Transcriptional Activity of TAL1 in T Cell Acute Lymphoblastic Leukemia (T-ALL) Requires RBTN1 or -2 and Induces TALLA1, a Highly Specific Tumor Marker of T-ALL*

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TAL1, which is frequently activated in T cell acute lymphoblastic leukemia (T-ALL), encodes lineage-specific basic helix-loop-helix (bHLH) proteins that bind specifically to E-box DNA motif upon dimerization with ubiquitous basic helix-loop-helix proteins E47 or E12. RBTN1 and RBTN2, also frequently activated in T-ALL, encode proteins only with tandem cysteine-rich LIM domains. We found that aberrant expression of TAL1 detected in 11 out of 14 T-ALL cell lines was invariably accompanied by that of either RBTN1 or RBTN2. Forced expression of TAL1 together with RBTN1 or RBTN2, but not TAL1 alone, strongly induced artificial reporter genes in a TAL1/RBTN-negative T-ALL cell line, HPB-ALL. Such collaborative transcriptional activity of TAL1 and RBTN was not, however, observed in non-T cell lines, suggesting further involvement of some T cell-specific cofactors. In this context, we carried out preliminary evaluation of a potential role of the T cell-specific GATA-binding protein, GATA3, in the transcriptional activity of TAL1 and RBTN. We also showed that expression of TAL1 and RBTN1 in HPB-ALL strongly induced TALLA1, a highly specific T-ALL marker whose positivity correlated 100% with ectopic expression of TAL1 among various T-ALL cell lines. Collectively, ectopic TAL1 and RBTN1 or -2, together with some endogenous T cell-specific cofactors like GATA3, constitute a highly collaborative set of transcription factors whose aberrant activity in T cells may lead to leukemogenesis by modulating expression of downstream genes such as TALLA1.

TAL1, also called SCL or TCL5, is a gene whose aberrant activation in the T cell lineage by recurrent chromosomal translocations, t(1;14)(p32;q11) and t(1;7)(p32;q35), precise 90-kilobase pair interstitial chromosomal deletions (tal4), and yet other undefined mechanisms is implicated as the major pathway for the development of T cell acute lymphoblastic leukemia (T-ALL).1 (1–3). TAL1 is normally expressed in erythroid, mastocytic, and megakaryocytic lineages of the hematopoietic system but not in T cells (4, 5). TAL1 encodes at least two polypeptides, full-length 42-kDa TAL1α (amino acid residues 1–331) and 22-kDa N-terminally truncated polypeptide TAL1β (amino acid residues 176–331) (6), both containing the basic helix-loop-helix (bHLH) motif, a DNA-binding and protein dimerization domain found in a number of transcription factors. TAL1 proteins, having no intrinsic DNA binding activity, dimerize with the ubiquitously expressed E2A gene products, E47 and E12 (7, 8), and the heterodimers bind to E-box elements (CANNTG) with a preferred sequence of AACAGAT-GGT (9). By using an artificial reporter gene containing multiple copies of the optimal TAL1/E2A binding sequence, transcriptional activity of the TAL1/E47 heterodimer was examined in transiently transfected murine C3H/10T1/2 fibroblasts (10). While the E47 homodimer strongly induced the reporter gene, the TAL1/E47 heterodimer was much less active, suggesting a negative regulatory role of TAL1 (10). So far, nothing is known about genes regulated by TAL1. Transcriptional activity of TAL1 in T cells has not been examined either. Transgenic mice expressing 42-kDa TAL1 in the T cell lineage did not develop tumors nor did mice irradiated and reconstituted with bone marrow cells infected with a recombinant retrovirus encoding TAL1 (11, 12). Such findings may suggest that additional cofactors are necessary for TAL1 to be oncogenic in T cells (13). RBTN1/TTG1 and RBTN2/TTG2 are genes that also were originally identified from recurrent chromosomal translocations in T-ALL, t(11;14)(p15;q11) and t(11;14)(p13;q11), respectively (14–16). They encode highly related proteins consisting of only two cysteine-rich zinc finger-like LIM domains, which are considered to mediate protein-protein interactions (17). RBTN1 and RBTN2 are mainly expressed in the brain and erythroid lineage cells, respectively, but not in normal T cells (18, 19). Transgenic mice expressing RBTN1 or RBTN2 in T cells developed tumors but at variable frequencies and only after long latency periods (~10 months) (20, 21), again suggesting a need for cofactors.

