Disruption of the SCL Gene by a t(1;3) Translocation in a Patient with T Cell Acute Lymphoblastic Leukemia

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Summary

SCL gene disruptions are the most common chromosomal abnormality associated with the development of T cell acute lymphoblastic leukemia (ALL). Such disruptions can be the result of t(1;14) and t(1;7) translocations, as well as a cytogenetically undetectable interstitial deletion of chromosome 1. We present here a case of T cell ALL with a t(1;3)(p34;p21) translocation that also disrupts the SCL locus and leads to dysregulated SCL gene expression. This translocation, similar to previously reported SCL gene disruptions, appears to have been mediated, at least in part, by the V(D)J recombinase complex, since cryptic heptamer recognition sequences, as well as nontemplated N region nucleotide addition, are present at the breakpoints. The t(1;3) also disrupts a region on chromosome 3 characterized by alternating purine and pyrimidine residues, which can form a Z-DNA structure, reported to be prone to recombination events. A previously undescribed, evolutionarily conserved transcript unit is detected within 8 kb of the breakpoint on chromosome 3. This report extends the spectrum of recognized SCL translocations associated with T cell ALL, and underscores the contention that dysregulated SCL expression may be a causal event in T cell ALL.

Nonrandom chromosomal translocations and deletions are frequently associated with acute leukemia. The most common structural disruption recognized in T cell acute lymphoblastic leukemia (ALL)[1] occurs within the SCL (also known as TCL5 or tal-1) (1-4) gene, a member of the basic domain, helix-loop-helix (bHLH) family of transcription regulators (5). Several recent surveys have indicated that the SCL gene is disrupted in ~30% of patients with childhood T cell ALL (6-8). This disruption results in the expression of SCL in T lymphocytes, a cell type where SCL expression normally appears to be repressed (3). To date, SCL rearrangement has been reported exclusively in T cell ALL, and not in other types of leukemias or lymphomas (7). The frequency and specificity of this disruption, along with the presumed dysregulated mRNA expression seen in the cells that undergo these changes, have led us and others to speculate that disruption and dysregulation of SCL may contribute to T cell leukemogenesis (1, 2, 4, 9).

Thus far, three types of SCL rearrangements have been reported to occur in T cell ALL: t(1;14)(p32;q11) and t(1;7)(p32;q11) translocations (1, 9-12), joining SCL to the TCRD and TCRB loci, respectively, and a microscopically undetectable interstitial deletion of chromosome 1 juxtaposing

SCL with the SIL gene (6, 13, 14). All these chromosomal abnormalities seem to be mediated, at least in part, by the V(D)J recombinase complex responsible for the generation of immunologic diversity. Evidence for involvement of the V(D)J recombinase complex includes the presence of nontemplated or N region nucleotide addition as well as exonucleolytic "nibbling" at the breakpoints, which typically occurs in the immediate vicinity of approximate heptamer/nonamer recognition sequences within the SCL locus. Recently, one of us (S. C. Raimondi) has described several rare, previously unreported, nonrandom translocations associated with childhood ALL (15). Two of these patients had, in their leukemic cells, t(1;3) translocations with breakpoints on chromosome 1 at bands p32-34. Since the human SCL gene has been mapped to this region, we studied DNA and RNA from these two cases to assess whether these translocations physically altered the SCL gene, and whether SCL was expressed in the malignant cells.

The leukemic cells of one of the two patients demonstrated both a breakpoint within the SCL locus and SCL mRNA expression. Sequence analysis of the breakpoint suggested that this translocation was also mediated, at least in part, through the action of the V(D)J recombinase system. A previously undescribed transcript unit was identified on chromosome 3, within 8 kb of the breakpoint.

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[1] Abbreviation used in this paper: ALL, acute lymphoblastic leukemia.

Materials and Methods

Cytogenic Analysis. Bone marrow (BM) samples were obtained from two patients with ALL enrolled in Total Therapy Studies at St. Jude Children's Research Hospital. The samples were prepared by a direct method, with or without short-term culture, to obtain metaphase spreads. A modified trypsin-Giemsa technique was used for chromosome banding (16). The identification and classification of chromosomes followed the International System for Human Cytogenetic Nomenclature (17). Written informed consent was obtained from the patients or parents; the investigations were approved by the institution's clinical trials review committee.

Nucleic Acid Extraction. DNA and RNA were extracted from viably preserved leukemic samples by the guanidium isothiocyanate method (18).

Southern and Northern Blot Analysis. DNA samples (10 μg) were digested to completion with restriction enzymes using the manufacturer's (Bethesda Research Laboratories, Gaithersburg, MD) recommended conditions and size fractionated on 0.8% agarose gels. The denatured DNA was transferred to a solid matrix (nitrocellulose; Schleicher & Schuell, Inc., Keene, NH) by the Southern method (19). RNA samples were size fractionated on 1.0% agarose/formaldehyde gels and transferred to nitrocellulose (18). Southern and Northern blots were hybridized to ³²P probes labeled by the random priming method (20), using "Prime-It" (Stratagene, La Jolla, CA) reagents and protocols. Hybridization was carried out as previously described (10); the blots were washed twice with 0.1× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.1% SDS at 52°C for 20 min and exposed to x-ray film at −70°C with an intensifying screen.

