scholar of the Leukemia and Lymphoma Society.

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[4] The abbreviations used are: B-ALL, B-precursor acute lymphocytic leukemia; T-ALL, T-lineage acute lymphocytic leukemia; CBF, core binding factor; NGFR, nerve growth factor receptor; GFP, green fluorescent protein; IRES, internal ribosome entry site; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; LTR, long terminal repeat; HR, hazard ratio.

of the injection. Leukemia transplantation to secondary recipients was by i.p. injection of 2×10^6 cells.

Analysis of Leukemic Cells. Cytospins of marrow or spleen cells were subjected to Wright's-Giemsa staining. Murine marrow or spleen cells (10^6) were stained with biotin-conjugated antibodies at 1 μg/10^6 cells for 30 min at 4°C, washed, incubated with streptavidin-conjugated R-phycoerythrin (BD PharMingen, San Diego, CA) for 30 min at 4°C, and fixed with 1.0% paraformaldehyde and 0.01 M HEPES (pH 7.4). Antibodies used recognized Sca-1 (Ly6A/E; stem cell), TCRβ (H57-597; T lineage), B-220 (RA3-6B2; B lineage), Mac-1 (M1/70; macrophage), Gr-1 (RB6-8C5; granulocyte), Ter-119 (erythroid), CD4 (GK1.5; T lineage), and CD8α (53-6.7; T lineage; all from BD PharMingen). Immunophenotypes were analyzed with a FACScan flow cytometer (Becton Dickinson).

Statistical Methods. The end point of this study was time to leukemia. Event time distributions were estimated by the method of Kaplan and Meier. HRs were obtained using the Cox model. When the Cox model did not converge, the log-rank *P* is presented. Statistical computations were performed using the SAS or EGRET PC packages (12, 13).

Results

Development of Retroviral Vectors. The control vector MING, which expresses only NGFR-GFP, and MING-TEL-AML1 are diagrammed in Fig. 1*A*. These vectors were transfected into CRE packaging cells along with pSV40-puro. After puromycin selection, NGFR-GFP-expressing cells were selected using NGFR antibody and magnetic microbeads. Two rounds of selection yielded a population of CRE-MING cells homogenously expressing NGFR, whereas four rounds of selection were required to purify CRE-MING-TEL-AML1 cells. TEL-AML1 was detected at the expected molecular weight in these cells, using either an AML1 or a TEL antiserum (Fig. 1*B*). GFP fluorescence was present uniformly in the CRE-MING cells but absent in the TEL-AML1 cells, a phenomenon observed previously with NGFR antibody selection of MING producer lines[5] and likely reflecting outgrowth of a clonal population carrying a mutation in the GFP segment of the integrated vector. The viral titers generated by the resulting two producer populations were compared by analyzing retroviral RNA in the CRE supernatants (Fig. 1*C*) and by transduction of NIH 3T3 cells (data not shown). Both assays indicate that the titer of the MING viral stock was approximately four times that of the TEL-AML1 vector. Based on quantification of 3T3 transduction, the titers of the MING and TEL-AML1 vectors were approximately 8×10^4/ml and 2×10^4/ml, respectively. CRE-MING cells transferred GFP expression to 27% of Sca-1+ marrow stem/progenitor cells in an *in vitro* assay, and MING vectors expressing CBFβ-SMMHC or E7 and having a 2-fold lower titer transferred GFP expression to 10% or 14% of Sca-1+ cells (14). Inactivity of the GFP segment in the CRE-TEL-AML1 producer line and lack of detectable surface NGFR in marrow cells transduced with the MING vector or any of its derivatives (14) precluded assessment of marrow transduction by CRE-TEL-AML1 cells. However, based on the titers of the TEL-AML1 and MING viral stocks, we estimate that these cells transduced approximately 7% of Sca-1+ marrow cells.

TEL-AML1 Induces ALL in Mice. The MING or TEL-AML1 vector was used to transduce marrow cells isolated from C57BL/6 mice, and unselected marrow was then transplanted into syngeneic recipients. Leukemia incidence for these two cohorts is shown in Fig. 2. Two leukemias developed in the nine mice transduced with the TEL-AML1 vector. Because the MING group had no leukemias, its HR *versus* the TEL-AML1 cohort could not be calculated, but the log-rank test shows a trend toward a significant difference between these groups ($P = 0.07$). As we will describe, no leukemias developed in a cohort of mice transplanted with $p16^{INK4a}p19^{ARF}(-/-)$ marrow transduced with the MING vector. The incidence of leukemia in the TEL-AML1 group is significant compared with the combined MING groups ($P = 0.01$).