In normal hematopoiesis TAL1 is considered to function in close collaboration with RBTN2 and a group of GATA binding zinc finger type transcription factors, GATA1 and GATA2 (19). This conclusion comes from their overlapping patterns of expression in developing erythroid cells (4, 5, 18, 22, 23), their proven physical interactions in vitro as well as in vivo (24–26), and a very similar abnormality in the embryonic erythropoiesis that was produced by disruption of each of these genes (27–30). It is thus conceivable that the activity of TAL1 in T cells as a transcriptional and oncogenic protein requires a similar set of transcriptional cofactors. Recently, efficient collaboration of TAL1 and RBTN2 in T cell oncogenesis has indeed been demonstrated in vivo by generating double transgenic mice (31).

Previously we identified a highly specific surface marker of T-ALL designated TALLA1 (32). TALLA1 is a new member of the tetraspans or transmembrane 4 superfamily and normally expressed in neurons, certain vascular endothelial cells, and certain epithelial cells but not at all in any hematopoietic cells.

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* The abbreviations used are: T-ALL, T cell acute lymphoblastic leukemia; bHLH, basic helix-loop-helix; TALLA1, T-ALL-associated antigen-1; RT, reverse transcription; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; HSV, herpes simplex virus; MBP, maltose-binding protein; GST, glutathione S-transferase; tk, thymidine kinase.

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including T cells (32). The highly frequent ectopic expression of TALLA1 in T-ALL suggests that its aberrant expression is closely related to leukemogenesis of T-ALL. We have not, however, detected any T-ALL-associated genetic changes involving the TALLA1 gene that is located on X chromosome. One possibility is that aberrantly activated transcription factors in T-ALL such as TALLA1 are responsible for ectopic expression of TALLA1. In the present study, we examined expression of TAL1, RBTN1, and RBTN2 in a panel of 14 human T-ALL cell lines and evaluated transcriptional activity of TAL1 and RBTN by monitoring expression of transfected artificial reporter genes and the endogenous TALLA1 gene. The transcriptional activity of TAL1 indeed requires RBTN1 or RBTN2 together with some other T cell-specific cofactors and induces the TALLA1 gene. Such a highly collaborative set of transcriptional factors may thus lead to development of T-ALL by modulating expression of downstream genes like TALLA1.

**EXPERIMENTAL PROCEDURES**

Cell Culture—All of the cell lines used in the present study were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. For details please see Takagi et al. (32). RT-PCR—Total RNA samples were prepared from various cell lines using Trizol reagent (Life Technologies, Inc.). The first strand cDNA synthesis was carried out from 1 µg of total RNA in 20 µl of reaction buffer using a RNA PCR kit (Takara Shuzo, Kyoto, Japan). PCR was then set up using 2 µl from the reaction buffer as template in a 50-µl PCR reaction buffer containing 2.5 units of Taq DNA polymerase (Stratagene). Amplification was carried out by denaturation at 94 °C for 1 min (5 min at the first cycle) and annealing/extension at 70 °C for 5 min. The number of amplification cycles was 30 for TAL1, RBTN1, and RBTN2; 27 for GATA3; and 35 for GATA1. Amplified products were electroblotted onto a nylon membrane (GeneScreen Plus; NEN). The membrane was baked at 80 °C for 3 h, prehybridized in a solution containing 50% formamide, 5x Denhardt's solution, 0.5% SDS, 10% dextran sulfate, and 0.1 M phosphate buffer (pH 7.2) for 3 h, and hybridized with a 32P-labeled oligomers or plasmid DNA insert overnight at 42 °C. The membrane was washed 5 times with 2x SSC/0.1% SDS at 50 °C and then washed 1 time in 0.1x SSC/0.1% SDS at 65 °C. The hybridized bands were detected with a phosphor imager (Molecular Dynamics). The oligonucleotide used as a probe for TAL1, RBTN1, and RBTN2 was 5’-CTACGCCTGCGCTCATGCTCTCAGCTCAGGATTTAGG-3’; 5’-TCACCAGCTGACAGGAGG-3’ and 5’-GCTCCATGCCGAGGCCATGGAGGTGAC-3’. PTN1, p53, and RBTN2 cDNA were subcloned into the EcoRI site of the multiple cloning site of the pCMV ΔNeo (Clontech). The resulting plasmids were transfected into a panel of T-ALL cell lines using Lipofectamin (Life Technologies, Inc.). The transfected cells were selected with 1 µg/ml puromycin. The pCMV ΔNeo plasmid was used as a control DNA to confirm transfection efficiency of each cell line. The expression of TAL1, RBTN1, and RBTN2 was determined using both Northern and Western blot analysis. The expression of TAL1 was also determined by real-time RT-PCR using a TaqMan system (Perkin-Elmer). The expression of RBTN1 and RBTN2 was determined by real-time RT-PCR using a SYBR Green system (Takara). The results were analyzed and expressed as fold increases relative to the control samples.

