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Genetics

Acute leukemias of different lineages have similar *MLL* gene fusions encoding related chimeric proteins resulting from chromosomal translocation

(cancer/leukemia/translocation/gene fusion/transcription factor)

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ABSTRACT **The *MLL* gene, on human chromosome 11q23, undergoes chromosomal translocation in acute leukemias, resulting in gene fusion with *AF4* (chromosome 4) and *ENL* (chromosome 19). We report here translocation of *MLL* with nine different chromosomes and two paracentric chromosome 11 deletions in early B cell, B- or T-cell lineage, or nonlymphocytic acute leukemias. The mRNA translocation junction from 22 t(4;11) patients, including six adult leukemias, and nine t(11;19) tumors reveals a remarkable conservation of breakpoints within *MLL*, *AF4*, or *ENL* genes, irrespective of tumor phenotype. Typically, the breakpoints are upstream of the zinc-finger region of *MLL*, and deletion of this region can accompany translocation, supporting the der(11) chromosome as the important component in leukemogenesis. Partial sequence of a fusion between *MLL* and the *AFX1* gene from chromosome X shows the latter to be rich in Ser/Pro codons, like the ENL mRNA. These data suggest that the heterogeneous 11q23 abnormalities might cause attachment of Ser/Pro-rich segments to the NH_2 terminus of *MLL*, lacking the zinc-finger region, and that translocations occur in early hematopoietic cells, before commitment to distinct lineages.**

Specific chromosomal translocations in acute leukemias suggest that a variety of oncogenes are activated by them, which lead to cancer. DNA cloning of translocation breakpoints from many acute lymphoblastic leukemias [ALLs; (B-cell or T-cell ALL) (B-ALL or T-ALL)] in humans (1) has revealed several different genes, and animal-model systems have confirmed that some of the identified genes can participate in tumorigenesis (2–7). Most of these genes seem able to elicit only tumors of a particular hematopoietic lineage, as for example *RBTN2*/Ttg-2 (8–13) or *TAL1*/SCL (14–16), which are only found in childhood T-ALL. This fact suggests that one effect of translocations in acute leukemia is to activate cellular genes that participate in determination of tumor lineage.

A different situation seems to pertain to the translocations involving the long arm of chromosome 11, band q23, which is prone to translocation with a variety of different chromosomes. Unusually tumors of lymphoid and nonlymphoid lineages have been described, with some correlation of translocation to lineage (17). For example, t(9;11)(p22;q23) mainly occurs in acute nonlymphoid leukemia (ANLL) but does occur in early B-ALL. The latter phenotype is more prevalent with the t(4;11)(q21;q23), but cases of ANLL have also been described with this translocation. Further the t(11;19)(q23;p11) is seen in early B-ALL, common-ALL (c-ALL), ANLL, and occasionally in T-ALL. Other translocations, such as t(10;11)(p11-15;q23) and t(11;17)(q23;q21), are found in ANLL tumors that are otherwise indistinguishable from those discussed above.

Recently, a gene on chromosome 11q23 designated *MLL* (also *ALL-1* and *HRX*) (18–23) has been described that is rearranged in t(4;11) and t(11;19) (18–22, 24–27). These translocations have been studied at the molecular level (18, 19, 21), and the breakpoints create a fusion of the *MLL* gene with a gene called *AF4* in the case of the t(4;11) (19) or *ENL* in t(11;19) (21). The *MLL* breakpoints seem restricted in both t(4;11) and t(11;19) around exon 7 and 8. No information on the comparable breaks in *AF4* or *ENL* genes is yet available. In this paper we determine the breakpoint within *MLL* of several additional translocations [namely, t(1;11), t(6;11), t(9;11), t(10;11), t(11;17), t(11;22)], two different t(X;11), and two interstitial deletions of 11q, as well as a panel of t(4;11) and t(11;19) patient samples. All major hematopoietic lineages are represented. Restricted breakpoints occur in *MLL* and *AF4* and *ENL*, irrespective of tumor phenotype. Our data suggest that gene fusion of *MLL* occurs in multipotent hematopoietic precursor cells.

