Low Incidence of TAL1 Gene Rearrangements in Adult Acute Lymphoblastic Leukemia: A Cancer and Leukemia Group B Study (8762)1


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ABSTRACT

TAL1 gene rearrangements have been described in approximately 25% of children with T cell acute lymphoblastic leukemia (ALL). Three percent of these rearrangements are the result of a reciprocal translocation, t(1;14)(p34;q11). The remainder of these rearrangements are submicroscopic and involve a nearly precise 90-kilobase pair deletion of the TAL1 gene. Detection of these submicroscopic rearrangements can be accomplished easily using Southern blot and PCR technology and may have potential value for monitoring disease during and following treatment. The incidence of TAL1 gene rearrangements in adults with ALL is unknown. In this pilot study, we performed Southern blot and PCR analysis in a group of newly diagnosed adult ALL patients to determine the incidence of TAL1 rearrangements. Thirty-three adults with T cell ALL were studied; of these, one patient had a t(1;14)(p34;q11) and molecular rearrangement of TAL1. No submicroscopic deletions of TAL1 were detected (95% confidence intervals, 0.000 and 0.106). Unlike pediatric T cell ALL, the incidence of TAL1 rearrangements in adult ALL appears to be very low.

INTRODUCTION

Specific recurring chromosomal translocations are characteristic of a number of human acute and chronic leukemias. These rearrangements are useful in the determination of diagnosis and therapeutic options, and as markers for monitoring residual disease. In contrast, cytogenetic studies have not uncovered a specific karyotypic defect associated with most cases of T cell ALL although a number of distinct chromosome translocations have been described, each of which occurs in a relatively small proportion of T cell ALL patients (1). One such translocation, the t(1;14)(p34;q11) is observed in only 3% of T cell ALL patients (2). As a result of this translocation, the TAL1 proto-oncogene (also called SCL or TCL5) is transposed from its normal position on chromosome 1 into the T cell receptor α/β chain complex on chromosome 14 (3–5).

Recently, rearrangements of the TAL1 locus that are not detectable by karyotypic analysis have also been described (6–8). These result from nearly precise 90-kilobase pair deletions of upstream untranslated TAL1 sequences which are mediated by site-specific recombination and result in the juxtaposition of TAL1 with a distinct transcribed locus, SIL. The net result of this rearrangement is that TAL1 mRNA expression becomes regulated by the SIL promoter and leads to inappropriate TAL1 expression (8, 9).

Studies indicate that TAL1 recombination is a relatively common feature of pediatric patients with T cell ALL and occurs in 16–30% of this population (6, 8, 10). Therefore, it may serve as a useful marker for this cancer. The incidence of TAL1 rearrangements in adults with ALL is unknown. As a pilot

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3 The abbreviations used are: ALL, acute lymphoblastic leukemia; FAB, French-American-British; CALGB, Cancer and Leukemia Group B.
study to investigate the potential clinical utility of TAL1 rearrangements as a molecular marker for adult T cell ALL, we analyzed retrospectively a group of newly diagnosed adults with T cell ALL treated on two CALGB protocols for the presence of these rearrangements.

PATIENTS AND METHODS

Leukemia cell specimens were obtained as part of a prospective trial examining molecular rearrangements in adult ALL (CALGB 8762). Samples were selected based on the availability of frozen material from the presenting (diagnostic) specimen. All patients were also entered into one of two CALGB treatment protocols (8811 or 9111) between September 1988 and September 1993 (11, 12). Thirty-three adult patients with T cell ALL and an additional 62 patients with non-T cell ALL (CD2−, CD5+) who were entered into these studies were examined retrospectively for rearrangements of the TAL1 gene using Southern blot and, in 27 of 33 T cell ALL cases, PCR analysis. The T cell ALL patients ranged in age from 16–57 years and included 14 females and 19 males. All patients had a morphological diagnosis of ALL as defined by the standard FAB criteria (13). The FAB classification of the study population showed: L1, 16 patients; L2, 14 patients; mixed leukemia, 1 patient. Two patients were not evaluable because the sample was not sent for central pathology review but were classified as ALL by the referring institution. All patients were immunophenotyped centrally as part of CALGB 8364, a prospective study of immunophenotyping in adult ALL (14, 15). Surface antigen phenotype was determined by means of cytofluorometric analysis with mAbs. The criterion for surface marker positivity was expression by at least 20% of the leukemic blast population. T antigen expression was defined as CD5 or CD2 reactivity; B antigen expression was defined as CD19 or CD20 positivity; and myeloid antigen expression as CD13, or CD33 positivity. Of the 33 T cell ALL patients, 21 expressed only T antigens, 8 expressed T and myeloid antigens, 3 expressed T and B antigens, and 1 expressed T, B, and myeloid antigens.