**RESULTS**

Regular Coexpression of TAL1 and RBTN1 or RBTN2 in T-ALL Cell Lines—Ectopic expression of the TAL1 gene in T-ALL is now known to occur much more frequently than previously considered from the incidence of gross chromosomal rearrangements involving the TAL1 gene locus (2). Similarly, ectopic expression of RBTN1 and RBTN2 in T-ALL may be more frequent than, or at least comparable to, the incidence of respective chromosomal translocations (3). Therefore, we examined 14 T-ALL cell lines for expression of RBTN1, TAL1, and RBTN2 together with that of E2A, whose expression represents an important event in the development of T-ALL (32). The results are summarized in Table 1. The expression of E2A was determined by real-time RT-PCR using a TaqMan system (Perkin-Elmer). The expression of TAL1, RBTN1, and RBTN2 was determined by real-time RT-PCR using a SYBR Green system (Takara). The results were analyzed and expressed as fold increases relative to the control samples.

**Transcription and CAT Assay—**The cDNAs for TAL1, RBTN1, RBTN2, and GATA3 were amplified by PCR using tk-F (5’-CGAGCGCCCGAGATGATGGTGCTGG-3’) and tk-R (5’-GCCGAGCGCCCGAGATGATGGTGCTGG-3’). Amplified cDNAs were cloned into pCR™II (Invitrogen) and digested with EcoRI. The EcoRI fragments were then cloned into pMlKneo or pMlKhyg. Recipient cells (33) have shown that the heterodimers of TAL1 and RBTN1 or 2 in T-ALL may be ectopic expression of the TAL1 gene in T-ALL (2). Importantly, the same 11 out of 14 T-ALL cell lines expressed neither of them. All of the 14 cell lines expressed the E2A gene, which was thus useful as an internal control. The complete concordance of aberrant expression of TAL1 and RBTN1 or RBTN2 in 11 out of 14 T-ALL cell lines was striking, supporting their interdependent transcriptional and oncogenic activity in T cells. It is also notable that the 11 T-ALL cell lines that coexpressed TAL1 and RBTN1 or RBTN2 are exactly the ones that are strongly positive for surface expression of TALLA1, a highly specific tumor marker of T-ALL (32).

**Transcriptional Activity of TAL1 Requires RBTN1 or RBTN2—**No natural target genes regulated by TAL1 are known yet. Hau et al. (9) have shown that the heterodimers of TAL1 and E47 or E12 specifically bind to an E-box motif in vitro and regulate expression of an artificial reporter gene.
containing multiple copies of the optimal TAL1/E2A binding motif in murine C3H10T1/2 fibroblasts (10). We therefore decided to use a similar artificial reporter gene system to examine transcriptional activity of TAL1 and RBTN in T cells. The HSV tk minimal promoter (34) was linked to the CAT structural gene in pCAT-Basic (Promega), making tk-CAT, and then a DNA segment containing four copies of the optimal TAL1/E2A-binding site (AACAGATGGT) (9) was inserted into the upstream sequence of the tk promoter, making tk-TAL1CS-CAT. HPB-ALL cells, which expressed E2A but not TAL1, RBTN1, or RBTN2 (Fig. 1), were used as a recipient. Due to alternative translation initiation sites, TAL1 proteins have at least two isoforms containing the bHLH motif, the 42-kDa TAL1α and an N-terminally truncated 22-kDa TAL1β (6). We therefore tested the activity of both polypeptides.

As shown in Fig. 2A, TAL1α or TAL1β alone did not significantly induce expression of the CAT reporter gene from tk-TAL1CS-CAT. RBTN1 or RBTN2 had no effect on the expression of the reporter gene either. In the presence of RBTN1 or RBTN2, however, TAL1 proteins strongly induced the reporter gene. The full-length TAL1α and the N-terminally truncated TAL1β were similarly effective in the presence of RBTN1 or RBTN2. Thus, RBTN1, which is expressed in the brain (18) and RBTN2, which is expressed in erythroid cells like TAL1 (19), both efficiently cooperated with TAL1. Unexpectedly, however, the reporter plasmid with only the tk minimal promoter and without 4 copies of the TAL1/E2A-binding motif (tk-CAT) was also nearly half as much induced by the coexpression of TAL1 and RBTN as tk-TAL1CS-CAT. Computer analysis revealed a single potential E-box element in the HSV tk minimal promoter and as many as 12 potential E-box elements in the pCAT-Basic plasmid itself. Some of these sequences may thus fortuitously function as a TAL1/E2A-binding site. The deletion of the potential E-box element in the tk promoter, however, did not affect induction of tk-CAT by the combination of TAL1 and RBTN (data not shown). The role of any of the E-box-like elements within pCAT-Basic in the induction by TAL1 and RBTN remains to be seen.