MATERIALS AND METHODS

DNA Analysis of Acute Leukemia Samples: Filter Hybridization. Ten micrograms of genomic DNA was digested with restriction enzyme, and the digestion products were separated on 0.8% agarose gels. The gels were blotted (28) onto nylon membranes and hybridized as described (28) with either the myeloid-lymphoid leukemia (MLL) genomic probe P4 (27) [containing exon 8, a 5′ MLL probe (EX5/GEB13) which was prepared by reverse transcriptase (RT)-PCR from RS411 cDNA extending from residues 3869 to 4443 of the published *MLL* sequence (19, 21], or a 3′ MLL probe (VAR8F/EX11B), which was also prepared by RT-PCR from RS411 cDNA, extending from residues 4318 to the *Bam*HI site, in exon 11, at residue 4609 of the published *MLL* sequence.

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Abbreviations: ALL, acute lymphoblastic leukemia; T-ALL, T-cell ALL; B-ALL, B-cell ALL; ANLL, acute nonlymphoid leukemia; RAP, reverse-anchored PCR method; MLL, myeloid-lymphoid leukemia; RT, reverse transcriptase.

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PCR and Sequence Analysis of MLL mRNA Fusions: RT-PCR of t(4;11) and t(11;19) Junctions. mRNA was prepared from frozen samples of leukemic cells by addition of 150 units of RNasin (Promega) before thawing. The cells were thawed as quickly as possible with continuous shaking and lysed with the addition of Tris·HCl (pH 7.5)/0.3 M RT/0.15 M NaCl/1.5 mM $MgCl_2$/0.5% Nonidet P-40. The nuclei were pelleted (these were kept to prepare DNA for genomic analysis), and RNA was prepared from the cytoplasmic fraction by adding SDS to 1.5% and phenol-chloroform extraction. The RNA-containing aqueous phase was concentrated by ethanol precipitation.

cDNA copies were prepared with total RNA using the primer N6CAS [5'-GATGCGGCCGCCTCGAGCTCANNNNN-3' (N = A, G, C, or T)] for reverse transcription at 42°C for 60 min with 3 μM primer and total RNA at 250 μg/ml in standard buffer (29). The N6CAS primer was used in preference to oligo(dT) priming because in some cases the RNA quality was low and the utilization of the randomizing primer facilitated heterogeneous priming sites along the length of the mRNA and also tagged each cDNA molecule with a specific priming site (complementary to primer CAS, 5'-GATGCGGCCGCCTCGAGCTCA-3') for subsequent PCR amplification. Amplification across the t(4;11) junction was done with PCR reactions (30) using EX5UNP (5'-CCTGAATCCAAACAGGCCACCACT-3', starting at position 3841 in the previous sequence) (21) plus AF4B1 (5'-GATGAGCTCACTGAGCTGAAGGTCGTCTTC-3', corresponding to residues 229–251 in Fig. 3 and incorporating a *Sac* I restriction site for cloning). Amplifications were done with the "touch down" method (31) by using the program 95°C for 1.5 min, reduction from 63°C to 56°C by 2° steps (1.5 min and two cycles at each temperature), 55°C for 1.5 min (14 cycles), and 72°C to a total of 30 cycles (2.5 min). A second round of 30-cycle PCR was done as above with EX5NP [5'-GATAAGCTTCCAGGAAGTCAAGCAAGCAGG-3', starting at position 3869 in the previous sequence (19, 21) and incorporating a *Hin*dIII restriction site for cloning] plus AF4B1. Amplification across the t(11;19) junction was done with PCR reactions using EX5UNP plus ENLB2 [5'-GATGGATCCACGAAGTGCTGGATGTCACAT-3', corresponding to residues 182–205 in the previous sequence (21) and incorporating a *Bam*HI restriction site for cloning]. Amplifications were done with the touch-down program. A second round of 30-cycle PCR was done with EX5NP plus ENLB2. The PCR products of the t(4;11) or t(11;19) junctions were cleaved with *Hin*dIII (EX5NP) and *Sac* I (AF4B1) or *Bam*HI (ENLB2) and cloned into Phagescript (Stratagene); the sequence of the junctions was obtained with *MLL* exon 5 (5'-CTCCTAGTGAGCCCAAG-3'), exon 5–6 (5'-CAGAATCAGGTCCAGAG-3'), exon 7 (5'-CAGATGGAGTCCACAGG-3'), or exon 8 (5'-TGGGAGGCTTAGGAATC-3') primers.

