RAPID COMMUNICATION

The t(9;14)(p13;q32) Chromosomal Translocation Associated With Lymphoplasmacytoid Lymphoma Involves the *PAX-5* Gene

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The t(9;14)(p13;q32) translocation is associated with approximately 50% of lymphoplasmacytoid lymphoma (LPL), a subtype of B-cell non-Hodgkin's lymphoma (NHL). We cloned the chromosomal breakpoint of der (14) from an LPL case (1052) and showed that it involved a junction between 9p13 and the switch μ region of the Ig heavy chain locus (*IgH*) on 14q32. Using a YAC contig spanning 1.5 megabase (Mb), we determined that the 9p13 breakpoint in one case (1052) mapped within a 270-kb restriction fragment containing two previously reported 9p breakpoints associated with a α-heavy chain disease case (MAL) and Ki-1 positive diffuse large cell lymphoma (DLCL) cell line (KIS-1). The same fragment also contained the *PAX-5* gene which encodes a B-cell specific transcription factor involved in the control of B-cell proliferation and differentiation. The breakpoints of KIS-1 and 1052 were mapped within the 5′ noncoding region of *PAX-5*, while the 9p13 breakpoint of MAL mapped 230 to 270 kb upstream to *PAX-5*. In all three cases, the translocation caused the juxtaposition of the *PAX-5* gene to the *IgH* locus in the opposite direction of transcription. When compared with six other DLCL cell lines lacking t(9;14)(p13;q32), the KIS-1 cell line showed an 11-fold overexpression of PAX-5 mRNA and a significantly reduced expression of the *p53* gene, which is normally regulated by PAX-5. Moreover, metaphase and interphase fluorescence in situ hybridization (FISH) analysis using a YAC clone spanning 1 Mb including the *PAX-5* as a probe identified chromosomal translocations in 5 of 7 cases carrying 9p13 translocations. These findings suggest that the *PAX-5* gene is the target of the t(9;14) in LPL whereby its expression may be deregulated by juxtaposition to *IgH* regulatory elements, thus contributing to lymphomagenesis.

NONRANDOM CHROMOSOMAL translocations are typically associated with lymphoid neoplasms where they involve a number of protooncogene loci.[1] In B-cell non-Hodgkin's lymphoma (NHL), translocations deregulate these proto-oncogenes by juxtaposition with regulatory elements of genes expressed normally in the cells, most frequently immunoglobulin genes (*Ig*).[2] Different chromosomal translocations are associated with specific subtypes of lymphoma, and substantial experimental evidence, including studies in transgenic mice, indicates that most these oncogenes contribute specifically to lymphomagenesis. For instance, t(8;14)(q24;q32), t(8;22)(q24;q11), and t(2;8)(p12;q24) lead to the deregulated expression of the *c-MYC* proto-oncogene by juxtaposition to one of the *Ig* loci in 100% of Burkitt's lymphoma.[3] Similarly, rearrangement and deregulation of the antiapoptotic gene, *BCL-2*, caused by t(14;18)(q32;q21) has been shown in 70% to 90% of follicular lymphomas.[4,5] In approximately 50% of mantle cell lymphomas, the t(11;14)(q13;q32) results in the overexpression of *BCL-1*(*Cyclin D1/PRAD1*), which regulates cell-cycle progression, by juxtaposition with the *IgH* gene.[6,7] The *BCL-6* gene, encoding a POZ-zinc-finger transcription factor, is altered by rearrangements or mutations involving its 5′ noncoding region in the majority of diffuse large cell lymphomas (DLCL) and less frequently in follicular lymphomas.[8,9]

The t(9;14)(p13;q32) is associated with 50% of lymphoplasmacytoid lymphoma (LPL)/immunocytoma (Revised European-American Classification of Lymphoid Neoplasms [REAL]; small lymphocytic lymphoma with plasmacytoid differentiation according to the Working Formulation[10,11]). This rare type of lymphoma is characterized by the infiltration of B cells displaying a lymphoplasmacytoid phenotype, the presence of cytoplasmic Ig and serum paraproteins, and an indolent clinical course followed by transformation into large cell lymphoma.[10] LPL are associated with translocations involving chromosome 9p13, which are "promiscuous" in that various chromosome loci including 14q32, 1q25, 3q27, 7q11, 12q13, 12q21, 19p13, and 9q13 can act as reciprocal translocation partners in different cases.[12] Two distinct breakpoint regions linking 9p11 and 9p13 sequencies to the Ig heavy-chain locus (*IgH*) on 14q32 have already been cloned from a case (MAL) of α-heavy chain disease[13] and a Ki-1 positive DLCL cell line (KIS-1),[14] respectively. However, the gene on 9p13 involved in these translocations has not been identified.

In further pursuit of the target gene involved in 9p13 translocation, we analyzed the breakpoint of a typical LPL case and compared its genomic location with two previously identified cases. This approach led us to identify that the translocation breakpoints at 9p13 of all three cases are located within a 270-kb region containing the *PAX-5*(paired homeobox-5) gene.[15,16] A YAC probe spanning this region could also identify chromosomal rearrangements in five out of seven (71%) cases with 9p13 translocations by fluorescence in situ hybridization (FISH) analysis. This structural

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analysis together with the finding of overexpression of the PAX-5 mRNA in the KIS-1 cell line suggest that the t(9;14)(p13;q32) translocation deregulates the expression of the *PAX-5* gene by juxtaposition to the *Ig* regulatory elements.

