**FVT-1, A Novel Human Transcription Unit Affected by Variant Translocation t(2;18)(p11;q21) of Follicular Lymphoma**

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**Variant t(2;18) and t(18;22) chromosome translocations observed in B-cell chronic lymphocytic leukemias and in follicular lymphomas have been reported to consistently involve the 5' region of the BCL-2 gene on chromosome 18 and various regions on the Ig light chain loci. We show here that a variant t(2;18)(p11;q21) translocation observed in a case of follicular lymphoma leads to the juxtaposition of a Jκ segment to a chromosome 18 transcriptional unit located 10 kbp upstream of the BCL-2 locus. The cDNA of this new evolutionarily conserved gene, termed FVT-1 for follicular-variant-translocation gene, codes for a putatively secreted protein of 36 Kd that is not homologous with any described protein. The FVT-1 gene is weakly expressed in all the analyzed normal hematopoietic tissues but a very high rate of transcription is observed in some T-cell malignancies and in phytohemagglutinin-stimulated lymphocytes. The proximity of FVT-1 to the BCL-2 locus suggests that in the t(14;18) currently observed in follicular lymphomas, both genes would participate in the tumoral process. © 1993 by The American Society of Hematology.**

**MATERIALS AND METHODS**

**Cells.** Various fresh samples of normal and tumoral human tissues, human cell lines of hematopoietic origin, and fresh samples of murine tissues were used in this study. Peripheral blood lymphocytes (PBL) were obtained from the blood of normal volunteers by centrifugation on Triosol Ficol (Seromed, Berlin, Germany). After monocyte depletion by adhesion on plastic Petri dishes, mitogen-stimulated lymphocytes were obtained by incubating PBL at a concentration of 2×10^6 cells/mL in the presence of phytohemagglutinin (PHA; Eurobio, Paris, France) diluted 1:200 in RPMI 1640 medium supplemented with 20% fetal calf serum plus 0.03% L-glutamin, 100 pg/mL of penicillin G and 100 μg/mL of streptomycin sulfate (Institut Merieux, Lyon, France). FVT-1 RNA level was then assessed at various times after PHA stimulation.

**DNA and RNA isolation and analysis.** High molecular weight DNA was extracted from fresh cells or frozen material following standard procedures. After digestion with appropriate endonucleases as recommended by the suppliers (Boehringer Mannheim GmbH, Mannheim, Germany), DNA fragments were electrophoresed on 0.8% agarose gels and transferred onto nylon filters.

Total cellular RNA was isolated from cultured cell lines or from frozen samples by the acid guanidinium thiocyanate-phenol-chloroform method. Poly(A^+) RNA was selected by chromatography on oligo(dT)-cellulose. For Northern blot analysis, 10 μg of total RNA, 2 or 5 μg of poly(A^+) RNA was size fractionated in formaldehyde-12% agarose gels and transferred onto nylon filters.

Preparation and analysis of DNA and cDNA libraries. DNA from the tumoral lymph node (CAM) was partially digested with Mbol restriction endonuclease. DNA fragments averaging 15 to 20 kbp in length were selected by sucrose gradient centrifugation and ligated to BamHI arms of the EMBL 3 phage (Stratagene, La Jolla, CA). Library screenings were performed with probes for Igk light chain joining region gene (Jκ), for Igk light chain constant region gene (Cκ), and with probe p140 mentioned in Fig 1. The recombinant positive clones were characterized by restriction endonuclease mapping and subclones were prepared in pUC18, pUC19 or Bluescript vectors.

Poly(A^+) RNA extracted from the IARC 171 Epstein-Barr virus (EBV)^* lymphoblastoid cell line was used to construct an oligo-dT primed human cDNA library in the Agt11 vector as recommended by the suppliers (Pharmacia, Uppsala, Sweden).

DNA probes and hybridization procedures. Jκ probe was a gift from Dr H. Zachau (München, Germany), probe Ct was a gift from Dr T.H. Rabbitts (Cambridge, UK), pB16 (BCL-2) probe was a gift from Dr Y. Tsujimot (Philadelphia, PA). Probe α-32P-labeling, prehybridization, hybridization, and washing conditions were performed as previously described.