Reverse-Anchored PCR (RAP) to Sequence der(11) Fusion Transcripts. To clone and sequence the der(11) fusion product, which consists of 5' *MLL* and 3' unknown sequences, cDNA, prepared with the N6CAS primer (see above), was used for PCR amplification reaction with CAS plus EX5NP primers for 30 cycles as above. This PCR product mixture was subjected to a further 30 PCR cycles with CAS primer plus either primer EX5NP (exon 5) or primer VAR7F (located at the end of exon 7, sequence 5'-GATAAGCTTCAGGATCAGAGTGGACTTTAAG-3') or primer VAR8F (located at the end of exon 8, sequence 5'-GATAAGCTTGCCAGTAGTGGGCATGTAGAG-3'), depending on the position of the breakpoint within *MLL* (determined by filter-hybridization data) and all having *Hin*dIII restriction sites to allow cloning of the PCR products. After the second round of amplification, the PCR reaction was passed through a G50 spin column, size-fractionated on agarose, digested with *Hin*dIII (primers EX5NP, VAR7F, and VAR8F) and *Not* I (primer CAS), cloned into Phagescript, and sequenced with an appropriate primer (see above). The *AF4* sequence was extended by RAP using the published *AF4* sequence (19) to design primers. The first round of PCR amplification used primer AF4UNP (5'-CCTACTCCAATGAAGTCCATTGTG-3', starting at position 2 in Fig. 3) and primer CAS; the second 30 cycles used primer AF4NP (5'-GATAAGCTTTGACAGCAATACATACGCCTAG-3', starting at position 67 in Fig. 3 and incorporating a *Hin*dIII restriction site for cloning) and primer CAS. The final PCR reaction was cleaved with *Hin*dIII (primer AF4NP) and *Not* I (primer CAS) and cloned into Phagescript. *AF4*-specific clones were detected by using the PCR product of primer AF4FP1 (5'-AAGTTTCCTTTCCCTACAAAGGAC-3', starting at position 108 in Fig. 2) and primer AF4B1, and sequence data were obtained with the primers AF4SP1 (5'-GAACGTCATCCATGCTC-3' starting at position 212 in Fig. 2) and AF4SP2 (5'-CGATCAGCACATTCCAG-3', starting at position 343 in Fig. 2). Double-stranded sequence was obtained by isolation of inserts from relevant clones and recloning in the appropriate M13. AFX1 clones were obtained by RAP with cDNA made from K45 (32) using the *MLL* exon 8 primer together with the CAS primer. The chromosome X origin of the derived cDNA sequence was confirmed with use of somatic cell hybrids carrying only human chromosome X on a rodent background (data not shown).

RESULTS

Rearrangements of *MLL* in Acute Leukemias. Although the *MLL* gene rearrangement has been shown in t(4;11) and t(11;19) (18–22, 24–27), there is no information on other forms of 11q23 abnormality. A range of tumors with 11q23 translocations were available, and these were analyzed for DNA rearrangement by using a probe (P4) containing exon 8 (27) of *MLL* (Fig. 1*B*). Representative *MLL* rearrangements in DNA prepared from tumor material, biopsied from acute leukemia patients with 11q23 translocations, are shown in Fig. 1*A*. All show rearranged bands when the DNA was digested with *Bam*HI, which encompasses exons 5–11 of *MLL* (18, 19, 21) just upstream of the zinc fingers of MLL (19, 21).