MATERIALS AND METHODS

Tumor biopsies and cell lines. The biopsy (case 1052, 79-year-old woman) sample analyzed was derived from a retroperitoneal lymph node diagnosed as LPL. Cytogenetic analysis showed that 16 of 16 metaphases had 46, XX, t(9;14)(p13;q32). Detailed clinical characteristics were previously reported.[10] The KIS-1 cell line derived from a Ki-1 positive DLCL with t(9;14)(p13;q32) was previously described.[14,17] The RC-K8 and LY1, 2, 3, 17, 18 cell lines were kindly provided by Dr I. Kubonishi (Kouchi Medical College, Kouchi, Japan) and Dr H.A. Messner (Ontario Cancer Institute, Toronto, Canada), respectively.[18,19]

Southern blot analysis. Southern blot analysis was performed as described previously.[20] A 1.3-kb *Eco*RI-*Eco*RI Cμ probe and a 6.6-kb *Bam*HI-*Hin*dIII JH probe were kindly provided by S.J. Korsmeyer (Washington University, St Louis, MO).

Cloning of chromosomal breakpoints. High-molecular-weight DNA from case 1052 was partially digested with *Sau*3AI and size-fractionated by using a low-melting point agarose gel. DNA fragments ranging from 15 to 23 kb were gel-purified, ligated into the λ dash II vector (Stratagene, La Jolla, CA) and in vitro packaged. Recombinant clones, 5×10^5 were screened by plaque hybridization using the Cμ and JH probes. A human placental DNA library purchased from Stratagene was used for the isolation of 9p13 germline sequences.

Polymerase chain reaction (PCR) primers, probe preparation, and YAC library screening. STS primers (sense 5′-GGAAATGCCCTCCTACTG-3′; antisense 5′-GACAAGCCAGTAGCTAAG-3′) were designed from the 9p13 sequence in the vicinity of MAL case's breakpoint.[13] The amplified 153-bp PCR product was also used as a probe (MAL). PCR primers (sense 5′-CACAGTGGCAACCTTCAG-3′; antisense 5′-TGAGAGACCTGGCTCTAG-3′) derived from the 9p13 sequence surrounding the 1052 breakpoint were prepared based on the sequence of the 1.5-kb *Hin*dIII fragment, which was also used as a probe (1052-1.5H) (Fig 1B). These PCR primers were used to screen a human CEPH YAC DNA pools (Research Genetics, Huntsville, AL). Extraction of YAC DNA was performed according to standard techniques.[21] *PAX-5* gene probes were prepared by reverse transcription (RT)-PCR using primer sets to amplify nucleotides 46 to 675 (PAX-5 5′: sense 5′-GTCCATTCCATCAAGTCC-3′; antisense 5′-GACTCCTGAATACCTTCG-3′) and 1,721 to 2,370 (PAX-5 3′: sense 5′-CATCACCGGACATCTTAG-3′; antisense 5′-CAGAAGTTCTGGCTGTAG-3′) of the PAX-5 cDNA sequence.[15] The pBS8.6BB clone containing pKIS probe, where the KIS-1 breakpoint is located, was described previously.[14]

FISH. Biotin labeling of YAC DNA, chromosome preparation, and FISH were performed as previously described.[22] The signals from the YAC were scored in metaphases and/or interphases from archival fixed suspensions of tumor chromosome preparations. In metaphase preparations, the der (9) and the der (14) chromosomes were identified and analyzed for the presence of hybridizing signal. Presence of the signal on both chromosomes at the breakpoint confirmed rearrangement. In interphase preparation, presence of three signals in a significant proportion of cells was considered as evidence of the chromosomal translocations involving signals represented by the YAC.

Isolation of YAC end clones and construction of the YAC contig. A vectorette-PCR method was used to isolate YAC end probes. Briefly, 500 ng of each YAC DNA were digested with *Rsa* I, *Dde* I, and *Bfa* I and ligated with specific vectorette linkers as described by Riley et al.[23] First-round PCR was performed using 224 primer (5′-CGAATCGTAACCGTTCGTACGAGAATCGCT-3′) and YAC vector-specific HYACD primer (5′-GGTGATGTCGGCGATATAGGCGCCAGCAAC-3′) for the right arm and HYACC primer (5′-GCTACTTGGAGCCACTATCGACTACGCGAT-3′) for the left arm. A seminested second-round PCR was performed using 224 primer and RA-2 primer (5′-TCGAACGCCCGATCTCAAGATTAC-3′) for the right arm and LS-2 primer (5′-TCTCGGTAGCCAAGTTGGTTTAAGG-3′) for the left arm to isolate each YAC end. Localization on chromosome 9 of gel-purified each YAC end probe was confirmed by hybridization to somatic cell hybrid DNAs containing human chromosome 9 (NIGMS Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ). Overlapping between YAC clones were evaluated by Southern hybridization.

Pulsed-field gel electrophoresis (PFGE) analysis. Yeast cells were embedded in agarose plugs by a standard method,[21] and analyzed by PFGE after restriction enzyme digestion using the CHEF DR II (Bio-Rad, Richmond, CA) as described previously.[24] The insert size of each YAC clone was determined by hybridization with total human DNAs to a nondigested plug. Mapping of the YAC insert was achieved by partial digestion of the YAC DNA followed by hybridization with both YAC end (right and left arms) probes. Double digestions were also used for detailed analyses.