Sequencing procedures and sequence analysis. Overlapping deletions of DNA and cDNA cloned into Bluescript SK (−) (Stratagene, La Jolla, CA).
Molecular cloning of the chromosomal translocation t(2;18)(p11;q21) breakpoints and of the normal chromosome 18 counterpart. Previously reported Southern blot analyses have shown that the chromosomal breakpoint on chromosome 2 occurred within or 5' of the Jκ segment in a case of follicular lymphoma (CAM). To analyze this rearrangement, a genomic phage library prepared from CAM cells was screened with a Jκ probe. Physical mapping of the bacteriophage recombinant clones allowed their separation into two distinct groups different from one another in their 5' part; the restriction map of their 3' end was identical and matched that of the germline Jκ-CK region. Southern blot analyses of interspecies somatic hybrids DNA using probes derived from the 5' region of these two types of clone (probes p516 and p140) (Fig 1) enabled us to state that one group of recombinant clones originated from the functionally rearranged Jκ light chain allele and that the others contained sequence originating from the chromosome 18. We were not able to isolate the germline Jκ light chain allele. The p140 probe was then used to isolate the chromosome 18q21 counterpart from a genomic phage library prepared from normal donor leukocytes, and three overlapping clones (CA2A1, CA131, and CA61) representing a 25-kpb region of chromosome 18 were isolated (Fig 1). Several unique DNA fragments that span this 25-kpb region were used as probes against Southern blots containing normal DNAs digested by EcoRI, HindIII, and BamHI restriction endonucleases. One of these fragments, pD15 probe in Fig 1, hybridized with the same bands as did the pB16 probe that maps to the 5' region of the BCL-2 gene. Furthermore, nucleotide sequence of the pD15 probe perfectly matches that of the BCL-2 5' region. These results clearly showed that, on chromosome 18, the breakpoint
was located at about 13 kbp upstream of the first exon of BCL-2.

Southern blot analysis using p140 probe detected a single rearranged fragment in addition to the germline band in EcoRI- and XhoI-cleaved CAM DNA. This rearranged fragment clearly cohybridized one of the two rearranged bands detected by Jk probe (Fig 2).

**Nucleotide sequence analysis of breakpoints.** We next determined the nucleotide sequence spanning the breakpoint on the der(18) chromosome and on the normal chromosome 18 counterpart (Fig 3). The break occurred within the J4 segment of the Jk region; the existence of eight extranucleotides at the junction (putative N region?) and of two heptamer-like consensus on chromosome 18 in the vicinity of the breakpoint suggests that this translocation might have resulted from an illegitimate chromosome recombination.

**Identification of a transcriptional unit on 18q21.** To identify transcripts that might arise from 18q21, we identified unique fragments that spanned 18q21 sequences and used them as probes against Northern blots containing RNA from CAM cells and from different human cell lines. The 1.4-kbp p140 probe (Fig 1) of chromosome 18 origin that remained on the der(18) hybridized to a transcript of approximately 2.3 kb present in poly(A) RNA extracted from CAM cells, IARC 171 lymphoblastoid cell line, and normal human spleen (Fig 4). This transcript can be detected in total RNA extracted from the various normal and tumoral human tissues and cell lines of hematopoietic origin being analyzed (Fig 5). It is noteworthy that the highest expression of the FVT-I transcript was observed in some T-cell malignancies and especially in large cell anaplastic lymphoma (“Ki-1 lymphomas”) with a T-cell phenotype and genotype (Fig 5). Furthermore, while FVT-I transcript is barely detectable in quiescent PBL, PHA stimulation results in an increase of FVT-I RNA with a peak at 18 hours (Fig 6).

Once total RNA extracted from follicular lymphomas carrying a t(14;18)(q32;q21) has been hybridized to the FVT-I cDNA probe, a weak 2.3-kb signal is observed (Fig 5). To check that expression of FVT-I in fresh samples of follicular lymphomas is not caused by the presence of normal T cells, we assessed its expression in poly(A) mRNA extracted from cell lines carrying t(14;18) translocation with a break in the

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**Fig 3.** Nucleotide sequence of the (t2;18) translocation and of the corresponding normal chromosomes 2 and 18. Slanted-horizontal lines under the sequence indicates the limits of the J4 light chain domain; single underlining identifies the heptamer-like sequences on 18; double underlining identifies the putative N-region on the der(18) chromosome. Slanted-horizontal lines above the sequence indicate the location of the heptamer and nonamer consensus on chromosome 2.