We carried out the same transfection assays using a B-ALL cell line, BALL-1. As shown in Fig. 2B, little transcriptional activity of TAL1 proteins on tk-TAL1CS-CAT or tk-CAT was observed in BALL-1 even in the presence of RBTN1 or RBTN2. Similar negative results were obtained with another B cell line Raji or a monocytoid cell line U937 (data not shown). It is therefore likely that the transcriptional activity of TAL1 proteins requires, besides RBTN, still other cofactor(s) specifically present in T cells.

**Fig. 1.** RT-PCR analysis for expression of TAL1, RBTN1, RBTN2, and E2A in human T-ALL cell lines. Total RNA was isolated from the indicated human T-ALL cell lines. For details of these cell lines, see Takagi et al. (32). RT-PCR was carried out for transcripts from TAL1, RBTN2, RBTN1, and E2A. Amplification products were electrophoresed on 1.5% agarose and stained with ethidium bromide.

**Effect of GATA3 on Transcriptional Activity of TAL1**—In the erythroid lineage cells, GATA1 and GATA2, the zinc finger GATA binding transcription factors are expressed at high levels (19), and in close collaboration with TAL1 and RBTN2 they play essential roles in the erythropoietic development and differentiation (27–30). Furthermore, Osada et al. (24) demonstrated direct binding of RBTN2 with TAL1 and GATA2. On the other hand, they observed little significant binding between TAL1 and GATA1 or GATA2 (24). These results suggest that RBTN2 mediates interactions between TAL1 and GATA proteins. Among the GATA proteins, GATA3 is known to be expressed very specifically in the T cell lineage (37). We confirmed this in Fig. 3 where RT-PCR detected the expression of GATA3 in all the T-ALL cell lines tested but not in any other hematopoietic cell lines including BALL-1. Since it was not known whether RBTN1 or RBTN2 was capable of binding to the T cell-specific GATA3, we also examined direct binding of RBTN1 and RBTN2 to GATA3. To do this, GATA3 was expressed as a GST fusion protein, while RBTN1 and RBTN2 were expressed as MBP fusion proteins. GST alone or GST-GATA3 was immobilized by glutathione beads and incubated with MBP alone, MBP-RBTN1, or MBP-RBTN2. Free proteins and bound proteins eluted by glutathione were analyzed by immunoblot with anti-MBP. As shown in Fig. 4, GST-GATA3 but not GST alone retained MBP-RBTN1 and MBP-RBTN2 but not MBP alone. Thus, both RBTN1 and RBTN2 are capable of binding to GATA3. We also examined direct binding between TAL1 and GATA3 by the same protocol using MBP-TAL1 fusion protein and GST-GATA3 fusion protein. We did not observe any direct binding between TAL1 and GATA3 (data not shown).

These results led us to examine the effect of GATA3 on the transcriptional activity of TAL1/RBTN in T cells and non-T cells. For the sake of convenience, we used only the reporter...
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FIG. 3. RT-PCR analysis for expression of GATA3 and E2A in various human hematopoietic cell lines. Total RNA was prepared from Jurkat, CEM, HPB-ALL, DND4.1 and Molt-4 (all T cell lines), BALL-1 and Raji (B cell lines), U937 (a monocyte line), HL-60 (a promyelocytic cell line), and K562 (an erythroleukemia cell line). For details of these cell lines, see Takagi et al. (32). RT-PCR was carried out for transcripts from GATA3 and E2A. Amplification products were electrophoresed on 1.5% agarose and stained with ethidium bromide.