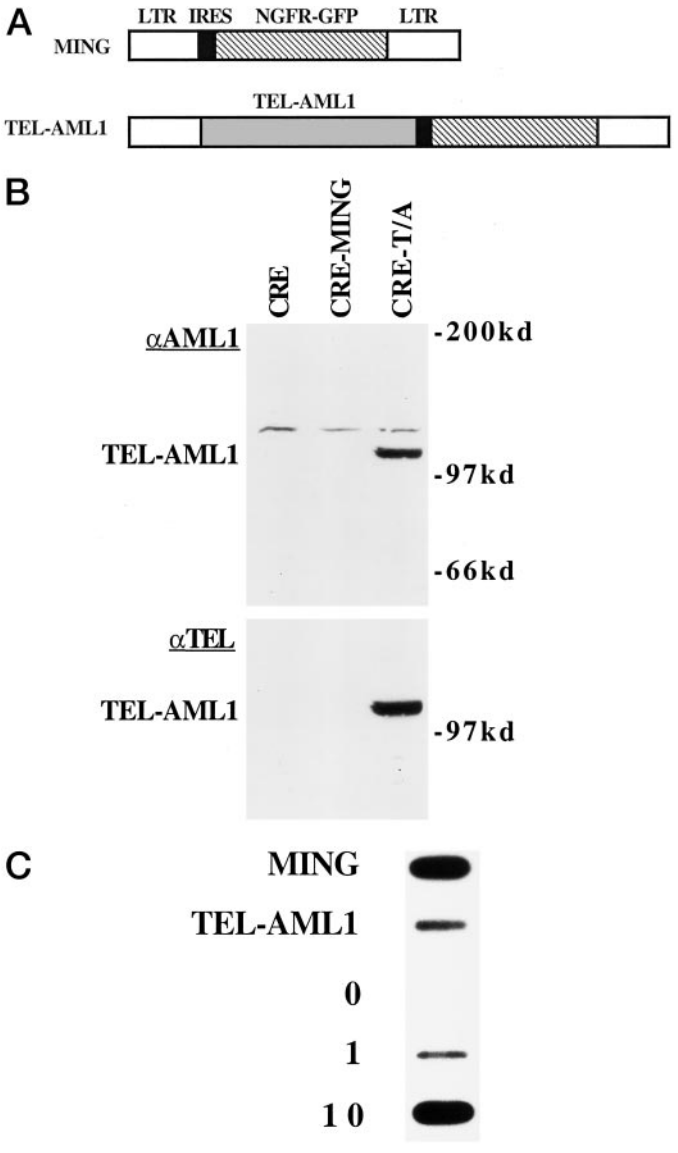

Fig. 1. Retroviral vector TEL-AML1 and a control vector. *A*, diagram of retroviral vectors. MING contains a murine stem cell virus LTR, followed by a polylinker, an IRES, the NGFR-GFP cDNA, and a second LTR. In MING-TEL-AML1, the IRES allows translation of both TEL-AML1 and NGFR-GFP from a single RNA species. *B*, total cellular protein extracts from 1×10^6 CRE, CRE-MING, and CRE-TEL-AML1 (*CRE-T/A*) cells were subjected to Western blotting using antiserum specific for AML1 (*top panel*) and TEL (*bottom panel*). The positions of protein molecular markers are also indicated. *C*, viral RNAs corresponding to 1 ml of CRE-MING or CRE-TEL-AML1 supernatants were subjected to slot blot analysis for viral titer using a LTR probe. Various amounts (0, 1, and 10 ng) of MING DNA were included on the filter for comparison.

The morphologies of the two TEL-AML1 leukemias, compared with normal spleen or bone marrow cells, are shown in Fig. 3. One leukemia, T/A-1 (T/A, TEL-AML1), was best detected in the spleen and had prominent nucleoli and visible cytoplasm. The second leukemia, T/A-2, homogenously replaced the bone marrow and demonstrated an open chromatin pattern and only a rim of dark cytoplasm. Both blast populations were significantly larger than the normal lymphocytes visible as the major population in the *top left panel*. Fluorescence-activated cell-sorting analysis demonstrated that T/A-1, which developed at 6.5 months, was $TCR\beta^+CD4^+CD8^-$ and negative for other antigens evaluated, a T-lineage ALL (T-ALL). T/A-2

[5] L. Cheng, unpublished observations.