Northern blot analysis and cDNA library screening. Total RNA was prepared by the guanidium thiocyanate method and poly(A) RNA was selected using Oligotex coated with poly(T) (Qiagen, Chatsworth, CA). Northern blot analyses were performed as described previously.[20] 1×10^6 plaques from a cDNA library constructed from the BJAB cell line were screened with the PAX-5 5′ cDNA probe.

DNA sequencing. DNA sequencing was performed by the dideoxy chain termination method using an automated sequencing system, ABI 473A (Applied Biosystems, Foster City, CA).

RESULTS

Molecular cloning of the t(9;14) breakpoint region in an LPL case. Southern blot analysis of *Bam*HI-digested DNA from case 1052 using the Cμ probe showed two rearranged bands (Fig 1A). One of these bands (8.0 kb) did not comigrate with the JH band in the *Bam*HI digest, suggesting the existence of a chromosomal breakpoint between the JH and Cμ loci of the *IgH* gene. To clone this fragment, a genomic library was constructed and screened with both Cμ and JH probes. As a result, we isolated a clone (A4) containing the *IgH* Sμ region juxtaposed to an unknown fragment (Fig 1B). FISH analysis showed that clone A4 hybridized to both 14q32 and 9p13 chromosomal loci on normal metaphase spreads (data not shown), indicating that it represented the breakpoint region of the der (14) chromosome. Localization to chromosome 9 was also confirmed by hybridization to a somatic cell hybrid panel with the 1052-1.5H probe (data not shown).

Three NHL-associated 9p13 breakpoints map within a 270-kb genomic region containing the PAX-5 *locus.* Restriction mapping of clone A4 showed that the sequences involved in the breakpoints of case 1052 and those previously reported in MAL and KIS-1 were independent from each other. To investigate the genomic distance between these three breakpoints, we isolated YAC clones spanning this region. For this purpose, we designed STS primers in the

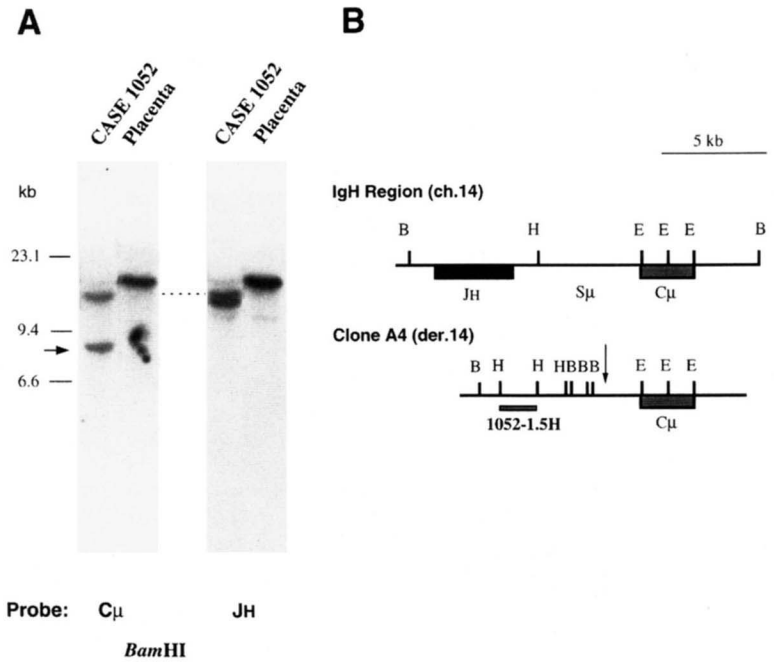

Fig 1. Molecular cloning of the breakpoint of case 1052 carrying t(9;14)(p13;q32) translocation. (A) Southern blot analysis of case 1052 DNA with Cμ and JH probes. Ten micrograms of high-molecular-weight DNA was digested with *Bam*HI, electrophoresed on a 0.8% agarose gel, and blotted. The filter was sequentially hybridized with Cμ and JH probes. The Cμ probe detected two rearranged bands of 14.0 kb and 8.0 kb. The 14.0-kb band comigrated with the JH rearranged band, suggesting it to be a productive rearrangement (shown by dashed line), whereas the 8.0-kb band does not show any comigration, suggesting that it may represent the derivative 14 chromosome (shown by an arrow). Size markers of λ/*Hin*dIII are shown on the left. (B) Restriction map of clone A4 representing derivative 14 chromosome is shown, together with the germline map of the *IgH* locus at 14q32. The arrow indicates the chromosomal breakpoint. B, *Bam*HI; H, *Hin*dIII; E, *Eco*RI.

vicinity of each breakpoint, and screened a CEPH YAC library. Overlapping YAC clones spanning 1.5 Mb were isolated and mapped to the 9p13 locus by FISH analysis (Fig 2, FISH data not shown). A representative YAC contig (Fig 2) was constructed based on the overlapping hybridization pattern given by YAC end probes. Using mapping by PFGE, we found that the three breakpoints were located within 270 kb which also contained the *PAX-5* gene, recently assigned to the 9p13 locus.[25] In addition, two of the breakpoints (case 1052 and KIS-1) mapped within the same 150-kb *Nru* I/*Not* I fragment (Fig 3), while the breakpoint of case MAL mapped 230 to 270 kb upstream to the *PAX-5* locus.