A summary of rearrangement positions for the various translocations [determined with different enzymes and additional 5' (EX5/GEB13) and 3' (VAR8F/EX11B) MLL probes, see *Materials and Methods*] is given in Fig. 1*C*, which shows the position of each rearrangement—e.g., t(1;11) sample 3 has a rearrangement within the *Bgl* II–*Eco*RI band. The majority of samples showed rearranged DNA bands when digested with *Bam*HI, although a few samples only showed rearrangements with *Hin*dIII, which places the rearrangement position upstream of *MLL* exon 5. We assume that these rearrangements represent the translocations (confirmed by sequencing the fusion mRNA junction in some cases); therefore, breakage within *MLL* appears to occur in the restricted region irrespective of translocation or tumor phenotype.

Deletion Can Accompany Translocations of *MLL*. There are a number of examples in which deletion of DNA apparently accompanies translocation, identified by rearrangement patterns found with the probe P4, the 5' probe (extending from exon 5 to exon 9), and the 3' probe (extending from exon 8 to exon 11). For example, the t(4;11) sample 411-22 has an mRNA fusion between exon 6 of *MLL* and position b of *AF4* (defined by sequence analysis, see below), yet the P4 probe does not detect a rearranged band in *Bam*HI or *Hin*dIII digests. Conversely, the 5' MLL probe detected rearranged bands with *Hin*dIII, *Bam*HI, and with *Bgl* II. Thus the region of *MLL* exon 8 (the P4 probe encompasses this exon) must have been lost during the chromosomal translocation, and the