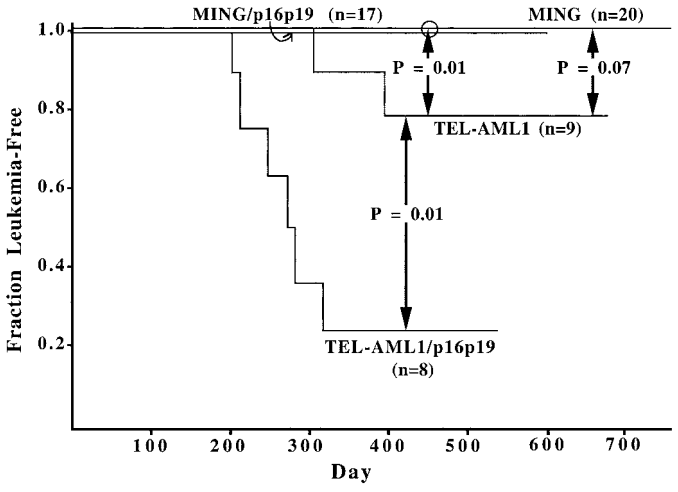

Fig. 2. TEL-AML1 accelerates formation of acute leukemia, alone or in cooperation with loss of $p16^{INK4a}p19^{ARF}$. Wild-type or $p16^{INK4a}/p19^{ARF}(-/-)$ marrow cells were transduced with the MING or TEL-AML1 vectors and then transplanted by tail vein injection into irradiated, syngeneic wild-type recipients. Shown are Kaplan-Meier estimates of the proportion of mice in each group remaining leukemia free at the indicated times. The MING and MING/p16p19 cohorts had been reported previously as control groups for experiments assessing leukemogenesis by CBFβ-SMMHC (14). The number of mice in each group (*n*) is indicated. The *curved arrow* indicates that the MING/p16p19 cohort is plotted just below the MING cohort; no leukemias developed in these groups. The *P*s shown were obtained by comparing the indicated groups using the log-rank test. The *small circle* indicates that the incidence in the MING/p16p19 and MING groups considered together was compared with that in the TEL-AML1 group in one analysis.

was only $B220^+$, a B-ALL. T/A-2 was transplanted into a secondary, nonirradiated recipient, and overt leukemia developed 3 months later. TEL-AML1 expression was detected by Western blotting in marrow cells isolated from the secondary recipient (Fig. 4, *top panel*). A small amount of expression was also detected in bone marrow cells from a TEL-AML1 mouse that died on day 490 without leukemia, but expression in the B-ALL was significantly higher. Endogenous TEL was not detected (data not shown). Fast Green staining (Fig. 4, *bottom panel*) indicates that the B-ALL sample was underloaded severalfold relative to the other samples.

Loss of p16p19 Cooperates with TEL-AML1 to Induce Acute Leukemia. The MING or TEL-AML1 vectors were also introduced into marrow cells isolated from C57BL/6 mice lacking the overlapping $p16^{INK4a}$ and $p19^{ARF}$ genes, and the transduced cells were then injected into irradiated, wild-type, syngeneic recipients. $p16^{INK4a}$ is a cyclin-dependent kinase inhibitor. Loss of murine $p19^{ARF}$ or human $p14^{ARF}$ is expected to increase Mdm2 and thereby decrease p53 activity. Deletion of the overlapping $p16^{INK4a}p19^{ARF}$ genes occurs in approximately 20% of B-ALL and 60% of T-ALL (15). The $p16^{INK4a}$ gene is also specifically inactivated by promoter methylation in 80% of high-grade lymphomas, but not in ALL cases (16). Leukemia incidence for these two cohorts is shown in Fig. 2. Leukemia developed in 6 of 8 TEL-AML1/p16p19 mice and in 0 of 17 of the MING/p16p19 cohort ($P < 0.0001$). Compared with the TEL-AML1 group, the TEL-AML1/p16p19 mice developed leukemia more rapidly and at a higher incidence (log-rank $P = 0.01$). The HR between these groups is also significant (HR = 6.3; 95% confidence interval, 1.3–32; $P = 0.03$). One of the TEL-AML1/p16p19 leukemias was