Relationship between 9p13 breakpoints and the PAX-5 *gene.* To precisely determine the genomic distance between the breakpoints and *PAX-5* gene sequences, we isolated the 5′ region of this gene by screening a cDNA library derived from the BJAB cell line. Two kinds of PAX-5 cDNA clones were isolated, which differed in their 5′ termini. One of the cDNA species was identical to that previously reported for PAX-5.[15] The other cDNA species, represented by phage λBJ-11, contained a different 5′ cDNA sequence (Fig 4A). Nucleotide sequencing showed that this clone contained a putative alternative in-frame translation initiation site (ATG), partly corresponding to Kozak's consensus sequence[26] (Fig 4B). The N-terminal sequences of the protein putatively encoded by this cDNA species differ from those previously reported for PAX-5, although the main functional domains of the protein remain conserved. Consistent with these two cDNA species, Northern analysis showed two alternatively spliced transcripts of 10 kb and 10.5 kb, using a PAX-5 probe derived from the 3′ end (Fig 4C). On the other hand, the 10-kb and 10.5-kb transcripts were specifically detected by the pKIS and 1.7B genomic probes, respectively, suggesting that the 5′ region of *PAX-5* contains two alternatively used first exons (1a and 1b in Fig 4D). Most B-cell lines (eg, RC-K8 and KIS-1) express both the 10-kb and the 10.5-kb transcripts, which use the first (ATG1) and second translation initiation site (ATG2), respectively. Only the 10-kb transcript is detectable in the RAMOS cell line by Northern analysis, although the 10.5-kb mRNA species was also identified by RT-PCR analysis (data not shown).

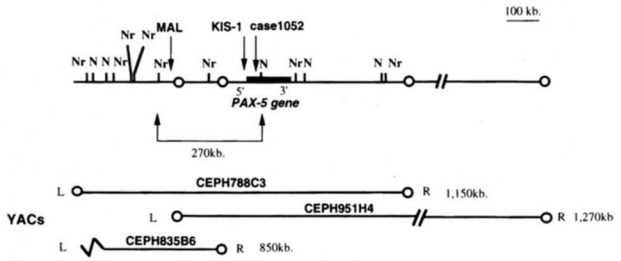

Fig 2. Mapping of three 9p13 breakpoints and *PAX-5* gene within 270 kb on YAC contig. Three representative Mega Yac clones spanning the three breakpoints and *PAX-5* gene are shown. Open circles at the termini of YACs indicate nonchimeric ends confirmed by hybridization to somatic cell hybrid DNA with vectorette-PCR products. Insert sizes were determined by PFGE and the orientation of both the right (R) and left (L) YAC arms are shown. Each arrow indicates the breakpoint locus involved in t(9;14)(p13;q32). N, *Not* I; Nr, *Nru* I.

The breakpoints of KIS-1 and case 1052 mapped 2.5 kb upstream to the ATG1 site and just 5′ to the ATG2 site within exon 1b (Fig 4B and D). In both cases, the *PAX-5* gene was juxtaposed to the *IgH* locus in the opposite direction to transcription as a result of the t(9;14) translocation. In case 1052, t(9;14) removed the enhancer μ (Eμ) element and the ATG1 site of *PAX-5,* while in the case of the KIS-1 cell line, the Eμ sequences and both *PAX-5* exon 1s remain on der (14).

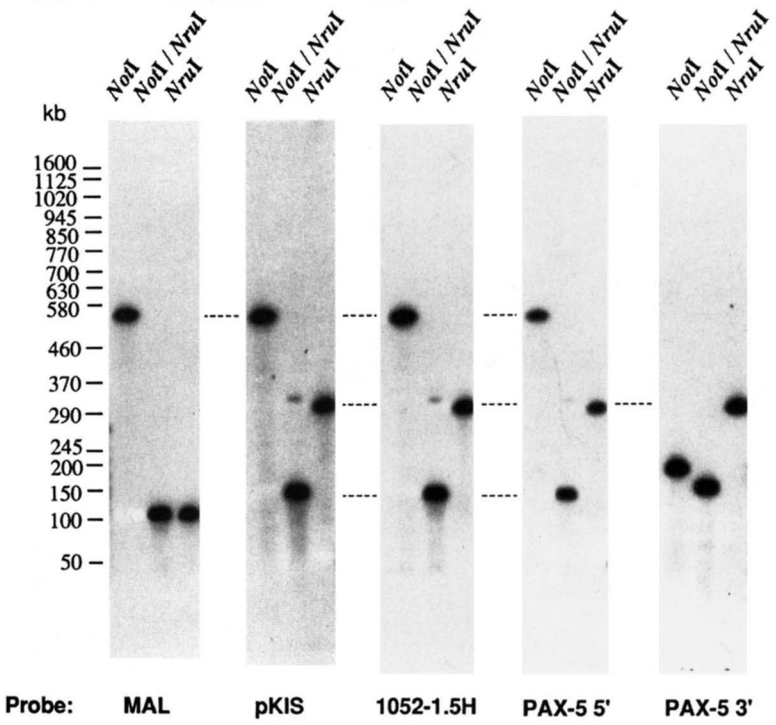

Fig 3. PFGE analysis of the CEPH 788C3. YAC plugs prepared from CEPH 788C3 were digested to completion with *Not* I, *Nru* I, and *Not* I/*Nru* I, and analyzed by PFGE. The same blot was hybridized sequentially with the probes indicated. Comigration of bands after hybridization with the probes is indicated by dashed lines. The size of the molecular-weight marker is shown on the left in kilobases.