Genomic Library Construction. Genomic DNA (2 μg) was digested to completion with EcoRI, extracted with phenol/chloroform, and ethanol precipitated. One-half of the EcoRI-digested genomic DNA was ligated to 1 μg of EcoRI-digested, purified, λ Dash (Stratagene) phage arms, in a total volume of 10 μl, at 4°C for 16 h. The naked recombinant phage DNA was packaged using Gigapack II (Stratagene) extracts and protocols. 10⁶ independent recombinant phage were plated with either SRB (Stratagene) or LE 392 (Stratagene) cells and incubated at either 39°C (SRB cells) or 37°C (LE 392 cells). Filter lifts, hybridizations, and plaque purification was performed as described (18).

DNA Sequence Analysis. Pertinent genomic fragments were subcloned into plasmid vectors (pBluescript II; Stratagene) and sequenced using oligonucleotide primers synthesized on an oligonucleotide synthesizer (380; Applied Biosystems, Inc., Foster City, CA) and Sequenase (U.S. Biochemical Corp., Cleveland, OH) reagents and protocols.

Results

The t(1;3)(p34;p21) in a T Cell ALL Patient Has an SCL Breakpoint. The clinical and laboratory features of the two patients with a t(1;3) in their leukemic blasts have been presented elsewhere (15), and are only briefly recapitulated here (Table 1). One of these patients (patient 1) had a T cell immunophenotype; the other (patient 2) had an early pre-B immunophenotype. The assignment of the breakpoints on chromosome 1 was difficult because the band definition of this 1p region is ambiguous (Fig. 1). We have previously characterized genomic and cDNA probes from two genes mapped to 1p32-34: SIL (14) and SCL (1, 3, 10). Southern blots of genomic DNA extracted from the leukemic blasts of both patients were hybridized to several probes from these two loci; the SCL probes (0.3B2, 0.3N, 2.2XX, and 1.0SX) are shown in Fig. 2 B. A 5′ SCL probe (0.3N) identified novel rearranged bands in BamHI, EcoRI, PstI, and HindIII digests of genomic DNA from patient 1's leukemic blasts; a unique 17-kb HindIII fragment, as well as the germline 21-kb band, are shown in Fig. 2 A. Comparison of the Southern blot data with a genomic SCL restriction map suggested that the breakpoint should be ~2 kb centromeric to the 0.3N probe. In agreement with this prediction, a probe (0.3B2 of Fig. 2) 4 kb upstream and centromeric of the 0.3N probe, and therefore presumably centromeric to the breakpoint, identified a unique 10-kb HindIII fragment in DNA from patient 1 in addition to the germline 21-kb fragment (Fig. 2). As this patient's karyotype demonstrated a balanced translocation, we presumed that the unique 17- and 10-kb HindIII fragments were derived from the two reciprocally translocated chromosomes.

Genomic Southern blots of leukemic blasts from the patient 2, which had a B cell precursor immunophenotype, failed

Table 1. *Clinical and Laboratory Features of Patients with t(1;3)(p34;p21)*

Patient	Karyotype	Age	Sex	WBC	Hgb	Platelet	Fab subtype	Immunophenotype	Liver/ spleen
		yr		*× 10⁹/liter*	*g/dl*	*× 10⁹/liter*			*cm*
1	46, XY, t(1;3)(p34; p21)/ 46, XY, − 13, , + der(13)t(13;?)(q34;?), t(1;3)(p34;p21)	16.1	M	163.0	16.1	131	L1	T-cell	8/9
2	45,X, − X,del(6)(q13q21), t(1;3)(p34;p21)	11.1	F	3.0	11.7	371	L1	Early pre-B	0/0