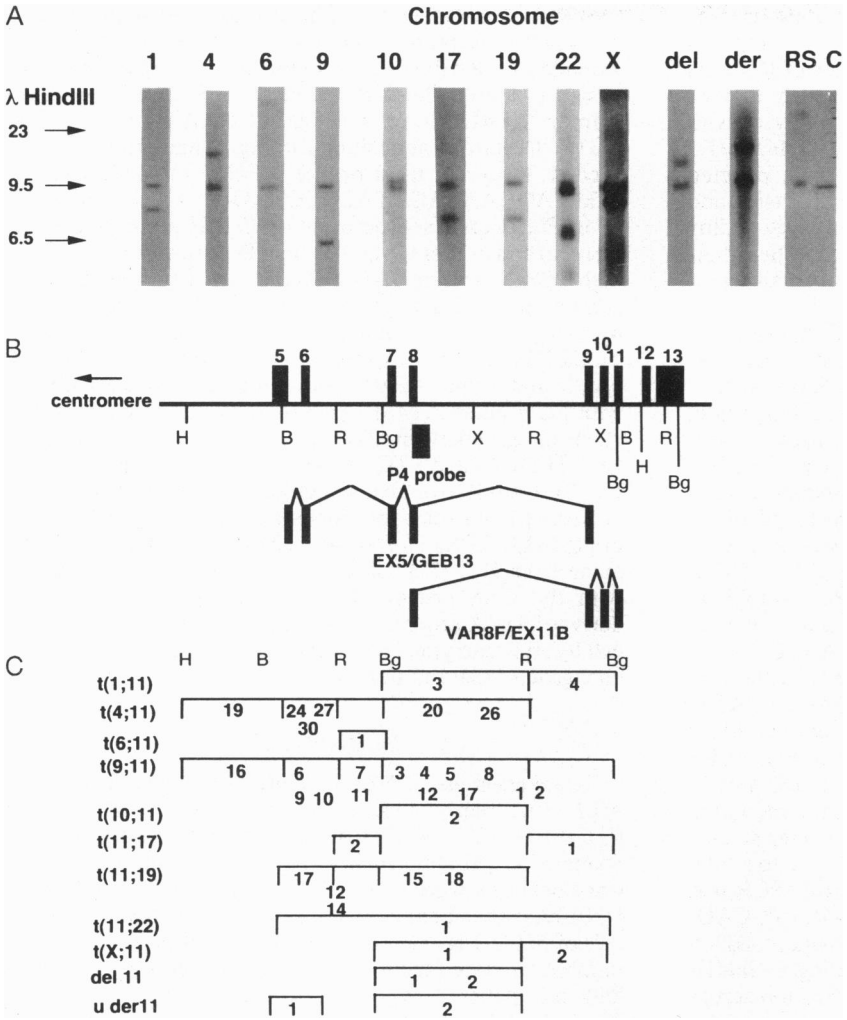

FIG. 1. DNA rearrangement of various 11q23 translocations detected with the 11q23 probe P4. (*A*) DNA was prepared from acute leukemias with each 11q23 translocation, and 10-μg aliquots were digested to completion with *Bam*HI, fractionated on 0.8% agarose, and transferred to nylon membranes. Hybridization was done with randomly labeled P4 probe, and fragment sizes were estimated by coelectrophoresis of λ DNA cut with *Hin*dIII. The map of *MLL* derived from published maps (19, 21) is shown at the bottom with the P4 probe location, and the two relevant *Bam*HI sites are shown. The exons encoding the zinc fingers of MLL are exons 8–11 (19, 21). Only samples for which no sequence data are available are shown. Samples are indicated by the following lanes: 1, 111-4; 4, 411-27; 6, 611-1; 9, 911-3; 10, 1011-2; 17, 1117-1; 19, 1119-17; 22, 1122-1; X, X11-2; del, DEL11-1; der, U11-1; RS, RS411; C, normal control DNA. (*B*) Restriction map of *MLL* based on previous maps (19, 21) together with the locations of P4 (27), 5′ MLL (EX5/GEB13), and 3′ MLL (VARF8F/EX11B) probes. The exons encoding MLL zinc fingers are exons 6–12. H, *Hin*dIII; B, *Bam*HI; Bg, *Bgl* II; X, *Xba* I; R, *Eco*RI. (*C*) Each line refers to the particular translocation, and the number refers to the patient number. Vertical lines indicate restriction sites that delineate the breakpoint positions.

rearrangement position lies between the *Bgl* II site and exon 6. The 3′ probe was also unrearranged, indicating that the deletion extends at least beyond this position in *MLL*. Other examples of t(4;11) samples that have no detectable rearrangement with the P4 probe are 411-24 and 411-30. Both of these pre-early B-ALL samples display rearrangements with the 5′ MLL probe but not with P4, placing the rearrangement between the *Hin*dIII and *Bgl* II sites that span exon 5. Similar examples of deletion are found with the ALL t(11;19) sample 1119-11 and the ANLL-M5 t(1;11) sample. In each case, the deletions encompass at least exon 8 and exon 11.

Consistent Translocation Breakpoints Within *AF4* and *ENL*. The phenotype of acute leukemia with 11q23 abnormality cannot be determined from the restricted breakpoints within *MLL* but the breakpoint on the other involved chromosome may be crucial. This was investigated using RT-PCR to determine the sequence at the fusion junction of a panel of t(4;11) and t(11;19) leukemias. Using known sequences for the *MLL*, *AF4*, and *ENL* genes (19, 21), PCR primers were designed that allowed amplification, cloning, and sequence analysis of the fusion mRNA. The AF4 mRNA fusion consistently occurs in one of three places (Table 1), which are indicated within the sequence of AF4 protein, shown in Fig. 2*A*, within only ≈250 bp of each other in the mRNA. Each fusion maintains the reading frame of *MLL*. The small variation found in *MLL* and *AF4* junctions is typified by samples 411-11 (ANLL), 411-5 (early B-ALL), 411-9 (common-ALL), and 411-2 (early B-ALL). Sample 411-5 also has