plasmid tk-TAL1CS-CAT in the following experiments. We first examined the effect of GATA3 overexpression on the transcriptional activity of TAL1α in HPB-ALL (Fig. 5A). Transfection of GATA3 had no direct effect on the expression of tk-TAL1CS-CAT. GATA3 also did not collaborate with TAL1α. On the other hand, GATA3 clearly augmented the collaborative transcriptional activity of TAL1α and RBTN1 or RBTN2 by 2–3-fold. We next carried out the same transfection experiment using BALL-1 (Fig. 5B). As already shown in Fig. 2B, coexpression of TAL1α and RBTN1 or -2 hardly induced expression of tk-TAL1CS-CAT. The combination of TAL1α and GATA3 was, however, found to induce the reporter gene to some extent even without RBTN1 or -2. The combination of TAL1α, GATA3, and especially RBTN1 further enhanced expression of the reporter gene to some extent. However, it should be noted that the levels of induction of the reporter gene in BALL-1 even by the full combination of TAL1α, GATA3, and RBTN1 or -2 were still far less than those seen in HPB-ALL by the combination of TAL1α and RBTN1 or -2. Therefore, the exact role of GATA3 as a cofactor in the collaborative transcriptional activity of TAL1α and RBTN1 needs further evaluation.

Induction of TAL1α in HPB-ALL Stably Transformed with TAL1α and RBTN1α—As mentioned above, the aberrant expression of TAL1α in T-ALL cell lines correlates 100% with strong expression of TAL1α, a highly specific tumor marker of T-ALL not detected on normal T cells (32). To examine the role of TAL1α and RBTN1 in the ectopic expression of TAL1α, we cotransfected HPB-ALL with the following combinations of expression plasmids: pMIKneo + pMIKhyg, pMIKneoTAL1α + pMIKhyg, pMIKneo + pMIKhygRBTN1, and pMIKneoTAL1α + pMIKhygRBTN1. Stable transformants were isolated by double selection with G418 and hygromycin B, and expression of TAL1α and RBTN1 in each clone was determined by RT-PCR (data not shown). Two representative clones from each combination were then examined for surface TALLA1 by indirect immunofluorescence staining and flow cytometry (Fig. 6A). HPB-ALL clones transformed with vectors only or those expressing either TAL1α or RBTN1 alone showed little surface expression of TAL1α. In sharp contrast, HPB-ALL clones coexpressing TAL1α and RBTN1 were strongly positive for TAL1α. The two clones coexpressing TAL1α and RBTN1 also contained a large quantity of TAL1α mRNA, whereas clones transfected with vectors only or those expressing either TAL1α or RBTN1 alone showed no such accumulation of TAL1α mRNA (Fig. 6B). These results strongly suggest that the collaborative transcriptional activity of TAL1α and RBTN1 indeed induces, either directly or indirectly, the TAL1α gene in T-ALL.

DISCUSSION

TAL1, TAL2, and LYL1, whose bHLH domains are most closely related to each other among >60 bHLH proteins so far described, are all identified as the genes activated in T-ALL by recurrent chromosomal translocations (38, 39). It is thus conceivable that these three bHLH proteins have a common mode of action in the leukemogenesis of T cells. RBTN1 and RBTN2, encoding closely related proteins containing only tandem LIM domains, represent another group of genes frequently activated by T-ALL-specific chromosomal translocations (14–16). Wadman et al. (26) reported that TAL1, TAL2, and LYL1 all interacted with RBTN1 and RBTN2. Furthermore, Larson et al. (31) recently reported that double transgenic mice expressing both TAL1 and RBTN2 in T cells developed T cell tumors much more efficiently than those expressing either gene alone (12, 20, 21).
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RBTN2 was coexpressed (Fig. 2). Third, TALLA1, a highly motifs in the upstream sequence (9) only when RBTN1 or moter with four copies of the optimal TAL1/E2A binding E-box reporter gene under the control of the HSV tk minimal promoter was efficiently induced T cell tumors (31) further underscores the importance of such collaborative transcriptional activity of TAL1 and RBTN in triggering the oncogenic process of T cells. Studies are now in progress to examine how the TALLA1 gene is induced in T cells by TAL1 and RBTN.

Unexpectedly, the reporter gene with the HSV tk minimal promoter but without the upstream optimal TAL1/E2A-binding elements was also quite efficiently induced by the coexpression of TAL1 and RBTN1 or -2 in HPB-ALL (Fig. 2). We have not yet examined the mechanism of this induction in detail. However, we may point out that there are a number of potential E-box-like elements in the original CAT plasmid (pCAT-Base), some of which may function as fortuitous TAL1-responsive elements. Alternatively, TAL1 and RBTN may affect, somehow in a highly T cell-specific manner (see below), the expression and/or activity of transcription factors such as Sp1 and CTF that are known to interact with the HSV tk minimal promoter (40). These possibilities are now under investigation.