Immunophenotyping was performed as previously described (15). Organomegaly was assessed by palpation of liver/spleen (in centimeters) below right and left costal margin, respectively.
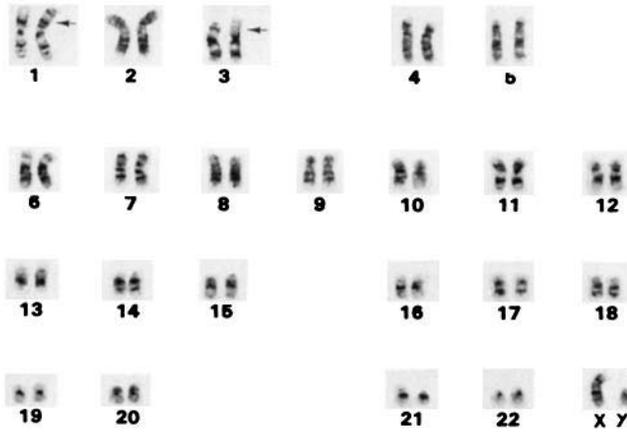
Figure 1. Karyotype of leukemic blasts from patient 1. The 1p and 3p breakpoints are indicated with arrows.
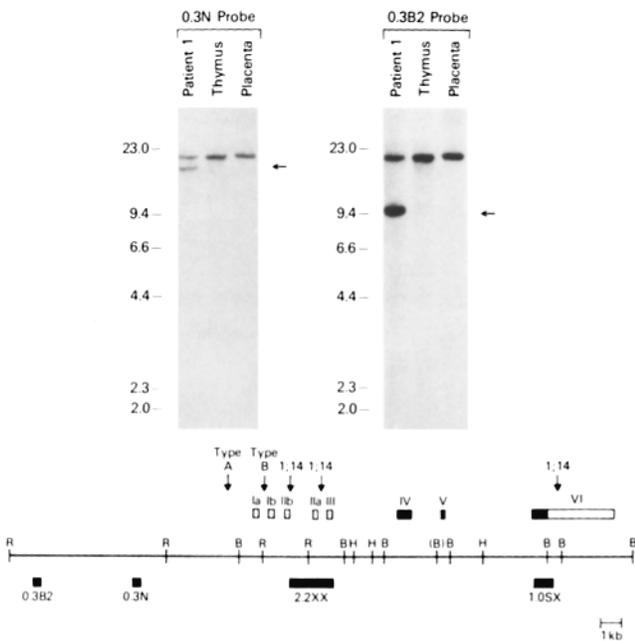
to demonstrate any rearranged bands when hybridized to the chromosome 1 probes described above (data not shown).

Isolation of the Germline and Translocated Alleles. A phage library created from genomic DNA from the leukemic blasts
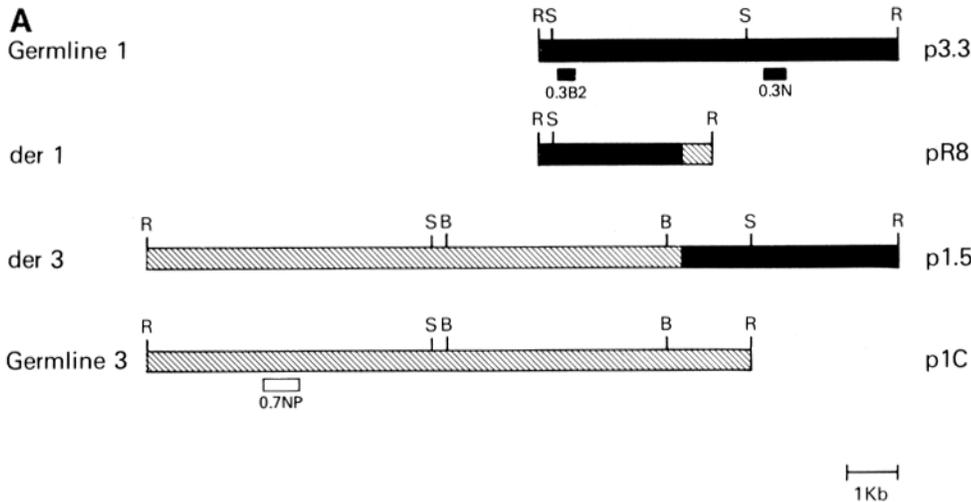

Figure 2. (*A*) Southern blots demonstrating rearranged bands in DNA from patient 1. Genomic DNA from patient 1, thymus, and placenta was digested with HindIII and Southern blotted. (*Left*) Southern blot hybridized to the 0.3N probe; (*right*) the same blot, stripped, and rehybridized to the 0.3B2 probe. The rearranged bands are indicated by arrows; size standards are in kilobases. (*B*) Restriction map of the germline SCL locus. The chromosome 1 centromere is to the left, the telomere is on the right. Exons Ia-VI are indicated with open boxes representing 5' and 3' untranslated regions, and filled boxes indicating coding sequences. The SCL breakpoints for SIL/SCL type A and B rearrangements (6, 7), as well as several t(1;14) translocations (1, 4, 9), are indicated with arrows; the breakpoint for a patient with a SCL disruption due to a t(1;7) is ~35 kb 3' of exon VI (12). Probes used (0.3B2, 0.3N, 2.2XX, and 1.0SX) for Southern hybridizations are indicated below the map. Restriction sites are indicated by: R, EcoRI; B, BamHI; and H, HindIII. The BamHI site in parentheses is known to be polymorphic.