The PAX-5 *gene is overexpressed in a cell line carrying t(9;14).* To determine whether the juxtaposition to *IgH* had any effect on *PAX-5* gene expression, we compared PAX-5 RNA levels in KIS-1 and other DLCL cell lines sharing the same histological subtype, but lacking t(9;14)(p13;q32) (Fig 5). Densitometric analysis of the hybridization bands showed an 11-fold overexpression in the KIS-1 cell line when compared with the mean value of the other six cell lines.

The p53 *gene, which is negatively regulated by* PAX-5, *is downregulated in an NHL cell line containing t(9;14) and overexpressing PAX-5.* The expression of the *p53* gene is physiologically modulated by PAX-5.[27] Because the KIS-1 cell line overexpresses PAX-5, we examined whether this was associated with p53 downregulation by analyzing p53 RNA levels in KIS-1 cells versus 6 control NHL lines. The results showed that p53 RNA expression is significantly repressed in KIS-1 cells (Fig 5).

Frequent detection of 9p13 breakpoint in LPL by FISH analysis. To investigate the frequency of the *PAX-5* gene involvement in B-cell NHL, we analyzed 20 LPL cases, including 2 cases with t(9;14) and a panel of 50 lymphoma cases representative of the main NHL subtypes by Southern blot analyses using the MAL, pKIS, and 1.7B probes. However, we did not detect any rearrangement (data not shown), suggesting that none of the probes used was representative of a rearrangement cluster. We then decided to use the CEPH788C3 YAC DNA as a probe for metaphase and/or interphase FISH analysis to examine a larger genomic region spanning the *PAX-5* gene. Seven B-cell NHL cases, including 3 LPL and 2 DLCL cases with t(9;14)(p13;q32) and 2 DLCL cases with other translocations involving 9p13 (Table 1) were selected for this analysis. The results show that 3 LPL and a DLCL case carrying t(9;14), together with 1 DLCL case with a t(3;9)(q27;p13) show rearrangements of 9p13 within the genomic region represented by the YAC clone (Table 1, Fig 6).

DISCUSSION

The t(9;14)(p13;q32) translocation is a recurrent chromosomal abnormality associated with a significant fraction of LPL cases and less frequently with other NHL subtypes.[10] Our results identify a chromosomal subregion at 9p13 which is involved in this translocation in most cases of LPL and provides evidence in a few cases that the chromosomal breakpoints directly involve the *PAX-5* gene. These results have implications for the consequences of t(9;14)(p13;q32) on PAX-5 expression, for the role of *PAX-5* in lymphomagenesis, and for the possible diagnostic use of 9p13 breakpoints in NHL diagnosis.

Consequences of t(9;14)(p13;q32) on PAX-5 *gene expression.* Our results show that 9p13 chromosomal breakpoints are located within a 270-kb genomic region which contains the *PAX-5* gene in three cases. In addition, the fact that the 9p13 breakpoints were detected by the 1-Mb YAC containing the *PAX-5* gene in 6 of 8 NHL cases by FISH analysis indicates that the breakpoints may be in relative proximity to the *PAX-5* gene in most LPL and DLCL cases. In analogy to the chromosomal translocations involving the *IgH* locus and the *BCL-1, BCL-2,* or c-*MYC* loci in other types of NHL,[28-30] it is conceivable that at these distances *IgH* gene regulatory elements such as *IgH* transcriptional enhancers and locus control regions[31] may deregulate *PAX-5* expression. This model is directly supported by the findings in case KIS-1 and case 1052, where the *IgH* locus

