T-Cell-directed TAL-1 Expression Induces T-Cell Malignancies in Transgenic Mice

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Abstract

The TAL-1 gene specifies for a basic domain-helix-loop-helix protein, which is involved in the control of normal hematopoiesis. In human pathology, the TAL-1 gene product is expressed in a high percentage of T-cell acute lymphoblastic leukemias in the pediatric age range; however, it has not been established whether the expression has a causal role in oncogenesis. In this report, we describe the phenotype of mice transgenic lines obtained by inducing tal-1 protein expression in lymphoid tissues using the LCK promoter.

The survival rate of tal-1 transgenic animals was much lower as compared with control mice. Histopathological analysis revealed lymphomas of T-cell type, often comprising a minor B-cell component. Some mice showed marked splenic lymphocyte depletion. Primary lymphocyte cultures showed partial independence from exogenous growth stimuli and of T-cell type, often comprising a minor B-cell component. Some mice showed marked splenic lymphocyte depletion. Primary lymphocyte cultures showed partial independence from exogenous growth stimuli and increased resistance to low-serum apoptosis. To further unravel the tal-1 oncogenic potential, a strain of tal-1 transgenic mice was crossbred with p53-/- mice; the survival rate in these animals was reduced by more than one-half when compared with that of tal-1 mice, and histopathological analysis revealed exclusively T-cell lymphomas.

Introduction

Transcription factors are often involved in tumorigenesis (reviewed in Refs. 1 and 2). The TAL-1 gene (also known as SCL or TCL-5), identified by analysis of t(1;14) (p32;q11) translocations in human T-ALL, codes for the tal-1 protein belonging to the family of BHLH domain transcription factors (3-5), which are involved in the control of cell growth and differentiation (6). The TAL-1 gene, although silent in normal adult T lymphocytes (7, 8), is constitutively activated in a high percentage of T-ALL by various mechanisms; these include chromosomal translocation, which has been reported in only 3% of T-ALL, or cis events, i.e., specific interstitial chromosomal deletions affecting the 5′ noncoding region of TAL-1 leading to transcriptional activation of the gene in approximately 25% of T-ALL (9, 10). Furthermore, a recent study has shown that TAL-1 is abnormally expressed in the leukemic cells of most patients with T-ALL; a trans-acting event may explain the active TAL-1 transcription in T-ALL cases without apparent TAL-1 gene alteration (11). Altogether, TAL-1 seems to be abnormally expressed in >60% of T-ALL cases (11).

Materials and Methods

Transgenic Mice

C57BL/6 mice and p53 homozygous recombinant mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and Taconics/GenPharm International (Mountain View, CA), respectively. All animals were bred and maintained in a pathogen-free environment at Jefferson Medical College, Thomas Jefferson University. All animal protocols were approved by the Institutional Animal Care and Use Committee, and NIH guidelines for animal care were followed throughout.

The construct LCK-TAL-1 was generated by insertion of a 1.2-kb fragment containing the coding region and a 3′ untranslated region of human TAL-1 cDNA (5). The TAL-1 cDNA was rendered BamHI-compatible on both ends and inserted into the unique BamHI cloning site downstream to the LCK promoter (26). The 3′ untranslated portion of this construct encompasses introns, exons, and polyA addition sites from the human growth hormone gene. The 6.8-kb LCK-TAL-1 fragment digested by SacII was used for microinjection.

Transgenic animals were generated according to standard procedures (27). Transgenic progeny were identified by Southern blotting of SacI-digested tail DNA using the entire LCK-TAL-1 fragment as a probe.

RT-PCR mRNA Analysis

Methodology for semiquantitative RT-PCR analysis has been reported previously (14). Briefly, total RNA extracted by the guanidinium isothiocya-
TAL-1 was inserted at the BamHI site downstream to the 3.2-kb LCK proximal promoter hormone constituting the 3' untranslated region.

To evaluate the expression of TAL-1, an aliquot of RT-RNA (~20 ng) of each sample and a mock reaction (negative controls) were amplified to exclude the presence of contaminant DNA.

The following 5', 3' primers and probe were used for TAL-1: 5' primer, 5'-ATGGTGCAGCTGAGTCCTCC-3'; 3' primer, 5'-TCTCATCCTGTGAGCCTC-3'; and probe, 5'-GATGCTCTTCCCTTTAGTTACCCACCA-AACAT-3'. The amplification procedure included denaturation at 95°C for 30 s, annealing at 54°C, and extension at 72°C for 45 s during 40 PCR cycles.