of patient 1 was screened with an SCL (0.3N) probe. 10⁶ recombinant phage were plated with the *Escherichia coli* strain LE 392 and screened with the 0.3N probe; no hybridizing plaques were identified. 10⁶ recombinants were then plated on SRB (Stratagene) cells, an *E. coli* strain that has modifications (mcrA⁻, mcrB⁻, and mrr⁻) reported to increase the yield of recombinant phage clones containing methylated DNA as well as increase the stability of nonstandard DNA structures. 10 independent hybridizing plaques were identified from this screen and purified. The hybridizing phage contained either a 7-kb (germline) or a 14-kb (rearranged) EcoRI fragment. These EcoRI fragments were subcloned into plasmids for further analysis. Fig. 3 *A* demonstrates that the restriction maps for these two fragments are colinear and then diverge centromeric to the SstI site, suggesting that the breakpoint falls just upstream of this restriction site. On Southern blots of patient 1 genomic DNA, a probe (0.7 NP, Fig. 3 *A*) ~8 kb centromeric to this breakpoint hybridized to this 14-kb rearranged fragment in addition to an 11.5-kb fragment. This probe hybridized to only the 11.5-kb fragment in human placental DNA, indicating that this was the germline EcoRI fragment. This 0.7 NP probe was mapped to chromosome 3 using a somatic cell hybrid panel (Table 2). The genomic library from patient 1 was screened with the 0.7 NP chromosome 3 probe in order to obtain the germline chromosome 3 sequences in the vicinity of the breakpoint. The 0.3B2 chromosome 1 probe, which identified the reciprocal translocation (Fig. 2), was used to isolate the reciprocally rearranged chromosomal region. Restriction maps of clones representing the germline and translocated alleles are shown in Fig. 3 *A*.

The 5' region of the SCL locus is proximal to the centromere, and the patient 1 breakpoint is in the 5' (centromeric) portion of the SCL locus. Thus, the 14-kb rearranged EcoRI fragment, which hybridized to the 0.7NP chromosome 3 probe and the 0.3N chromosome 1 probe, consists of chromosome 3 sequences at its centromeric portion and chromosome 1 sequences at its telomeric portion, and therefore represents the derivative 3 chromosome. The reciprocally rearranged 3.5-kb EcoRI fragment identified by the 0.3B2 probe represents the derivative 1 chromosome.

Nucleotide Sequence of the Germline and Translocated Chromosomes. The nucleotide sequence of the junctional region of the two derivative and two germline chromosomes is shown in Fig. 3 *B*. There are several noteworthy features of the sequence surrounding the breakpoint. The translocation was not a perfectly conservative reciprocal translocation, as a 65-bp sequence from chromosome 1 had been duplicated, and is found on both the derivative 1 and 3 chromosomes as well as the germline chromosome 1. In addition, an ~600-bp sequence from chromosome 3 had been deleted. The 65-bp sequence on germline chromosome 1 is flanked by close matches (5/7 and 6/7 nucleotides) for the consensus V(D)J recombinase heptamer recognition sequence (CAC A/T GTG). The 3' heptamer has an AT-rich nonamer-like motif 23 bp downstream, while the 5' heptamer is associated with a nonamer motif 12 bp upstream. This alignment (nonamer-spacer-

A

Figure 3. (*A*) Restriction map of the translocated alleles. Germline chromosome 1, derivative chromosomes 1 and 3 from the t(1;3), and germline chromosome 3 EcoRI fragments were subcloned into pBluescript (plasmids p3.3, pR8, p1.5, and p1C, respectively). The restriction maps are oriented with the centromere on the left, and the telomere on the right. Filled bars indicate chromosome one sequences, and hatched bars indicate chromosome three sequences. Probes 0.3B2, 0.3N, and 0.7NP are indicated. Restriction sites are indicated by: R, EcoRI; S, SstI; and B, BamHI. (*B*) Nucleotide sequence at the breakpoint(s). Chromosome 1 sequences are unboxed; chromosome 3 sequences are boxed. The 65-bp duplicated region of chromosome 1 is offset by gaps. The heptamer motifs are in bold type and underlined. The nontemplated nucleotides added at the breakpoints are indicated in lower-case letters. The inverted repeat (6/7 or 10/14 nucleotides) region at the derivative 3 junction is indicated (+). The ~600 bp of chromosome 3 deleted by the translocation event is offset by slashes. The tract of alternating purine and pyrimidine (Pu/py) residues disrupted by the derivative 1 breakpoint is evident.
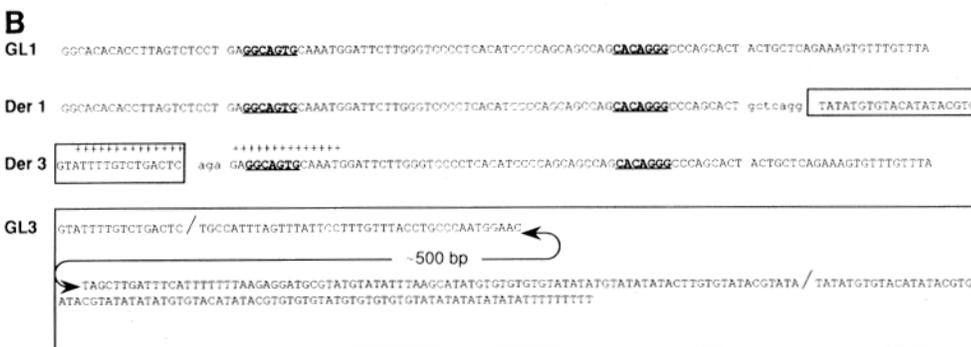
heptamer coding sequence heptamer-spacer-nonamer) is reminiscent of the sequences flanking D (diversity) regions in the TCR δ and β loci. There are short stretches of nontemplated nucleotides, consistent with N-region addition, seen at both the derivative 1 and 3 breakpoint junctions. The germline chromosome 3 and the derivative 1 sequences diverge within a 94-bp tract of alternating purine and pyrimidine residues that is predicted to form a region of "Z" DNA, reported to be prone to recombination events (21–24). There are thus two distinct breakpoints on the germline chromosome 3 separated by ~600 bp, which has been deleted, presumably at the time of translocation. At the junction of chromosome 3 and 1 sequences on the derivative 3 chromosome an inverted repeat is formed, with 6/7 and 10/14 nucleotides forming perfect homology (Fig. 3 *B*).