Cytogenetic analysis was performed as part of CALGB 8461, a prospective study of chromosomes in acute leukemia (16). Analysis of banded chromosomes from bone marrow or, if in aspirable, blood taken at diagnosis was performed in CALGB institutional cytogenetic laboratories. A minimum of 20 cells were analyzed in each case and all karyotypes were reviewed centrally. Karyotypes were designated according to the International System for Human Cytogenetic Nomenclature (17).

Bone marrow (28 cases) or blood (5 cases) samples were available for molecular analysis by Southern blot. DNA was extracted from cryopreserved or fresh bone marrow or blood cells using standard methods (18). DNA enzymatic digests were performed with a HindIII restriction endonuclease (New England Biolabs), electrophoresed on 0.8% agarose gels, transferred to nylon membranes (Genescreen Plus; New England Nuclear), and hybridized with a radiolabeled genomic DNA probe (probe 2.2X; Ref. 8). This probe recognizes a 21-kilobase germline HindIII fragment and a 4.8-kilobase germline BamHI fragment that delineate the major breakpoint cluster region of the TAL1 gene.

Amplification of the common TAL1 deletions was accomplished using the PCR with pairs of published oligonucleotide primers (10, 19). As an internal positive control to verify the integrity of the DNA samples, another set of primers was used to amplify germline fragments (19). DNA derived from T cell ALL cell lines RPMI 8402 and MOLT 16, which contain TAL1 deletions, served as positive controls for the PCR reactions (19). One-half to 1 μg high molecular weight DNA was amplified in a 50-μl reaction consisting of 200 mM each dNTP, 1.5 mmol/liter MgCl2, 250 ng of each oligonucleotide primer, and 1.25 units AmpliTaq polymerase (Cetus). Amplification was accomplished in a Perkin Elmer/Cetus Corp. thermal cycler for 60 cycles. The denaturing step was at 94°C for 1 min. The annealing step was at 61°C for 1 min and the extension step was at 72°C for 2 min. An extension step at 72°C for 7 min followed the 60 cycles of amplification. PCR products were analyzed on 2% agarose gels stained with ethidium bromide. The amplified TAL1 germline fragment was 250 base pairs; the rearranged TAL1 fragments from the MOLT 16 and RPMI 8402 cell lines were 172 and 159 base pairs, respectively.

RESULTS

A total of 33 adult patients with T cell ALL were studied for molecular rearrangements of the TAL1 gene by Southern blot. Adequate cytogenetic data were available for 22 of these patients, of which 12 had a normal karyotype. A single patient was found to have a TAL1 rearrangement by Southern blot (Fig. 1). This patient was a 38-year-old woman with T cell ALL, L1 morphology, and a presenting WBC count of 4.3/μl. Immunophenotypic analysis for this patient showed that the leukemic cells were CD2−, CD4−, CD7−, and CD3−, CD10−, CD13−, CD19−, CD33−. Her karyotype was 46,XX,t(1;14)(p34;q11),del(9)(p21p2?4),deb(9)?deb(9)(p13p22),t(9;i6)(p22;q22) [22]/46,XX[12], indicating that her TAL1 rearrangement was the result of a t(1;14). A second patient with the karyotype 46,XY,t(1;14)(p13;?1?q11) was included in this series. The breakpoints in this case were not clearly defined and this patient did not have a molecular rearrangement of TAL1.

No submicroscopic deletions of TAL1 were detected (95% confidence intervals, 0.00 and 0.106). Twenty-seven of the 33 T cell ALL patients, including the 2 with the t(1;14), were also examined by PCR for TAL1 microdeletions. None were positive (Fig. 2). The primers used for PCR detect the majority of submicroscopic 90-kilobase deletions of TAL1, but will not detect TAL1 rearrangements occurring as a result of the t(1;14) since the majority of these translocation breakpoints occur approximately 4 kilobases downstream of the TAL1 deletions.

In addition to the T cell ALL patients described here, 62 newly diagnosed adults with non-T cell ALL who were enrolled on the same treatment and laboratory protocols described above were studied using Southern blot analysis. None of these patients had a TAL1 rearrangement.