The expression of TAL-1 was amplified within the linear range by 40 PCR cycles (i.e., the cycle number allowed a linear cDNA dose response).

Fig. 1. LCK-TAL-1 transgene construct. A 1.2-kb cDNA (closed arrow) to human TAL-1 was inserted at the BamHI site downstream to the 3.2-kb LCK proximal promoter (open box). Introns, exons, and poly(A) addition sites (hatched boxes) of human growth hormone constitute the 3' untranslated region.

Each sample was electrophoresed in a 2% agarose gel, transferred to a nitrocellulose filter, and hybridized with an internal oligomer as probe. An aliquot of RNA (~20 ng) of each sample and a mock reaction (negative controls) were amplified to exclude the presence of contaminant DNA.

The following 5', 3' primers and probe were used for TAL-1: 5' primer, 5'-ATGGTGCAGCTGAGTCCTCC-3'; 3' primer, 5'-TCTCATCCTGTGAGCCTC-3'; and probe, 5'-GATGCTCTTCCCTTTAGTTACCCACCA-AACAT-3'. The amplification procedure included denaturation at 95°C for 30 s, annealing at 54°C, and extension at 72°C for 45 s during 40 PCR cycles, i.e., within the range of linear amplification. RT-RNA from the K562 cell line was used as an internal positive control in each PCR.

**Nuclear and Total Cell Extracts Preparation**

Nuclear extracts from the Jurkat cell line were obtained as described by Condorelli et al. (14).

For EMSA, total cell extracts from spleen and thymus of adult mice were prepared from single-cell suspensions lysed in a buffer containing 20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 2.5 mM DTT, 25% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml each of leupeptin and aprotinin. Cells were kept on ice for 20 min, frozen at ~70°C, and thawed once. The suspension was vigorously mixed and centrifuged for 10 min at 13,600 × g in a microcentrifuge.

For Western blot protein assay, single-cell suspensions of spleen and

**Fig. 2** A, semiquantitative RT-PCR of tal-1 mRNA from spleen (Lanes 1, 3, 5, 7, and 9) and thymus (Lanes 2, 4, 6, 8, and 10) of nontransgenic (wt, wild type; Lanes 1 to 4) and transgenic Lck-tal-1 (Lanes 5–10) mice (top). Three Lck-tal-1 transgenic lines were evaluated: line 1849 (Lanes 3 and 7), line 1856 (Lanes 4 and 8), and line 1859 (Lanes 9 and 10). GAPDH gene was used for normalization (bottom). The controls for semiquantitative RT-PCR analysis are detailed in "Materials and Methods." The K562 erythroleukemia cell line was used as an internal positive control. Representative results from three independent experiments are shown. The TAL-1 filter was autoradiographed for 10 h, and the GAPDH filter was autoradiographed for 3 h. B, tal-1 Western blot analysis of proteins from spleen (Lanes 1–4) and thymus (Lanes 5–8) of nontransgenic mice (Lanes 1 to 4) and transgenic lines 1849 (Lanes 3 and 7) and 1856 (Lanes 4 and 8). Analysis was performed with an equivalent amount (20 μg) of total cell extract from spleen and thymus of transgenic and nontransgenic mice. Nuclear extract from the Jurkat cell line (20 μg) was used as a positive control. The molecular weight marker is indicated in thousands (kd). Representative results from two independent experiments are shown. Filters were developed by ECL detection and exposed to X-ray film for 20 min (spleen, Lanes 1–4) and 2 min (thymus, Lanes 5–8).

To evaluate the expression of TAL-1, an aliquot of RT-RNA (~20 ng) was amplified within the linear range by 40 PCR cycles (i.e., the cycle number allowed a linear cDNA dose response).