Leukemic Blasts from Patient 1 Express High Levels of SCL mRNA. While we do not detect SCL mRNA in peripheral T lymphocytes or thymocytes from normal individuals, T cell ALL cell lines and leukemic blasts from T cell ALL patients often express easily detectable levels of SCL mRNA; samples that demonstrate SCL breakpoints uniformly express high levels of SCL mRNA (1, 2, 7, 9, 13). We used Northern blot analysis to investigate whether the leukemic blasts from either of these two patients with t(1;3) expressed an SCL transcript. The patient with an SCL disruption (patient 1)

expressed SCL at easily detectable levels. In contrast, patient 2, with a germline SCL locus, did not express levels of SCL mRNA that could be detected by Northern blot analysis (Fig. 4).

A Novel Transcript Unit Is Detected within 8 kb of the SCL Breakpoint. In an effort to detect a transcript unit on chromosome 3 disrupted by the translocation, we isolated probes from chromosome 3 flanking the SCL breakpoint detected in patient 1. However, all of the probes isolated from the region immediately centromeric or telomeric of the breakpoint contained highly reiterated sequences, and therefore were not useful for screening Northern blots. Reduced stringency hybridization demonstrated that a single-copy chromosome 3 probe (0.7NP), located 8 kb centromeric to the breakpoint, was conserved across species (monkey, mouse, hamster, chicken; data not shown), and therefore a likely candidate gene. Two hybridizing mRNA species, of ~4.0 and ~2.5 kb, were expressed at varying levels in RNA from thymus and the T cell lines SUPT1 (25) and Molt4 (26) (Fig. 5). This probe also hybridized to a 2.5-kb mRNA present in leukemic blasts from patient 1 (data not shown). Nucleotide sequence analysis of a partial cDNA from an SUPT1 library and comparison with the GenBank and EMBL databases reveals that this gene has not been previously cloned and reported (P. D. Aplan and I. R. Kirsch, unpublished results).

**Table 2.** *Somatic Cell Hybrid Mapping of the t(1;3)(p34;p21) Breakpoint*

Cell line	Hybridization to 0.7 NP	Percentage of cells with human chromosome:																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
GM/10253	+	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM/NA09926	+	69	75	75	65	2	88	85	69	0	68	0	2	77	73	93	2	81	75	84	96	2	4	2	0
GM/NA09927	+	69	83	75	77	0	93	79	73	0	82	0	0	77	79	90	0	81	73	87	89	0	0	0	0
GM/NA09928	+	0	84	58	0	48	32	0	66	0	2	0	0	4	76	92	0	98	0	28	0	70	82	0	78
GM/NA09929	+	0	0	61	59	0	43	2	49	0	0	33	49	0	59	2	0	96	0	2	31	0	0	2	0
GM/NA09930	+	0	34	62	4	12	0	26	4	0	0	6	22	56	82	12	0	86	78	0	22	82	76	6	8
GM/NA09933	+	50	0	84	16	54	76	92	54	0	6	0	50	84	78	92	0	88	70	80	32	94	88	0	32
GM/NA09935	+	0	0	52	10	28	12	0	0	0	8	0	22	74	72	0	0	93	59	0	9	91	71	0	0
GM/NA09937	+	0	0	54	38	0	62	54	70	0	4	0	42	0	70	60	0	96	66	0	0	0	0	0	0
GM06318B	–	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
GM10479	–	0	0	0	0	0	0	0	0	0	0	0	0	0	88	0	0	0	0	0	0	0	0	0	0
GM/NA09925	–	74	24	0	74	76	60	82	78	0	0	4	68	6	86	78	14	98	96	46	84	0	76	0	0
GM/NA09931	–	0	0	0	0	26	0	78	0	0	46	0	64	0	100	0	0	100	0	0	78	90	0	0	14
GM/NA09932	–	0	0	0	68	86	46	0	80	0	2	28	26	0	0	0	0	96	0	2	0	92	0	0	0
GM/NA09934	–	0	50	0	0	83	79	4	87	0	0	77	87	0	2	89	0	90	89	0	91	89	2	0	0
GM/NA09936	–	0	0	0	18	0	46	70	10	0	16	34	0	2	88	2	0	100	0	44	24	0	18	0	0

Human/rodent somatic cell hybrid DNA was obtained from the NIGMS Human Genetic Mutant Cell Repository (Coriell Institute for Medical Research, Camden, NJ), digested with EcoRI, and Southern transferred. Parental human (NAIMR91), Chinese hamster (NA10908), and mouse (NA00347A) DNA were digested as controls. The blot was hybridized to the 0.7NP probe, and each cell line was scored for the presence or absence of the germline 11.5-kb EcoRI fragment. The percentages of cells with human chromosomes represent averages of cytogenetic analysis for at least 25 cells examined at both first passage and final harvest. A perfect concordance between presence or absence of human chromosome 3 and the 0.7NP probe is evident in this panel.