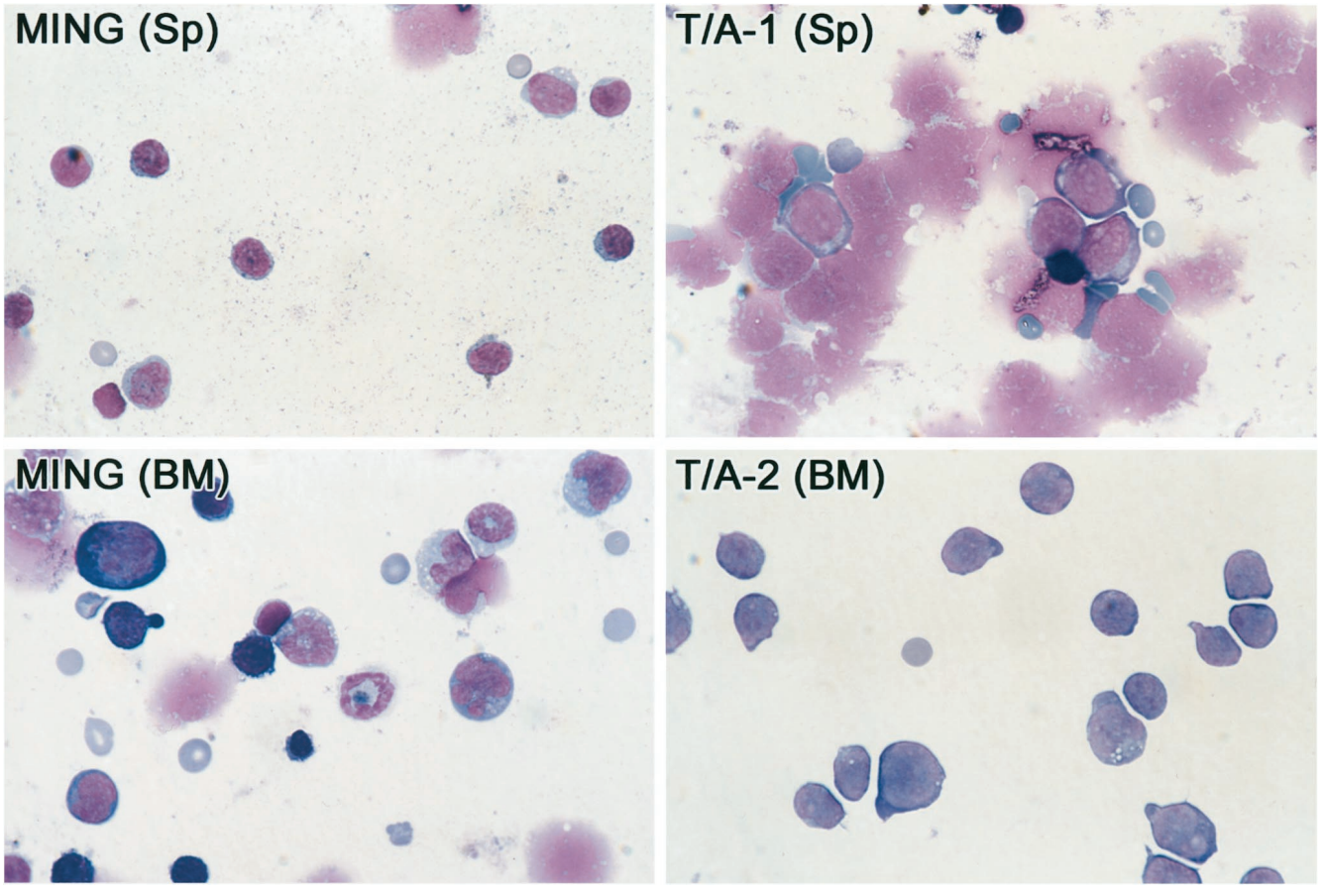

Fig. 3. Morphology of two TEL-AML1 leukemias. Spleen or marrow cells were cytospun and subjected to Wright's-Giemsa staining. *Top left panel*, normal spleen cells from a mouse transplanted with marrow transduced with the MING vector. *Top right panel*, spleen cells from a TEL-AML1 mouse (T/A-1) that developed T-ALL. *Bottom left panel*, normal bone marrow cells from a MING mouse. *Bottom right panel*, marrow cells from a TEL-AML1 mouse (T/A-2) that developed B-ALL.

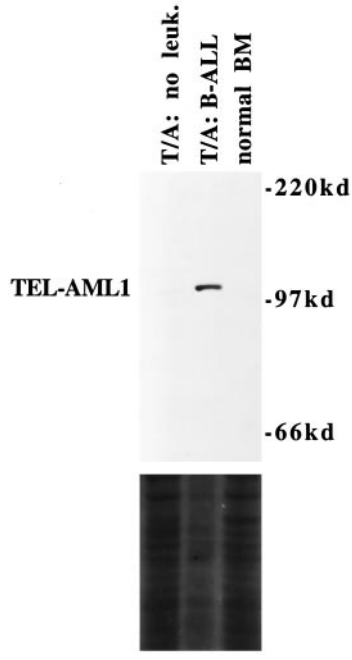

Fig. 4. TEL-AML1 is expressed in a murine B-ALL. Total cellular protein from 5×10^6 bone marrow cells from three mice were subjected to Western blotting using TEL antiserum (*top panel*). The protein samples were derived from a TEL-AML1 mouse that did not develop leukemia (*T/A: no leuk.*), from TEL-AML1 mouse T/A-2, which developed B-ALL (*T/A: B-ALL*), and from a normal C57BL/6 mouse (*normal BM*). The positions of protein molecular markers are also indicated. Before staining with antibody, the blot was stained with Fast Green to assess total protein loaded in each lane (*bottom panel*).