DISCUSSION

TAL1 rearrangements have been described recently as a common genetic alteration in children with T cell ALL and occur in up to 25–30% of these patients; approximately 3% of these occur as a result of a t(1;14) translocation and up to 25% occur as a result of recombinase-mediated deletion of upstream
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**Fig. 1** Demonstration of TAL1 gene rearrangement by Southern blot analysis in an adult patient with T cell ALL. DNA enzymatic digests with BamH1 (left) and HindIII (right) and of blood and/or bone marrow samples from five adults with ALL (Lanes 1–7). Blots were hybridized with the 2.2XX genomic fragment of the TAL1 gene. Germinal fragments of 21 kilobases (HindIII) and 4.8 kilobases (BamH1) are shown. Patient 4 has a rearrangement of TAL1 which is detected with both enzymatic digests in blood (Lane 5) and marrow (Lane 6). This patient also had the t(1;14).

TAL1 sequences (2, 8, 10). This makes TAL1 the most frequently occurring molecular rearrangement detected in pediatric T cell ALL. These tumor-specific rearrangements can be used to monitor minimal residual disease in these patients and, because each patient has a unique deletion junction, contamination problems that are common to other PCR-based assays can be minimized (19). For these reasons, we performed a pilot study to determine the incidence of TAL1 deletions in adults with T cell ALL. Of 33 adult patients, we found only 1 patient with a rearrangement of TAL1 which was the result of the t(1;14)(p34; q11) and no patients with a submicroscopic deletion involving TAL1. These results are similar to those obtained by Griesinger et al. (20) and confirm our earlier observations in a smaller series of patients (21).

Unlike the situation in pediatric T cell ALL, the occurrence of TAL1 rearrangements in adult T cell ALL appears to be very low. The reasons for this difference in the adult compared to the pediatric population are not clear. Although our sample size was small, with a reported incidence of subkaryotypic TAL1 deletions of approximately 25–30% in pediatric T cell ALL, the fact that we did not detect any submicroscopic TAL1 deletions may be significant. In the largest published pediatric series, which was a retrospective analysis of patients with T cell ALL, the only presenting clinical feature that was significantly associated with TAL1 rearrangements was a higher median WBC count (207,000/μl versus 115,000/μl; Ref. 10). The median WBC count in our patient population was much lower; 23,000/μl (range, 1,000–162,400/μl). In the pediatric series, the presence of CD2 and absence of CD10 expression were significantly associated with TAL1 alterations. CD2 expression and lack of CD10 were also seen in the single patient in our series with a TAL1 rearrangement; however, of the remaining 26 patients in our series who were examined for both CD2 and CD10 antigens, 8 were CD2− and CD10− but lacked the TAL1 rearrangement. Eight of 33 patients in our series had at least one myeloid marker. The leukemic blasts of children with rearrangements involving TAL1 have been reported to express myeloid-associated antigens in some cases (2). Although no specific association with FAB morphology has been reported, approximately one half of the adults in our study had L1 morphology which is found in the majority of pediatric T cell ALL. Our data demonstrating a lack of submicroscopic TAL1 gene rearrangements in adult T cell ALL, coupled with the observation that recurring cytogenetic abnormalities involving the T cell receptor appear to be more common in pediatric than adult T cell ALL (22, 23), suggest that the pathogenesis of T cell ALL in adults and pediatric patients may differ.

Detection of TAL1 rearrangements has not been shown to have a prognostic value in the pediatric population; however, DNA-based PCR assays may have great utility for monitoring minimal residual disease in these patients (19). On the basis of the low incidence of these rearrangements in our pilot study, we suggest that there may be little utility to these assays in adult T cell ALL.

The lack of detection of submicroscopic TAL1 rearrangements in the adult population does not necessarily preclude a
Incidence of TALI Gene Rearrangements in Adult ALL

The germline PCR fragment and the rearranged fragments of TALI not all of the T cell ALL cell lines that lack gross subtle mutations in genomic sequences, in regulatory sequences, (MOLT 16) and 159 (RPMI 8402) base pairs are visible. Morphism within the TALI rearrangements (24). Recently, Bash et al. (25) utilized a polymorphism within the TALI mRNA and a reverse transcriptase-PCR assay to detect the presence of TALI mRNA in pediatric T cell ALL patients who had no evidence of TALI gene rearrangement. Their results support the concept that TALI gene expression may be a result of both primary gene alteration and of secondary trans-activation of the TALI locus. These results also suggest that TALI expression in pediatric T cell ALL is even more common than previously described and underscores its potential importance in leukemogenesis of this disease. Such mechanisms of TALI expression in adult T cell ALL have yet to be reported, but may be worthy of study.

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