Discussion

One mechanism by which chromosomal translocations associated with lymphoid neoplasms are thought to transform cells is by "activating", or dysregulating a known or presumptive cellular proto-oncogene. SCL gene disruptions are the most common structural abnormality yet recognized to occur in T cell ALL (6, 7). With this work, we have reported the initial characterization of a new kind of translocation affecting the SCL gene: a t(1;3)(p34;p21). Similar to the previously reported SCL translocations, the t(1;3) translocation disrupting SCL reported here is found in a T cell (as opposed to B cell or myeloid) acute leukemia, results in SCL expression (which
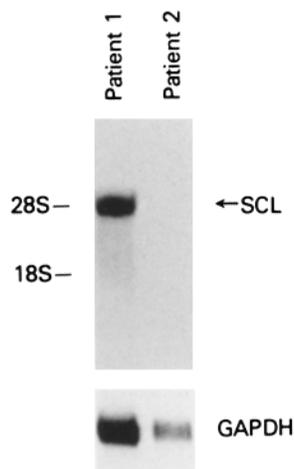
Figure 4. SCL mRNA expression. Total RNA from the leukemic blasts of patients 1 and 2 was size fractionated, Northern blotted, and hybridized to an SCL cDNA probe (0.9 PX of reference 10). A broad band of 4.5–5.0 kb, similar in size to the normal SCL message, is seen in the Patient 1 lane. Migration of the 28S and 18S ribosomal RNA is indicated. Integrity of the RNA is demonstrated by rehybridization to a GAPDH probe.
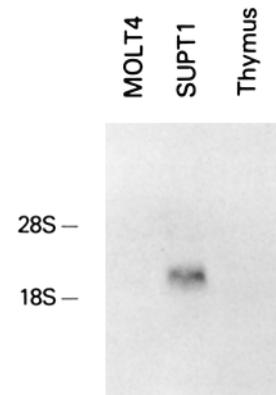
Figure 5. The chromosome 3 probe identifies a transcript. Total RNA from thymus or the T cell lines Molt4 and SUPT1 was size fractionated, Northern blotted, and hybridized to a nonreiterated chromosome 3 probe (0.7NP) from the vicinity of the breakpoint. Migration of the 28S and 18S ribosomal RNA bands is indicated. Bands of ~4.0 and ~2.5 kb are indicated by arrows. Although difficult to appreciate, a band of 2.5 kb is clearly seen in both the Molt4 and thymus lanes on the original autoradiograph.

is not usually detected in T cells), and seems to have been mediated, at least in part, by the action of the V(D)J recombinase complex, without involvement of a TCR locus. The other case with a t(1;3) that we evaluated had a B cell precursor immunophenotype and showed neither SCL gene rearrangements (by conventional genomic Southern blot analysis) nor SCL mRNA expression, suggesting a distant breakpoint at the molecular level and strengthening the observation that SCL gene disruptions are highly specific for T-lineage leukemias.

A well-known mechanism for chromosomal translocation associated with lymphoid neoplasms juxtaposes an antigen receptor (Ig or TCR) gene on one chromosome with a known or presumptive cellular proto-oncogene (such as c-myc or bcl-2) present on the other chromosome (see reference 27 for review). The frequent involvement of antigen receptor loci, which physiologically undergo intralocus V(D)J gene rearrangements in the process of generating immunologic diversity, has led many to speculate that the translocations associated with lymphoid neoplasms are the result of abnormal genomic rearrangements mediated by aberrant V(D)J recombinase activity (see reference 28 for review). Often, although not always, hallmarks of normal V(D)J recombinase activity (29), such as heptamer (sometimes associated with nonamer) signal sequences, nontemplated or "N" region nucleotide addition, and exonucleolytic "nibbling", are found in the vicinity of the breakpoints. In several cases, including one involving the SCL gene (12), where heptamer-like sequences have not been found in the vicinity of the breakpoints, investigators have noted tracts of alternating purine and pyrimidine residues (Pu/Py) within 900 nucleotides of the breakpoints (12, 21, 30). It has been proposed that the alternating Pu/Py residues, which can exist as left-handed or Z DNA, may also be prone to recombination events (possibly targeted because of tertiary chromatin structure) (21–24), and may serve as a functional alternative to heptamer recognition sequences in the context of V(D)J recombinase-associated translocations (21).