**Fig. 3** A, EMSA of TAL-1/E2A complexes in total cell extract of spleen (S, Lanes 15–35) and thymus (T, Lanes 17–37) of nontransgenic (Lanes 15 and 17) and transgenic lines 1849 (Lanes 25 and 27) and 1856 (Lanes 35 and 37), as compared with control Jurkat cells. The canonical tal-1/E2A heterodimer is marked; tal-1/E protein complexes are indicated by asterisks. B, EMSA of tal-1/E2A heterodimers (asterisks) in thymus total cell extract of nontransgenic mice line 1849 (Lanes 3, 4, and 5); nontransgenic mice extract is shown in Lane 2, whereas Jurkat leukemia T cell extracts were used as positive control for the canonical tal-1/E2A heterodimer (Lane 1). The protein complexes in transgenic thymocytes were supershifted (arrow) by incubation with anti-tal-1 serum and partially abrogated with anti-E2A serum (Lanes 4 and 5, respectively), as compared to the corresponding preimmune serum (Lane 3).
Thymus and spleen cells from wild-type and Lck-Tal-1 transgenic mice were analyzed for FACS and cell proliferation. Western blot analysis was performed with Tal-l rabbit antiserum. Electrophoresis was conducted at 180 V for 2–3 h at 4°C. In some binding reactions, the extract was preincubated for 15 mm at room temperature with 1 μg of TAL-1 rabbit antiserum. The E2A stranded oligonucleotide probe contained the preferred sequence for DNA binding. Western blot and EMSA were performed as described by Condorelli et al. (14). EMSA was performed as reported.

**Histological and Immunohistochemical Analyses**

Histological and histochemical analyses were performed on lymphoid tissues fixed in 10% buffered formalin and processed for paraffin embedding by Pathology Associates International (Frederick, MD). Serial sections were stained with H&E using standard procedures. Immunohistochemical analyses were performed on fixed tissues as described by Bindl et al. (28). Immunoperoxidase-conjugated anti-mouse CD3 and CD45R mouse monoclonal antibodies were purchased from PharMingen.

**Results**

**Transgenic Mice.** We developed a transgenic mouse model to assess the effects of Tal-1 expression in lymphoid tissues. A transgenic construct was generated by inserting a human TAL-1 cDNA downstream of the LCK promoter (Refs. 26 and 29; Fig. 1). Seven founders bearing LCK-TAL-1 were identified by Southern blot analysis (data not shown), and corresponding lines were established. Each line was examined by Western blot (see below) and RT-PCR (results not shown). Three lines (1849, 1856, and 1859) were further characterized, two of which were extensively studied (1849 and 1856). Tal-1 mRNA was present in the spleen and thymus of transgenic mice but not in wild-type animals, as assessed by RT-PCR and Western blot analysis (Fig. 2). Transgenic mice express a 42-kDa Tal-1 protein.

**Table 1** Membrane antigen phenotype of thymus and spleen cells from wild-type and Lck-Tal-1 transgenic mice (lines 1849 and 1856)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Strain/Line</th>
<th>Cell no. × 10⁶</th>
<th>CD3⁺ (%)</th>
<th>CD4⁺/CD8⁺ (%)</th>
<th>CD4⁺ (%)</th>
<th>CD8⁺ (%)</th>
<th>CD45R⁺ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>C57 Black</td>
<td>22.3 ± 3.6⁶</td>
<td>25.0 ± 3.2</td>
<td>92.8 ± 1.1</td>
<td>96.75 ± 1.03</td>
<td>96.0 ± 0.40</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1849</td>
<td>20.0 ± 3.1</td>
<td>34.3 ± 4.2</td>
<td>87.5 ± 1.4</td>
<td>97.0 ± 2.0</td>
<td>96.0 ± 1.0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1856</td>
<td>31.7 ± 4.0</td>
<td>26.5 ± 2.8</td>
<td>83.4 ± 3.4</td>
<td>92.83 ± 2.27</td>
<td>87.0 ± 8.05</td>
<td>ND</td>
</tr>
<tr>
<td>Spleen</td>
<td>C57 Black</td>
<td>68.8 ± 13.7</td>
<td>22.1 ± 2.0</td>
<td>ND</td>
<td>15.4 ± 1.3</td>
<td>12.6 ± 1.4</td>
<td>50.3 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>1849</td>
<td>58.0 ± 14.4</td>
<td>32.0 ± 1.8⁸</td>
<td>ND</td>
<td>17.6 ± 1.3</td>
<td>14.8 ± 1.2</td>
<td>37.6 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>1856</td>
<td>55.8 ± 11.3</td>
<td>39.1 ± 2.0⁰</td>
<td>ND</td>
<td>22.9 ± 1.0⁰</td>
<td>17.7 ± 1.2</td>
<td>41.3 ± 3.1</td>
</tr>
</tbody>
</table>

* Mean ± SE values.
* P < 0.05 when compared with control values by ANOVA.
* P < 0.01 when compared with control values by ANOVA.