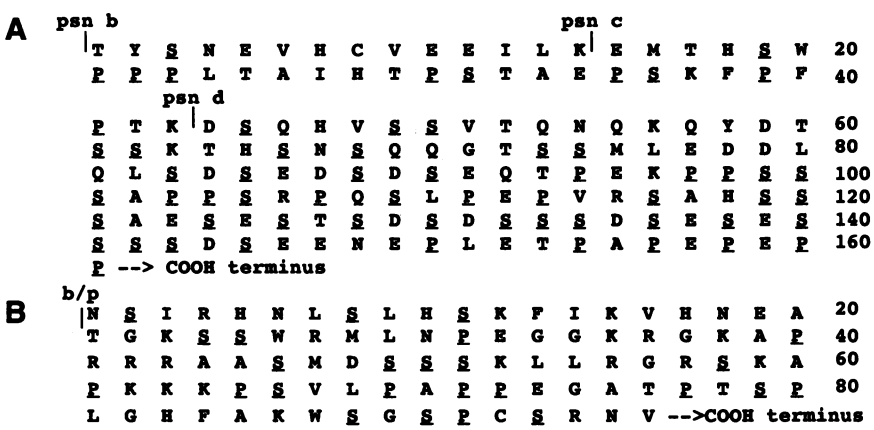

FIG. 2. Partial derived protein sequence obtained from RAP from *AF4* AFX1 cDNA. (*A*) cDNA was prepared by reverse transcription of mRNA from RS411 cells, and PCR amplification was done as described. AF4 clones were identified by hybridization with a PCR probe that corresponds to nt 108–251. Fusion positions (psn) b, c, and d are indicated by vertical bars. Amino acid translation is in the single-letter code, and serine or proline residues are underlined. (*B*) cDNA was prepared by reverse transcription of mRNA from K45 cells. Amino acid translation is in the single-letter code, and serine and proline residues are underlined. Vertical bar at NH_2 terminus indicates position of the fusion of *AFX1* with *MLL* from the der(11) chromosome (b/p).

Table 1. Translocation data of *MLL* in t(4;11)

t(4;11)(q21;q23)		Breakpoint		Probe		
411	Patient	*MLL*	*AF4*	P4	5′	3′
2	Pre-B-ALL	7	d			
3	Pre-pre-ALL	7	b			
4*	Pre-pre-B-ALL	6	b			
5*	Pre-B-ALL	6	b			
6*	Pre-pre-ALL	6	b			
7*	Pre-pre-B-ALL	7	c			
8	Pre-B-ALL	8	b			
9	Pre-pre-ALL	8	c			
10	Pre-B-ALL	7	c			
11	ANLL-M5	7	b			
12	Pre-pre-B-ALL	8	b			
13	Pre-pre-B-ALL	6	b			
14	Pre-pre-ALL	7	b			
15*	Pre-pre-B-ALL	7	b			
16	Pre-pre-B-ALL	7	b			
17	ANLL-M2	nd	nd	R	R	R
19	ALL-L1	nd	nd	R	R	R
20	ALL-L1	nd	nd	R	R	R
21	ALL-L1	nd	nd	G	R	G
22	Pre-pre-B-ALL	6	b	G	R	G
23	Pre-B-ALL	6	b	R	R	R
24	Pre-pre-B-ALL	nd	nd	G	R	G
25	Pre-pre-B-ALL	6	b			
26	Pre-pre-ALL	nd	nd	R	R	R
27	Pre-pre-B-ALL	nd	nd	R	R	R
28	Pre-pre-B-ALL	6	b	R	R	R
30	Pre-pre-B-ALL	nd	nd	G	R	G
32	Pre-pre-B-ALL	7	b			
33*	Pre-pre-B-ALL	6	c			
34	Pre-pre-ALL	6	b			