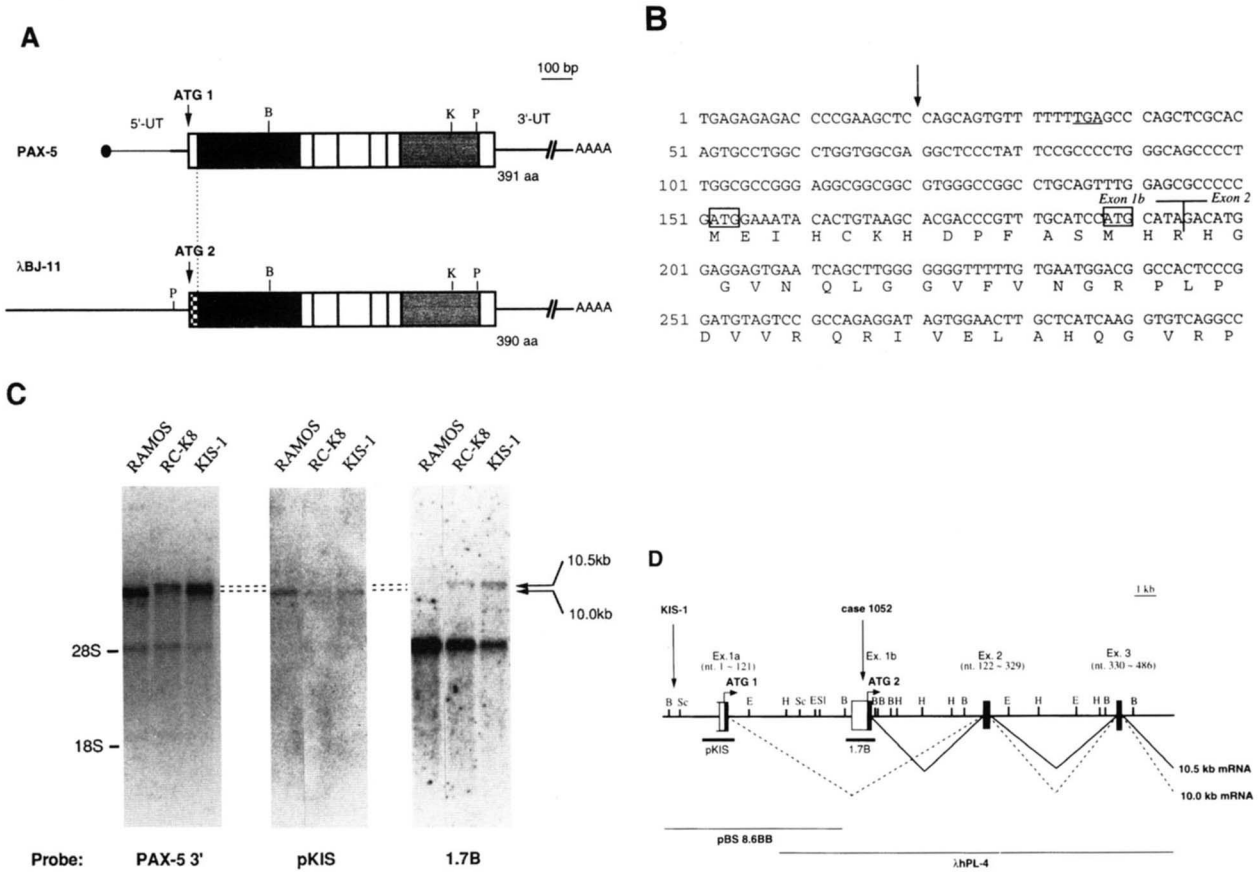

Fig 4. Alternative translation initiation sites of the *PAX-5* gene and the detailed location of the chromosomal breakpoints. (A) Two kinds of PAX-5 cDNA structure. Comparison of the λBJ-11 clone containing a putative alternative translation initiation site (ATG2) to that previously reported in PAX-5 cDNA which contained the ATG1 site only. Coding regions are boxed. Black boxes, bright and dark gray dotted boxes represent paired domains, serine/threonine rich domains, and serine/threonine/proline rich domains. Untranslated 5′ and 3′ regions (UT) are indicated. B, *Bam*HI; K, *Kpn* I; P, *Pst* I. (B) Partial cDNA sequence of the λBJ-11 clone containing ATG2 and the deduced amino acid sequence. Two possible translation initiation sites of this clone are boxed and the preceding in-frame stop codon is underlined. Case 1052's breakpoint is indicated by the arrow. (C) Alternative usage of exon 1 gives rise to two different sized transcripts of 10.0 and 10.5 kb in the B-cell lineage. Northern analysis of the three B-cell lines using three different PAX-5 probes. 2.5 μg poly(A) RNA from RAMOS and RC-K8 cell lines and 1.0 μg poly(A) RNA from KIS-1 cell line were loaded. The filter was sequentially hybridized with 3′ PAX-5 cDNA probe and two genomic probes containing exon 1a (pKIS) and 1b (1.7B), respectively. Both the PAX-5 3′ and the 1.7B probes cross-hybridize to remnant 28S ribosomal RNA. RAMOS, Burkitt's lymphoma cell line; RC-K8, DLCL cell line; KIS-1, Ki-1 positive DLCL cell line with t(9;14). (D) Genomic structure of the 5′ region of the *PAX-5* gene and the location of breakpoints of KIS-1 and case 1052 are indicated. Nucleotide numbers shown above each exon are the same as in the PAX-5 cDNA sequence reported by Adams et al.[15] Open and closed boxes indicate noncoding and coding regions of *PAX-5*, respectively. ATG1 in exon 1a and ATG2 in exon 1b are shown by horizontal arrows. Alternative splicing generates 10.5-kb and 10.0-kb transcripts as shown below the map. The vertical arrows indicate the chromosomal breakpoints of KIS-1 and case 1052. B, *Bam*HI; Sc, *Sac*I; E, *Eco*RI; H, *Hin*dIII; SI, *Sal* I.

is directly juxtaposed to the *PAX-5* gene in the opposite direction of transcription, and by the striking overexpression of the PAX-5 mRNA in the KIS-1 cell line. In addition, the observation that the 9p13 locus seems to be disrupted also in reciprocal translocations involving partner chromosomes other than 14q32 (see case 1615 in Table 1) suggests that multiple chromosomal regions may contribute to PAX-5 deregulation perhaps by juxtaposing different regulatory elements, a mechanism analogous to that observed for chromosomal translocations involving the *BCL-6* gene in DLCL.[8]

Role of PAX-5 in lymphomagenesis. The *PAX* gene family include a number of transcription factors which share homologous DNA binding and dimerization domains, designated as paired domains, and are characterized by a highly conserved α-helical structure at the N terminus.[32,33] Through transcriptional regulation of downstream genes, *PAX* genes control embryonal development and organogenesis. In addition, various *PAX* genes have been implicated in oncogenesis.[34] The *PAX-3* and *PAX-7* genes have been shown to fuse to fork head domain gene (*FKHR*) by t(2;13)(q35;q14) and t(1;13)(p36;q14) translocations in alveolar rhabdomyosarcoma.[35,36] The *PAX-2* and *PAX-5* genes have been found highly expressed in Wilms' tumor,[37] and in brain tumors such as medulloblastoma and glioblastoma,[38] respectively, although the mechanism responsible for their altered expression has not been identified.