Sequence analysis of this t(1;3) shows several of the hallmarks of normal V(D)J recombination, such as heptamer signal sequences on chromosome 1 in the vicinity of both the derivative 1 and 3 breakpoints, along with nontemplated N region nucleotide addition at the breakpoints. Although the germline chromosome 3 does not have heptamer sequences near the breakpoints, there is a remarkable run of 94 consecutive alternating purine and pyrimidine residues, which is disrupted by the derivative 1 breakpoint (Fig. 3 B). Therefore, consistent with previous reports, it is possible that this transloca-

tion was mediated, at least in part, by the V(D)J recombinase aberrantly targeting two cryptic heptamer recognition sequences on chromosome 1 and a region of Z DNA formed by Pu/Py tracts on chromosome 3. The 65 nucleotides of chromosome 1 bracketed by the derivative 1 and 3 breakpoints may have been duplicated in the process of repairing a staggered nick on chromosome 1 generated by the translocation. In addition, the presence of an inverted repeat sequence precisely at the derivative 3 breakpoint suggests that this region of homology may have targeted the derivative 3 breakpoint on chromosome 3. Similar to the mechanism proposed for the SIL/SCL rearrangement, a site-specific deletion that juxtaposes the SIL gene with SCL in 16–26% of patients with T cell ALL (6, 13), the V(D)J recombinase complex can be implicated in the generation of this t(1;3) translocation. This reinforces the notion that when the V(D)J recombinase complex is active, cryptic heptamer sequences may target aberrant V(D)J recombination events between nonantigen receptor genes.

This t(1;3)(p34;p21) translocation has identified a previously undescribed transcript unit on chromosome 3. Since the chromosome 3 transcript identified by the 0.7 NP probe is expressed in thymus and T cell lines (Fig. 5), it is possible that expression of SCL is being driven by the promoter/enhancer of the chromosome 3 gene. This situation would be similar to the other SCL translocations and deletions, where SCL expression seems to be dysregulated by promoter/enhancer elements of the TCRD (1, 2, 9), TCRB (12), or SIL (13, 14, and P.D. Aplan, D.P. Lombardi, and I.R. Kirsch, unpublished results).

With this report, there are now at least four general types of SCL disruptions recognized to be associated with acute leukemia; the SIL/SCL rearrangement resulting in a cytogenetically undetectable interstitial deletion of chromosome 1, and translocations between SCL and TCRD [t(1;14)], TCRB [t(1;7)], and an uncharacterized transcribed locus on chromosome 3p21. The similarities among these genomic rearrangements are striking. All these rearrangements are strongly associated with T cell (as opposed to B cell or myeloid) leukemias, all seem to be mediated, at least in part, by the V(D)J recombinase complex, all juxtapose SCL with a gene normally expressed in thymocytes, and all result in inappropriate expression of SCL mRNA. Though we have yet to demonstrate a direct tumorigenic effect of SCL, it is tempting to speculate that SCL is acting as a dominant oncogene in T cell ALL.

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References

1. Begley, C.G., P.D. Aplan, M.P. Davey, K. Nakahara, K. Tchorz, J. Kurtzberg, M. Hershfield, B.F. Haynes, D.I. Cohen, T.A. Waldmann, and I.R. Kirsch. 1989. Chromosomal translocation in a human leukemic stem-cell line disrupts the T-cell antigen receptor delta-chain diversity region and results in a previously unreported fusion transcript. *Proc. Natl. Acad. Sci. USA.* 86:2031.

2. Finger, L.R., J. Kagan, G. Christopher, J. Kurtzberg, M.S. Hershfield, P.C. Nowell, and C.M. Croce. 1989. Involvement of the TCL5 gene on human chromosome one in T-cell leukemia and melanoma. *Proc. Natl. Acad. Sci. USA.* 86:5039.

3. Begley, C.G., P.D. Aplan, S.M. Henning, B.F. Haynes, T.A. Waldmann, and I.R. Kirsch. 1989. The gene SCL is expressed during early hematopoiesis and encodes a differentiation-related binding motif. *Proc. Natl. Acad. Sci. USA.* 86:10128.

4. Chen, Q., J.-T. Cheng, L.-H. Tsai, N. Schneider, G. Buchanan, A. Carroll, W. Crist, B. Ozanne, M.J. Siciliano, and R. Baer. 1990. The tal gene undergoes chromosome translocation in T-cell leukemia and potentially encodes a helix-loop-helix protein. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:415.

5. Murre, C., P.S. McCaw, and D. Baltimore. 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell.* 56:777.

6. Brown, L., J.-T. Cheng, Q. Chen, M.J. Siciliano, W. Crist, G. Buchanan, and R. Baer. 1990. Site-specific recombination of the tal-1 gene is a common occurrence in human T cell leukemia. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3343.

7. Aplan, P.D., D.P. Lombardi, G.H. Reaman, H.N. Sather, G.D. Hammond, and I.R. Kirsch. 1992. Involvement of the putative hematopoietic transcription factor SCL in T-cell acute lymphoblastic leukemia. *Blood.* 79:1327.

8. Carroll, A.J., W.M. Crist, M.P. Link, M.D. Amylon, D.J. Pullen, A.H. Ragab, B.R. Buchanan, R.S. Wimmer, and T.J. Vietti. 1990. The t(1;14)(p34;q11) is nonrandom and restricted to T-cell acute lymphoblastic leukemia: A Pediatric Oncology Group study. *Blood.* 76:1220.