Patient number and disease diagnosis are shown with RT-PCR sequence or filter hybridization data [using P4, 5′ (EX5/GEB13) or 3′ (VAR8F/EX11B) MLL probes; see Fig. 1]. Only restriction enzyme data are given for those t(4;11) samples for which sequence data are unavailable (except in those cases where a deletion is also detected), and only *Bam*HI data are indicated. Breakpoint junctions of *MLL* are indicated by numbers corresponding to known exons, and breakpoint junctions of *AF4* are indicated by letters presumed to be exon boundaries. G, unrearranged; R, rearranged; nd, not determined by RT-PCR sequence.
*Adult leukemia.

an extra CAG codon that appears between *MLL* and the previously reported *AF4* break (position b) in a t(4;11) sample (19), suggesting a minor variation in splice junction. Sample 411-2 breaks at an unusual *AF4* position, and this could be an aberrant splice to within an *AF4* exon; the reading frame of the fusion protein is nonetheless maintained.

The *ENL* breakpoint position is completely conserved in all tumors analyzed (designated position b, see Table 2) and is identical with that reported (21). Indeed, in three different lineage tumors, the *MLL* exon 7 is joined to *ENL* position b. The cumulative data in Table 2 show fusion of exons 6, 7, and 8 of *MLL* with position b of *ENL*, even though *ENL* is associated with several phenotypes at diagnosis.

The *AFX1* Gene Encodes a Serine-Proline-Rich Sequence. The complete mRNA sequence of the chromosome 19 fusion partner *ENL* has been reported (21), showing a sequence rich in serine and proline codons. To investigate the possible generality of this finding, partial sequence from the fusion mRNA of *MLL* and *AFX1* resulting from t(X;11)(q13;q23) and a partial extension of the sequence of *AF4* (19) were obtained. A 3′-RAP was designed (see *Materials and Methods*) to allow the der(11) transcripts to be amplified and sequenced [the der(11) was the primary target because current evidence suggests that this is crucial to tumor occurrence

Table 2. Translocation data of the *MLL* gene in t(11;19)

t(11;19)(q23;p11)		Breakpoint		Probe		
1119	Patient	*MLL*	*ENL*	P4	5′	3′
1	Pre-B-ALL	8	b			
2	Pre-B-ALL	7	b			
3	Pre-B-ALL	8	b			
4	Pre-B-ALL	8	b			
5	Early B	8	b			
6	Pre-pre-B-ALL	8	b			
8	Nk	6	b			
9	Pre-pre-B-ALL	8	b			
10	ALL-L1	nd	nd	R	R	R
11	ALL	nd	nd	G	R	G
12	ALL-L1	nd	nd	R	R	R
13	ML/LBL	nd	nd	R	R	R
14	ALL-L1	nd	nd	R	R	R
15	T-cell lymphoma	nd	nd	R	R	R
17	ANLL	nd	nd	R	R	R
18	T-ALL	7	b			

Only restriction enzyme data are given for those t(11;19) samples for which sequence data are unavailable (except in those cases where a deletion is also detected), and only *Bam*HI data are indicated. Breakpoint junctions of *MLL* are indicated by numbers corresponding to known exons, and breakpoint junctions of *ENL* are given as letters presumed to be exon boundaries. G, unrearranged; R, rearranged; nd, not determined by RT-PCR sequence.

(33)]. In this way, cDNA extending from exon 5 of *MLL* to unknown sequence from the der(11) chromosome was obtained (Fig. 2). AF4 protein sequence was from cDNA made from RS411 cells (Fig. 2*A*) and AFX1 was from K45 cDNA (Fig. 2*B*). Both sequences indicate proteins rich in serine-proline residues. This is similar to the situation with the *ENL*-derived protein (21) and raises the possibility that Ser-Pro-rich sequences are a common feature of the proteins joined to MLL in the acute leukemias with 11q23 abnormalities.