Surprisingly, TAL1 even in the presence of RBTN1 or RBTN2 hardly induced the artificial reporter genes in non-T cells (Fig. 2). This suggests that the collaborative transcriptional activity of TAL1 and RBTN1 or -2 further requires some other cofactors specifically present in T cells. The fact that tumor-specific translocations involving TAL1, RBTN1, and RBTN2 are highly restricted to T-ALL and not observed in any other types of tumors (3) also supports that some T-cell specific cofactors are essential for the collaborative oncogenic activity of TAL1 and RBTN. In erythroid cells, TAL1 and RBTN2 are known to function in close collaboration with GATA1 or GATA2, the members of GATA-binding zinc finger type transcription factors (19). It is thus possible that the T cell-specific member of the GATA proteins, GATA3 (37) (Fig. 3), may be one of such cofactors in T cells. We showed that RBTN1 and RBTN2 were capable of directly binding to GATA3 in vitro (Fig. 4). We further demonstrated that overexpression of GATA3 in HPB-ALL augmented the collaborative transcriptional activity of TAL1 and RBTN1 or RBTN2 (Fig. 5). In BALL-1, TAL1 with and even without RBTN collaborated with GATA3 to induce the reporter gene (Fig. 5). These results may support that GATA3 is a cofactor for the collaborative transcriptional activity of TAL1 and RBTN in T cells. However, it should also be noted that the observed collaborative transcriptional activities of TAL1, GATA3, and RBTN1 or -2 in BALL-1 were far less than those seen by the combination of TAL1 and RBTN1 or -2 in HPB-ALL. Furthermore, we found that a dominant negative mutant of GATA3 KRR (35) had no inhibitory effect at all on the collaborative transcriptional activity of TAL1 and RBTN in HPB-ALL (data not shown). The KRR mutant, which has three amino acids KRR (305–307) in the region between the two zinc fingers mutated to alamines, was shown to be totally inactive on a reporter gene linked to multiple GATA3-binding sites and to block transactivation of the same reporter gene by wild type GATA1, GATA2, or GATA3 (35). However, the results with the KRR mutant still do not formally exclude the possi-

**Fig. 6. Induction of TALLA1 in HPB-ALL by TAL1α and RBTN1.** HPB-ALL was transfected with indicated combinations of expression vectors, and stable transformants were isolated. Expression of TAL1α and/or RBTN1 in each clone was determined by RT-PCR (data not shown). Two representative clones for each combination were then analyzed. A, surface expression of TALLA1. Clones were stained for surface TALLA1 by an indirect immunofluorescence staining method using monoclonal anti-TALLA1 (B2D) (32) and analyzed by flow cytometry. Background profiles were determined by staining with fluorescein isothiocyanate-labeled anti-mouse IgG only. B, Northern blot analysis for TALLA1 transcripts. Total RNA samples (15 μg each) from the same clones were analyzed by Northern blot using the 32P-labeled probe for TALLA1 (32). The same filter was reprobed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

RBTN2, which are devoid of intrinsic DNA binding activity, may thus function as an essential cofactor for TAL1 and related bHLH proteins in their transcriptional and oncogenic activity in T cells. In the present study, we have demonstrated that the transcriptional activity of TAL1 in T cells indeed requires RBTN1 or RBTN2.

First, we found that all the T-ALL cell lines expressing TAL1 do coexpress either RBTN1 or RBTN2 with 100% concordance (Fig. 1). Second, forced expression of TAL1 in a T-ALL cell line HPB-ALL strongly activated transcription from an artificial reporter gene under the control of the HSV tk minimal promoter with four copies of the optimal TAL1/E2A binding E-box motif in the upstream sequence (9) only when RBTN1 or RBTN2 was coexpressed (Fig. 2). Third, TALLA1, a highly
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bility of GATA3 as a cofactor because the KRR mutation may not affect the ability of GATA3 to function as such. Nevertheless, all of these observations may argue against a critical role of GATA3 in the collaborative transcriptional activity of TAL1 and RBTN1 or -2 in T cells. Thus, there may be more relevant cofactors for the TAL1/RBTN transcriptional complex in T cells besides or even independent of GATA3.

Obviously further studies are needed to define cofactors for TAL1 and RBTN in T cells and to identify downstream genes whose expression in T cells is modulated, like TAL1 and RBTN in T cells and to identify downstream genes besides or even independent of GATA3.

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