**Table 2** Proliferation of T and B cells from thymus and spleen of control and Lck-tal-1 transgenic mice after ConA or IL-2 and LPS stimulus, respectively

<table>
<thead>
<tr>
<th>Organ</th>
<th>Strain/Line</th>
<th>ConA</th>
<th>IL-2</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>C57 Black</td>
<td>1.80 ± 0.54</td>
<td>1.31 ± 0.22</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1849</td>
<td>1.77 ± 0.89</td>
<td>1.13 ± 0.21</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1856</td>
<td>1.14 ± 0.04</td>
<td>1.04 ± 0.07</td>
<td>ND</td>
</tr>
<tr>
<td>Spleen</td>
<td>C57 Black</td>
<td>254.8 ± 52.0</td>
<td>42.8 ± 0.34</td>
<td>35.89 ± 9.61</td>
</tr>
<tr>
<td></td>
<td>1849</td>
<td>76.8 ± 22.3⁸</td>
<td>22.2 ± 0.57⁹</td>
<td>16.43 ± 5.29</td>
</tr>
<tr>
<td></td>
<td>1856</td>
<td>91.7 ± 24.1³</td>
<td>12.6 ± 0.07⁰</td>
<td>22.88 ± 5.39</td>
</tr>
</tbody>
</table>

* P < 0.05 when compared to control values by ANOVA.
* P < 0.01 when compared to control values by ANOVA.

**Table 3** Histopathology analysis of tal-1 transgenic mice

<table>
<thead>
<tr>
<th>Transgenic line</th>
<th>1849</th>
<th>1856</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice analyzed</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Incidence of lymphoid hyperplasia</td>
<td>33.3%</td>
<td>30%</td>
</tr>
<tr>
<td>Incidence of lymphomas</td>
<td>66.7%</td>
<td>60%</td>
</tr>
<tr>
<td>Cell type of lymphomas</td>
<td>T (+ minor B component)</td>
<td>T (+ minor B component)</td>
</tr>
<tr>
<td>(in 80% of tumors)</td>
<td>(in 80% of tumors)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 4.** Survival curve of transgenic and control mice: □, wild-type mice; ○, transgenic mice line 1849; ■, line 1856.
T LYMPHOMAS IN TRANSGENIC tal-1 MICE

protein in the thymus and spleen (Fig. 2B); the expression in the thymus is higher than in the spleen, consistent with the difference in T-cell number in these organs. In fact, LCK is expressed at higher level in immature than mature T cells (30, 31).

Ectopic tal-1 Forms a DNA Binding Complex with E2A Protein in Lck-tal-1 Mice. It has been shown both in vivo and in vitro that tal-1 has the ability to bind DNA only when it associates with E2A protein (14, 20, 21). Furthermore, we observed that in diverse cell lines, ectopic expression of the TAL-1 gene product exerts both proliferative and antidifferentiative effects, which depend on both the basic and HLH domains (25) necessary for binding to DNA and E2A protein, respectively.

To investigate whether tal-1 expressed in T cells was able to form a complex with E2A protein and bind efficiently to its DNA consensus sequence, EMSA was performed with total extracts obtained from thymus or spleen of transgenic mice (Fig. 3). In the spleen, a band closely comigrates with the canonical tal-1/E2A protein complex in Jurkat cells; in thymus, this band was more clearly visible, together with an additional faster migrating band, whereas no protein complex was detectable in control mice (Fig. 3A). To confirm whether the components of this complex included tal-1 and E2A proteins, nuclear extracts were preincubated with anti-tal-1 (spleen and thymus) or anti-E2A serum (thymus), and EMSA was performed (Fig. 3B and results not shown). In both cases, the bands corresponding to the tal-1/E2A complex were either decreased or supershifted, thus showing the presence of these proteins in the nuclear complex. The intensity of the radioactive bands was higher in the thymus than in the spleen, thus in agreement with Western blot results (Fig. 3A).

Effects of tal-1 Expression on T-Cell Maturation and Function. FACS analysis was performed in 3–7-week-old mice to investigate whether tal-1 expression alters T- or B-cell development, as determined by the percentage of CD3\(^+\), CD4\(^+\)/CD8\(^+\), CD4\(^+\), CD8\(^+\), and CD45R\(^+\) cells (Table 1). In transgenic thymus, we observed a borderline, nonsignificant increase or decrease in the percentage of CD3\(^+\) or CD4\(^+\)/CD8\(^+\) cells, respectively, as compared with control mice. In the transgenic spleen: (a) the percentage of CD3\(^+\) and CD4\(^+\) cells was increased (although nonsignificantly for the latter parameter in the 1849 line), as compared with control mice; and (b) the number of CD8\(^+\) or CD45R\(^+\) cells showed a borderline but not significant increase or decrease, respectively. The morphological profile of the FACS scatter did not show gross abnormality when transgenic animals were compared with controls (data not shown).