DISCUSSION

Rearrangements of *MLL* in Diverse Translocations. Cytogenetic analysis of acute leukemias has revealed a large number of translocations involving 11q23 in hematopoietic tumors of several phenotypes (17). The *MLL* gene on chromosome 11, band q23, is involved in these translocations as judged by fluorescence *in situ* hybridization (25), filter hybridization (20, 22, 24, 26, 27), and gene cloning (18, 19, 21). In the latter, it was shown that the translocations t(4;11) and t(11;19) break the *MLL* gene and genes on chromosomes 4 and 19, called *AF4* and *ENL*, respectively (19, 21). In this paper, rearrangement of *MLL* (taken to indicate position of a chromosomal translocation) is shown in eight different 11q23 translocations, not including t(4;11) and t(11;19) cases, and two deletions.

A combination of different restriction enzymes in hybridizations allowed the *MLL* rearrangements in the various tumors to be fairly precisely defined (19, 21) (see Table 1 and Fig. 1*B*). All samples with rearrangements in *MLL* have breaks around exons 5–11 of the gene. This result means that the der(11) chromosome, thought crucial to tumor development (33), will lack the zinc fingers of MLL encoded by exons 8–12 (18, 19, 21). The frequency of deletions within the *MLL* gene, accompanying the chromosomal translocations, might render invalid conclusions about noninvolvement of the *MLL* region if only one probe and one enzyme digestion are used for the assessment. From a practical standpoint, the lack of variation in the chromosomal translocation breakpoints means that both diagnosis and assessment of minimal residual disease may be aided by RT-PCR methods.

Tumor Phenotype and *MLL* Translocations. The most frequent translocations of 11q23 are the t(4;11), t(11;19), and t(9;11). Rearrangement of all but one of these samples [a t(4;11) sample, 411-17, which was diagnosed as ANLL] was observed (Table 1). There is a tendency for each of these three major translocations to be found in particular lineages—for example, the t(9;11) is prevalent in ANLL-M5, whereas B-cell lineage tumors are mainly associated with the chromosome 4 and 19 partners. The precise position of the *MLL* rearrangement revealed no obvious correlation to cell type of the tumor. The group of ANLL t(9;11) cases has rearrangements between exon 4 and exon 9 with most clustered around exons 6–9, as do the predominantly pre-B-ALL tumors with t(4;11) (see Fig. 1*C*). Using a RT-PCR-based procedure, we found involvement of *MLL* exon 7, although fusions of exons 6 and 8 also occur. No obvious distinction could be drawn between breakpoints in the infant, childhood, or adult t(4;11) tumors, and neither the *MLL* nor *AF4* or *ENL* breakpoints entirely correlates with tumor phenotype. This fact is well illustrated for t(11;19) in which B-cell, T-cell, and nonlymphocytic lineages are afflicted by identical fusion points.

The data indicate that the various 11q23 chromosomal abnormalities occur in multipotent cells with the potential to differentiate along the various hematopoietic pathways. In most examples, the *MLL* fusion from the der(11) chromosome will lack part, or all, of the zinc fingers. In addition, in some cases, deletions of the zinc fingers occur, suggesting that this loss, normally associated with der(11), is the crucial consequence of the translocations. The mechanism of the deletions at 11q23 is unknown, but nonreciprocal translocations in acute leukemias are unusual (34).

The previous sequence data on *ENL* (21) and the data presented here on *AFX1* and *AF4* show that these genes encode proteins with Ser-Pro-rich regions. Clearly, examining different fusion proteins to establish the generality of this observation is important. It is nonetheless possible that the general consequence of 11q23 translocation in acute leukemias is attachment of a Ser-Pro-rich sequence at the COOH terminus of a truncated MLL. The der(11) protein product may, therefore, have the DNA-binding A·T hook region of MLL attached to one of a series of related serine/proline sequences (possibly transcriptional activation domains), giving a protein that may bind DNA and interact with the transcriptional machinery to support gene activation.

Note Added in Proof. Complete sequences of *AF4* and *AF9* (35, 36) show that these genes encode Ser-Pro-rich proteins.

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