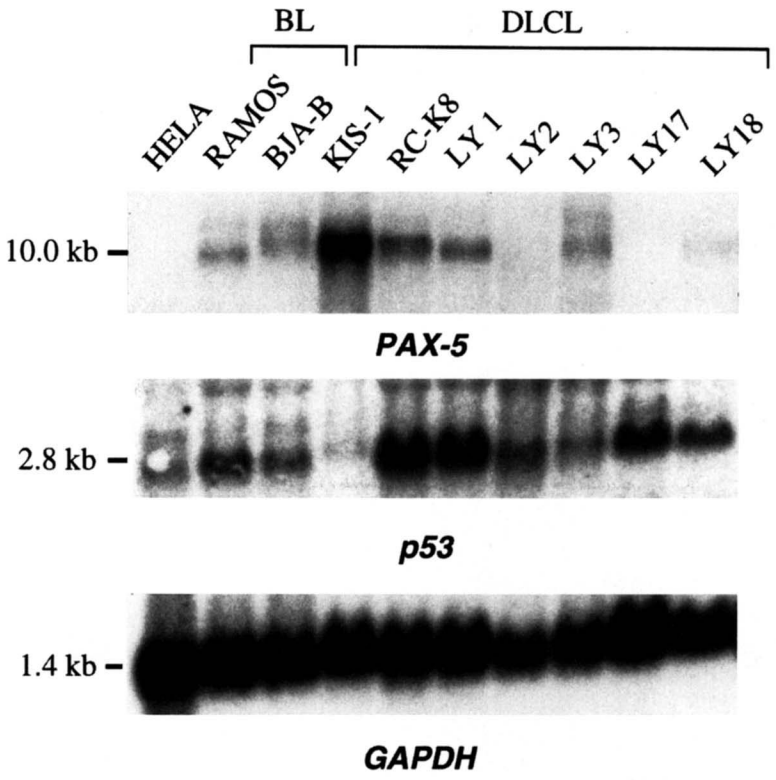

Fig 5. PAX-5 mRNA is overexpressed in KIS-1 cell line carrying t(9;14). A 10-μg aliquot of total RNA was loaded onto each lane, blotted, and hybridized with PAX-5 3′ and p53 cDNA probes, together with GAPDH probe to control for the amount of RNA. All the cell lines except for HELA, which acted as the negative control for PAX-5 expression, are of B-cell lineage. Histological subtype of the original patients' sample are indicated on the top. BL, Burkitt's lymphoma; DLCL, diffuse large cell lymphoma.

The *PAX-5* gene was cloned as a human homolog of the sea urchin *TSAP* (*t*issue-*s*pecific *a*ctivator *p*rotein), and turned out to be the gene encoding 50-kD BSAP (B-cell lineage specific activator protein).[15] PAX-5 is expressed in fetal brain and liver during the embryonic period and, after birth, is exclusively expressed in B lymphocytes and testis. Several observations suggest that PAX-5 plays a significant role in the control of B-cell proliferation and differentiation. Its loss of function results in a maturation arrest at the pro-B cell stage[39] and its overexpression results in proliferation of splenic B cells.[40] On the other hand, treatment with antisense oligonucleotides strikingly reduces lipopolysaccharide-induced proliferation of splenic B cells and spontaneous proliferation of B-lymphoma cell lines.[40] Furthermore, *PAX-5* gene expression is upregulated by the product of the E2A proto-oncogene,[41] whereas PAX-5 itself downregulates the expression of p53.[27] Taken together, these observations suggest that PAX-5 is crucial in the control of proliferation of mature B cells and that its deregulated expression may contribute to abnormal proliferation and thus to lymphomagenesis.

Table 1. Rearrangement of YAC(CEPH788C3) Signal in Tumors With 9p13 Chromosomal Translocations

Case No.	Histology	9p13 Aberration	YAC Status	
			Metaphase	Interphase
1052	LPL	t(9;14)(p13;q32)	Rearranged	Rearranged
1113	LPL	t(9;14)(p13;q32)	Rearranged	Rearranged
1178	LPL	t(9;14)(p13;q32)	NT	Rearranged
1800	DLCL	t(9;14)(p13;q32)	NT	Not rearranged
96080706	DLCL	t(9;14)(p13;q32)	Rearranged	Rearranged
1615	DLCL	t(3;9)(q27;p13)	NT	Rearranged
1068	DLCL	t(7;9)(q32;p13)	NT	Not rearranged

Abbreviation: NT, not tested.