likely a T-ALL because the thymus was grossly enlarged, one was a Mac-1^+Gr-1^+ AML, and the phenotypes of the four other leukemias in this group could not be determined because the mice were found dead between observation periods with grossly enlarged but necrotic spleens (190, 300, 440, and 580 mg; normal spleen, 80 mg). Mice with T-ALL invariably have enlarged thymuses, whereas this organ was not enlarged in these four mice.

Discussion

Using two different approaches, expression in wild-type or in $p16^{INK4a}p19^{ARF}(-/-)$ marrow cells, we have provided evidence indicating that TEL-AML1 contributes to leukemic transformation. This finding is of particular significance because loss of TEL expression or mutation in one or both AML1 alleles occurs in leukemias lacking TEL-AML1. Whereas our results indicate that TEL-AML1 participates in transformation, we cannot rule out the possibility that t(12;21) also contributes to the formation of B-ALL by disrupting a *TEL* and an *AML1* locus. It is possible that the murine leukemias that developed in this study harbor alterations in the endogenous *TEL* or *AML1* genes. TEL-AML1 but not endogenous TEL was detected in the one murine TEL-AML1 leukemia analyzed, mimicking human leukemias with t(12;21) (6–8). In future experiments, we will analyze additional TEL-AML1 leukemias for genetic alterations that may cooperate in leukemogenesis. It will also be of interest to determine whether leukemias obtained are monoclonal, especially when TEL-AML1 is combined with loss of the *p16p19* genes.

Our findings also indicate that TEL-AML1 and loss of the $p16^{INK4a}/p19^{ARF}$ genes functionally cooperate during leukemogenesis. Deletion of the $p16^{INK4a}/p19^{ARF}$ genes in pediatric B-ALL is common (15). Inhibition of CBF blocks myeloid and lymphoid differentiation in normal marrow and in 32D cl3 myeloblastic cells and slows apoptosis of Ba/F3 cells in response to DNA damage (10, 17, 18). On the other hand, inhibition of CBF also slows G_1 progression in myeloid or lymphoid cells (10). Exogenous cyclin-dependent kinase 4 or cyclin D2 overcomes delayed cell cycle progression of 32D cl3 and Ba/F3 cells resulting from blockade of CBF transactivation (10), and c-Myc acts similarly (19). These findings suggest that hematopoietic cells expressing CBF oncoproteins might be blocked at an early stage of differentiation but are incapable of proliferation. We previously demonstrated that loss of the *p16p19* genes or coexpression of E7 cooperates with CBFβ-SMMHC to induce acute leukemia (14), and we now find that deletion of the *p16p19* genes similarly cooperates with TEL-AML1. This result supports our proposal that mutations that accelerate G_1 potentiate the ability of CBF oncoproteins or AML1 mutations to block differentiation or apoptosis (5, 10, 14). Deletion of p16p19 might allow lymphoid stem cells to proliferate despite the presence of TEL-AML1 and might even enable increased levels of TEL-AML1, allowing more effective blockade of differentiation or apoptosis. In the future, transduction of marrow isolated from recently developed mice lacking either $p16^{INK4a}$ or $p19^{ARF}$, but not both, will allow assessment of whether TEL-AML1 cooperated with alteration of the Rb or p53 pathways in our TEL-AML1/p16p19 cohort.

TEL-AML1 contributed to the formation of a B-ALL but also to other leukemia phenotypes in this study. Perhaps this diversity reflects the expression profile of the murine stem cell virus retroviral promoter together with a difference between murine and human progenitor/stem cells with regard to lineage-specific susceptibility to additional mutations capable of cooperating with TEL-AML. We expect that future studies in which TEL-AML1 is expressed specifically in immature B cells will yield a more restricted pattern of leukemia phenotypes. Nevertheless, our findings support the conclusion that TEL-AML1 contributes to the formation of human B-ALLs harboring t(12;21).

Acknowledgments

We thank D. Bodine for advice regarding marrow transduction, W. Wang for technical assistance, and N. E. Sharpless and R. A. DePinho for the $p16^{INK4a}/p19^{ARF}(-/-)$ mice.

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