To assess whether TAL-1 interferes with the proliferation of T cells in thymus or spleen, primary cultures of these cells were incubated with appropriate amounts of IL-2 and ConA, and mitotic response was evaluated on the basis of the \[^{3}H\]thymidine incorporation (Table 2).

Fig. 5. Representative lymphoblastic lymphoma in transgenic mice (strain 1849). A. H&E staining. Immunohistochemical staining of lymphoma localized in the liver with a peroxidase conjugated anti-CD3 (B–C) or anti-CD45R antibody (D).

Fig. 6. Low FCS starvation culture of thymic cells from wild-type (○) or Lck-tal-1 (●; line 1849) mice. Mean values are presented; bars, SE.
The effects of these stimuli were sharply decreased in the splenic cells of transgenic mice, as compared with control animals; the apparent decrease observed for thymic cells was not significant. Transgenic B cells stimulated by LPS showed a borderline nonsignificant decrease of incorporation values, when compared with controls (Table 2).

Survival of Lck-tal-1 Mice. To determine the potential oncogenic effects of tal-1 expression in T cells, we grouped 30 animals for each strain (1849 and 1856) into two cohorts, which were followed for 70 weeks, together with a cohort of control, age-matched mice derived from control littermates (Fig. 4). The differences in survival were striking; most of the transgenic animals died by the end of the observation period, while control mice were alive.

Histopathological analysis was performed on moribund or dead mice (Table 3; Fig. 5). The presence of lymphoid hyperplasia and/or frank lymphoma was evaluated. In both lines, the number of lymphoid tumors was high and were combined with prevalent lymphoid hyperplasia. Most lymphoid tumors expressed the CD3 antigen, as evaluated by histochemical analysis; the type of tumors varied from lymphocytic lymphoma to lymphoblastic lymphoma. Particularly, 80% of tumors expressed a mixed T and B phenotype, as evaluated by the positivity for both CD3 and CD45R (CD45R⁺ cells ranged from <5 to 50% in some tumors). Approximately 20% of tumors were of T origin only. Whether B-cell proliferation is determined by a lack of T-cell function or by tal-1 expression in B cells has not been established. Our results show a potent effect of tal-1 on T-cell tumorigenesis in transgenic mice.

Interestingly, thymic cells from transgenic mice, cultured in low FCS concentration without exogenous growth factors or mitogens, showed a significantly enhanced survival, as compared with wild-type cells (Fig. 6). Standard FACS analysis following propidium iodide staining showed that the number of apoptotic cells was consistently lower in transgenic than wild-type cell cultures (data not shown).

Effects of p53 Heterozygosity on Survival of Lck-tal-1 Mice. Many potential oncogenes fully express their proliferative potential only when the cell cycle checkpoints are altered (32, 33). To definitively establish whether TAL-1 is an oncogene, it was necessary to show that TAL-1 increases its oncogenic potential in these altered conditions, e.g., in the presence of loss of a p53 allele. Thus, p53⁻⁻⁻/TAL-1⁻⁻ mice were cross-bred with tal-1⁻⁻⁻ mice from strain 1849. A number of F₁ mice was obtained with the genotype tal-1⁻⁻⁻/p53⁻⁻⁻. A cohort of 30 animals was established and compared with two other cohorts with the same number of animals bearing the genotype p53⁻⁻⁻ or tal-1⁻⁻⁻, which had been obtained by crossing the homozygous tal-1⁻⁻⁻ or p55⁻⁻⁻ mice with wild-type animals. The mice were followed for approximately 70 weeks; most of tal-1⁻⁻⁻/p53⁻⁻⁻ animals were dead by that age, thus in striking contrast with the other two cohorts of mice (Fig. 7). Histopathological analysis, conducted on 22 tal-1⁻⁻⁻/p53⁻⁻⁻ mice, showed lymphomas of T origin in all cases (Table 4; Fig. 8).