**Fig 4.** Identification of a transcriptional unit on chromosome 18q21. Five micrograms of poly(A) RNA extracted from the indicated tissues and cell line were loaded in each lane, size-fractionated in formaldehyde-agarose gel, transferred onto nylon filters, and hybridized to the p140 probe. IARC 171 is a human B-cell EBV lymphoblastoid cell line. The scale is in kilobases.

**Fig 5.** Northern blot analysis of FVT-I expression in tissues and cell lines of hematopoietic origin. Ten micrograms of total RNA was loaded in each lane and hybridized to the FVT-I cDNA probe. The cell lines used in this study are of B origin (RAMOS, IARC 970, UD 53), of myelomonocytic origin (U937, HL60) and of T origin (JURKATT, MOLT-4). K-199 is a cell line established from a large anaplastic T-cell lymphoma and HL1-1 a cell line established from a large anaplastic B-cell lymphoma. Fresh normal or pathologic samples were also used: LN, reactive lymph node; ML-1, large anaplastic T-cell lymphoma; ML-2, B-cell non-Hodgkin’s lymphomas; ML-3 to ML-7, follicular lymphomas; T-CLL, T-cell chronic lymphocytic leukemias; T-ALL, T-cell acute lymphoid leukemias.
major breakpoint region of the BCL-2 gene locus. As shown in Fig 7, a normal 2.3-kb FVT-I transcript is detected as well. However, we cannot specify if FVT-I transcript arises either from the normal or from the translocated allele in these lymphomas and cell lines analyzed.

Cloning and sequencing of the human FVT-1 cDNA. Polyadenylated RNA was used as a template to construct a λgt11 cDNA library that was screened with the p140 probe mentioned in Fig 1. Nine positive clones were isolated, their restriction mapping and their partial sequences showed that they all coded for the same protein. The longest cDNA clone was sequenced in both strands by generating progressive deletions and by dideoxy sequencing procedure (Fig 8). The sequence exhibits an open reading frame coding for a peptide of 332 amino acids, predicted molecular weight 36 Kd, and a 1,200-nt long 3′ untranslated region. The latter region is rich in A/U nucleotides (62.5%) and contains two AUUUA motifs; all elements are implicated in the posttranscriptional regulation of genes with unstable transcripts such as certain proto-oncogenes and cytokines. The 3′ untranslated region of FVT-1 is also characterized by the presence of a 80-nt long (CT)-rich pyrimidine tract including a TCTCTCTCTG tandemly repeated polynucleotide. The 5′ part of the cDNA (nucleotide 1 to 307) is characterized by a high G + C level (68%) and corresponds to a CpG island. This CpG island accounts for the existence, S' of BCL-2, of a second cluster of rare-cutting restriction site as previously observed. There is an in-frame terminator codon (TGA) 87 bases upstream of the initiation codon. None of the FVT-I cDNA clones contained a poly(A) tail. However, because the clones were obtained from an oligo-dT primed cDNA library, it is likely that all the cDNA clones were initiated from the numerous poly(A) stretches present within the 3′ end of the mRNA. When a genomic clone encompassing the terminal FVT-I exon had been sequenced and compared with the longest FVT-I cDNA clone, an AATAAA polyadenylation signal was noted 113 nucleotides 3′ of the end of the cDNA clone. These data indicate that the 3′ end of a full-length FVT-I cDNA clone is, in all likelihood, 20 to 30 nucleotides 3′ of this polyadenylation signal. This assumption is in agreement with the observed size of the FVT-I transcript.

Comparison of the FVT-I cDNA sequence with those in data bases (EMBL and GenBank, NBRF and SWISS PROT, February 1992) did not show any significant homologies. The hydropathic profile of fvt-1 obtained by the Kyte and Doolittle program showed a unique region of hydrophobicity (amino acids 1 to 24) at the N terminus of the protein with a potential cleavage site at position 25. fvt-1 is unlikely to be a membrane protein as no further hydrophobic region, which could span the membrane, is found; this suggests that it is secreted.

Comparison of the genomic DNA sequence with the 3′ end of the cDNA sequence allowed us to specify the boundaries of the last FVT-I exon (Fig 8) and to demonstrate that the break on chromosome 18 occurred within the last intron of FVT-I (Fig 1). As a result of the translocation process, the FVT-I gene is thus disrupted and the promoting region plus the 5′ part of the coding sequence is juxtaposed to the Vκ-Jκ region of the κ light chain gene in a tail-to-tail configuration. The complete genomic sequence and organization of the FVT-I locus have not been determined so far; however, preliminary results show that the gene contains more than four exons extending over about 40 kbp.