9. Bernard, O., P. Guglielmi, P. Jonveaux, D. Cherif, S. Gisselbrecht, M. Mauchauffe, R. Berger, C.-J. Larsen, and D. Mathieu-Mahul. 1990. Two distinct mechanisms for the SCL gene activation in the t(1;14) translocation of T-cell leukemias. *Genes Chromosomes & Cancer.* 1:194.

10. Aplan, P.D., C.G. Begley, V.L. Bertness, M. Nussmeier, A. Ezquerra, J. Coligan, and I.R. Kirsch. 1990. The SCL gene is formed from a transcriptionally complex locus. *Mol. Cell. Biol.* 10:6426.

11. Chen, Q., C.Y.-C. Yang, J.T. Tsan, Y. Xia, A.H. Ragab, S.C. Peiper, A. Carroll, and R. Baer. 1990. Coding sequences of the tal-1 gene are disrupted by chromosome translocation in human T cell leukemia. *J. Exp. Med.* 172:1403.

12. Fitzgerald, T.J., G.A.M. Neale, S.C. Raimondi, and R.M. Goorha. 1991. c-tal, a helix-loop-helix protein, is juxtaposed to the T-cell receptor beta chain gene by a reciprocal chromosomal translocation: t(1;7)(p32;q35). *Blood.* 78:2686.

13. Aplan, P.D., D.P. Lombardi, A.M. Ginsberg, J. Cossman, V.L. Bertness, and I.R. Kirsch. 1990. Disruption of the human SCL locus by "illegitimate" V(D)J recombinase activity. *Science (Wash. DC).* 250:1426.

14. Aplan, P.D., D.P. Lombardi, and I.R. Kirsch. 1991. Structural characterization of SIL, a gene frequently disrupted in T-cell acute lymphoblastic leukemia. *Mol. Cell. Biol.* 11:5462.

15. Raimondi, S.C., E. Privitera, D.L. Williams, A.T. Look, F. Behm, G.K. Rivera, W.M. Crist, and C.-H. Pui. 1991. New recurring chromosomal translocations in childhood acute lymphoblastic leukemia. *Blood.* 77:2016.

16. Williams, D.L., A. Harris, K.J. Williams, M.J. Brosius, and W. Lemonds. 1984. A direct bone marrow chromosome technique for acute lymphoblastic leukemia. *Cancer Genet. Cytogenet.* 13:239.

17. Harnden, D.G., and H.P. Klinger. 1985. ISCN: An International System for Human Cytogenetic Nomenclature; published in collaboration with *Cytogenet. Cell Genet.* Karger, Basel, Switzerland. 117 pp.

18. Davis, L.G., M.D. Dibner, and J.F. Battey. 1986. Basic Methods in Molecular Biology. Elsevier Science Publishing Co., Inc., New York. 388 pp.

19. Southern, E.M. 1975. Detection of specific DNA sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503.

20. Feinberg, A.P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6.

21. Boehm, T., L. Mengle-Gaw, U.R. Kees, N. Spurr, I. Lavenir, A. Forster, and T.H. Rabbitts. 1989. Alternating purine-pyrimidine tracts may promote chromosomal translocations seen in a variety of human lymphoid tumours. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:2621.

22. Rich, A., A. Nordheim, and A.H.-J. Wang. 1984. The chemistry and biology of left-handed Z-DNA. *Annu. Rev. Biochem.* 53:791.

23. Wells, R.D. 1988. Unusual DNA structures. *J. Biol. Chem.* 263:1095.

24. Holliday, R. 1989. Untwisting B-Z DNA. *Trends Genet.* 5:355.

25. Hecht, F., R. Morgan, B.K.M. Hecht, and S.D. Smith. 1984. Common region on chromosome 14 in T-cell leukemia and lymphoma. *Science (Wash. DC).* 226:1445.

26. Huang, C.C., Y. Hou, L.K. Woods, G.E. Moore, and J. Minowada. 1974. Cytogenetic study of human lymphoid T-cell lines derived from lymphocytic leukemia. *J. Natl. Cancer Inst.* 53:655.

27. Rabbitts, T.H., and T. Boehm. 1991. Structural and functional

chimerism results from chromosomal translocation in lymphoid tumors. *Adv. Immunol.* 50:119.

28. Tycko, B., and J. Sklar. 1990. Chromosomal translocations in lymphoid neoplasia: A reappraisal of the recombinase model. *Cancer Cells.* 2:1.

29. Lieber, M.R. 1991. Site-specific recombination in the immune system. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 5:2934.

30. Thirman, M.J., R.C. Burnett, J.D. Rowley, and M.O. Diaz. 1992. LCK gene involvement in the t(1;7)(p34;q34) from the T-cell acute lymphoblastic leukemia cell line HSB-2: analysis of LCK gene expression. *Proc. Am. Assoc. Cancer Res.* 33:358.