Discussion

Data presented here show that TAL-1 is highly oncogenic when ectopically expressed in lymphoid cells of transgenic mice. In fact, all animals died of lymphoid malignancies within 1 year. Most of the tumors were of T lineage, presumably reflecting the selective expression of LCK in T cells (26). This contrasts with the finding that CD2-tal-1 mice were not affected by lymphoid malignancy (24). The discrepancy might lie in the different promoter used in our experiments, which may cause a different timing and/or level of tal-1 expression.

tal-1 protein associates in vivo and in vitro with another nuclear factor in addition to E2A protein, Lmo-2 (34, 35). Similarly to TAL-1, the LMO-2 gene is rearranged and overexpressed in T lymphoid tumors with t(11;14) (p13;ql 1) (36–38), whereas it is not expressed in normal T cells (37). In normal tissue development, Lmo-2 is expressed together with TAL-1, especially in erythroid cells (39). Furthermore, selective expression of Lmo-2 in T cells driven by the CD2
promoter cassette induces lymphomas (40). It has, therefore, been suggested that the physical interaction between tal-1 and Lmo-2, which may be functionally relevant in normal hematopoiesis (34, 35), also takes place in tumor cells and is causal in the genesis of tumors; in fact, although transgenic CD2-tal-1 mice do not develop tumors, cross-breeding of these animals with CD2-Lmo-2 mice determine a shortening of survival due to a decrease of the tumor-free life interval (41). In this experimental setting, therefore, tal-1 serves as a tumor promoter, without having per se an oncogenic capacity. Our data, in contrast, show that tal-1 alone can generate T-cell tumors, without the inducing action of other oncogenes.

The oncogenic effect of TAL-1 is strikingly increased when its ectopic expression takes place in mice with a p53+/− background. In fact, in tal-1/−/p53+/− mice, the mortality rate was remarkably enhanced as compared with that of p53+/− mice. Furthermore, all p53+/−/tal-1/−/ mice died of disseminated T-cell lymphoma. The effect of p53 absence on murine cancerogenesis is well established (42). Regarding p53 heterozygosity, it has been shown that the survival rate in these animals, approximately 50% after 80 weeks of age, is much higher than in control p53+/− mice (43). In our experiments, all p53 heterozygous mice bearing a Lck-tal-1 transgene died by 40 weeks of age, thus shortening by more than one-half the survival, as compared with the homozygous parental strain of tal-1 mice. Remarkably, a more extended survival was observed in the control p53+/− mice. Therefore, tal-1 accelerates tumorigenesis in this condition. A similar acceleration of tumorigenesis, although relatively reduced, has been reported when p53+/− mice were crossed with mice expressing the Wnt-1 oncogene selectively in the mammalian gland (44). Furthermore, it has been shown that c-myc and raf oncogenes are able to immortalize hematopoietic cells in the absence of p53 (32). Our group observed that TAL-1 gene transfer in the 32D murine hematopoietic progenitor cell line bearing a temperature-sensitive p53 gene causes apoptosis at permissive temperatures but favors cell proliferation upon p53 inactivation (25). Therefore, it is apparent that TAL-1 acts on immortalization similar to c-myc or other oncogenes.

Furthermore, our results show that tal-1 expression in T-cell culture has an anti-apoptotic effect. This finding is in line with other observations, which unraveled the anti-apoptotic capacity of tal-1 in Jurkat (45) and 32D (25) cell lines and might also explain the absence of hematopoietic lineage differentiation in tal-1−/− embryonic stem cells (17).

Our results also suggest that E2A plays a role in T-cell homeostasis. It has been shown by homologous recombination in mice that the E2A gene is not essential for T-cell development; in fact, while B-cell development was blocked, no effect was detected in T cells (46, 47). We show that in transgenic T cells, tal-1 is part of a DNA binding complex that contains a protein supershifted by anti-E2A antisera, probably an E2A-related protein. It may be hence speculated that a gene within the E2A family is expressed and exerts a regulatory role in the proliferation-differentiation of T cells.

In conclusion, our data indicate that TAL-1 is per se an oncogene, which has a role in human T-cell leukemogenesis, rather than representing in T-ALL a gene with no proliferative potential (24) or a tumor promoter (41).

Acknowledgments

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12. Porcher, C., Swat, W., Rockwell, K., Fujiwara, Y., Alt, F. W., and Orkin, S. H. The E2A helix-loop-helix DNA binding domain is not essential for T-cell development; in fact, while B-cell development is blocked, no effect is detected in T cells (46, 47). We show that in transgenic T cells, tal-1 is part of a DNA binding complex that contains a protein supershifted by anti-E2A antisera, probably an E2A-related protein. It may be hence speculated that a gene within the E2A family is expressed and exerts a regulatory role in the proliferation-differentiation of T cells.

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