The FVT-1 gene is highly conserved in evolution. Zoo blot analysis using a probe encompassing nucleotides 1 to
320 of the cDNA sequence (Fig 8) showed that sequences homologous to \( V_{F-J} \) can be detected in the mouse and chicken genome (Fig 9). It must be also pointed out that a transcript with approximately the same size as the human \( V_{F-J} \) gene with oncogene \( MYC \) can rearrange at the 5' or 3' regions of the light chain genes, respectively. However, very similar situation is observed in \( \lambda \) and \( \kappa \) light chain genes, respectively. However, in some instances these tumors contain variant translocations with breakpoints located more distant from \( MYC \) in the \( PV T \) locus that extends from 57 kbp to more than 200 kbp downstream of \( MYC \).

**Discussion**

It has been shown that in Burkitt's lymphomas the proto-oncogene \( MYC \) can rearrange at the 5' or 3' regions of the gene with \( IGH \) and \( IgL \) light chain genes, respectively. However, in some instances these tumors contain variant translocations with breakpoints located more distant from \( MYC \) in the \( PV T \) locus that extends from 57 kbp to more than 200 kbp downstream of \( MYC \). A very similar situation is observed in follicular lymphomas where the t(14;18) translocations consistently involve the 3' end of \( B C L - 2 \) and the variant translocations t(2;18) and t(18;22) its 5' end. To make the analogy more complete, we report here that the breakpoint of a variant translocations t(2;18) in a follicular lymphoma fall within a transcriptional unit that we termed \( DISCUSS \)

upstream of the first ATG, the ATTTA motifs and the candidate polyadenylation site (AATAAA). The arrow shows the beginning of the last exon.
signal peptide in its amino acid sequence, and the presence, in the cDNA, of a 3' end characteristic of genes with unstable transcripts suggest that FVT-1 could be a new cytokine mainly expressed by activated normal T cells and by some T-cell malignancies. Further experiments are indeed necessary to support this assumption.

We have previously reported that in the follicular lymphoma studied in this work,8 the BCL-2 gene was overexpressed at the mRNA and protein levels, probably as a consequence of the t(2;18) translocation. The present work makes this assumption more likely because we show that, in this translocation, the BCL-2 gene is juxtaposed to the κ light chain locus in the vicinity of the 5’κ gene enhancer. On the other hand, the FVT-1 gene seems unlikely to play a role in this lymphoma as the translocation results in the disruption of the FVT-1 gene and should lead to the constitution of a chimeric gene where the Vκ-Jκ domains and the 5’ region of the FVT-1 gene are in a tail-to-tail configuration on the der(2) chromosome. However, we were not able to detect any fusion transcript with the Jκ probe and cDNA FVT-1 probes. Because of its proximity to the BCL-2 gene, the FVT-1 gene could be involved in the t(14;18) translocation usually observed in follicular lymphomas. Effectively, in the t(14;18) translocation with break at the 3’ end of BCL-2, the FVT-1 and BCL-2 genes are juxtaposed to the Ig heavy chain locus and might both be deregulated as a result of the same mechanisms. The finding that FVT-1 is expressed in t(14;18) associated lymphomas and cell lines analyzed could be an argument in favor of this hypothesis. Further studies are necessary to specify whether expression of FVT-1 in t(14;18) containing tumoral cells results from its juxtaposition to cis-acting sequence from the IgH locus or not.

With regard to the recombination mechanism involved in this t(2;18) translocation, the existence of two heptamer-like consensus on chromosome 18 in the vicinity of the breakpoint and the presence of a putative N region at the chromosomal junction suggest that this translocation might have resulted from an illegitimate chromosome recombination.22 However, the d(CAT)-rich pyrimidine tract located 2 kbp from the breakpoint could also be implicated in the translocation process. Such a stretch, which can adopt a triple helical structure,23 has been involved in recombination, replication, and gene expression; the resulting change in DNA conformation within this region could enhance its accessibility to the recombinational enzymes, leading to the chromosome translocation. Finally, this work points out that the breakpoints of the t(2;18) variant translocations in follicular lymphomas do not always cluster within the BCL-2 VCR region. Thus, FVT-1 probes constitute additional molecular tools in an attempt to widely identify all the breaks occurring on chromosome 18q21 in B-cell chronic lymphocytic leukemias and follicular lymphomas.

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