In this study we have also identified alternative transcripts and translation initiation sites that had not been previously reported for the *PAX-5* gene. Interestingly, the amino acid sequence (MEIHCKHDPFASMH) encoded by the novel exon 1b identified in this study is 92% homologous to that (MDMHCKADPFSAMH) encoded by exon 1 of the *PAX-2* gene,[37] suggesting that this domain may have functional significance. Alternatively spliced mRNAs encoding different open reading frames are found in the same (class III) *PAX* family genes including *PAX-2* and *PAX-8*,[42,43] and in the *PAX-8* gene such alternative forms are developmentally regulated and generate isoforms with different transactivation properties. These observations suggest that the two PAX-5 mRNAs (10.0 kb and 10.5 kb) which are expressed in most B-cell lines may encode functionally distinct proteins, whose role in normal B-cell development and possibly lymphomagenesis requires further investigation.

Diagnostic implications. Alterations of the *PAX-5* gene represent the first molecular lesion found in association with LPL. However, the low frequency of this rearrangement detected by conventional Southern analysis suggests the possibility that the breakpoints at 9p13 may be dispersed within a several-hundred kilobase range or the existence of multiple breakpoint cluster regions. Metaphase and/or interphase FISH analysis of NHL samples using the 1-Mb CEPH788C3 YAC spanning the *PAX-5* gene as a probe may complement

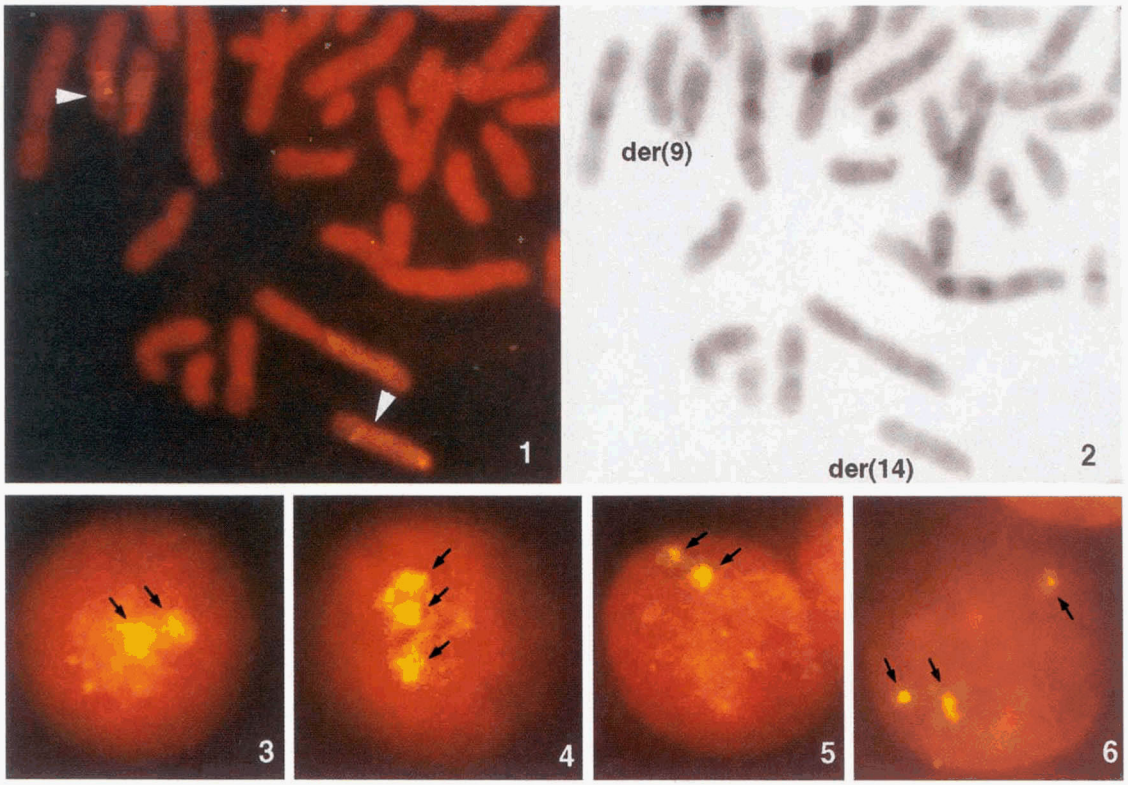

Fig 6. Rearrangement of nonchimeric YAC(CEPH 788C3) in lymphomas with 9p13 chromosomal translocations. (1 through 4) Case 1113. (1 and 2) A partial metaphase of a tumor cell showing hybridization signals at the breakpoints of der (9) and der (14) chromosomes (arrowheads). (1) and the corresponding DAPI image showing a G-banding like staining of chromosomes (2). (3 and 4) Interphase nuclei from the same case showing two signals in a normal nucleus (3) and three signals in a tumor nucleus (4). (5 and 6) Interphase nuclei from case 1052 showing two signals in a normal nucleus (5) and three signals in a tumor nucleus (6). Positive signals in interphase FISH analysis are indicated by arrows.

or substitute conventional cytogenetic analysis for detecting these chromosomal abnormalities. In addition, this approach may facilitate the detection of cytogenetically undetectable 9p13 rearrangements. Finally, 9p13 rearrangements, which are common in LPL, but occasionally present in DLCL, may represent useful clonal markers to determine whether some LPL cases can progress into DLCL.

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