Molecular Cloning of the Breakpoint for 3q27 Translocation in B-Cell Lymphomas and Leukemias

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Reciprocal exchanges between chromosomal region 3q27 and three loci of the Ig genes have been reported in cases of B-cell type non-Hodgkin’s lymphoma. We have cloned a region containing a breakpoint junction of 3q27 from a cell line established from a patient with Burkitt’s lymphoma carrying t(3;22)(p27;q11). The region cloned was shown to contain an Ig λ light chain gene fused to a gene on chromosome 3q27. This finding was subsequently confirmed by fluorescence in situ hybridization. Extra nucleotides were present at the joining site. The heptamer-like and nonamer-like sequences separated by an intervening 24 bp were present in the region corresponding to the breakpoint of 3q27, suggesting that a misrecombination in Ig gene rearrangement may be involved in the translocation. South-ern blot analysis with a 3q27-specific probe showed rearrangements in three additional patients with B-cell malignancies with the t(3;14)(q27;q32). The breakpoints of all four cases clustered within a limited 3-kb region on chromosome 3q27. The region of 3q27 involved in the translocation was designated as the BCL5 locus. The transcripts from the BCL5 locus were detected in normal tissues and hematopoietic cell lines, and the increased expression of transcript of aberrant size was detected in the established cell line carrying t(3;22). These observations suggest that a gene located at 3q27 is involved in the translocation and that its deregulation plays a role in the malignant transformation of B cells.

MATERIALS AND METHODS

Cell line. An Epstein-Barr virus (EBV)-negative lymphoid cell line, MD901, was established from cells in the pleural effusion of a patient with Burkitt’s lymphoma. Immunologic stainings of the cell line showed positive staining for surface Ig μ, κ, and λ chains. In cytogenetic analysis, t(3;22)(q27;q11) and t(8;22)(q24;q11) were observed in 20 out of 20 metaphases analyzed and diagnosed as a “variant type” Burkitt’s lymphoma. The other cell lines, Raji, Ramos, Daudi, EB3, and MOLT4, were obtained from the Japanese Cancer Research Resources Bank (JCRB; Tokyo, Japan) or the Institute for Fermentation, Osaka (IFO; Osaka, Japan). Raji, Ramos, Daudi, and EB3 are the B-cell lines derived from Burkitt’s lymphomas. These cell lines contain typical chromosomal translocation t(8;14) for Burkitt’s lymphoma, but were not reported to have 3q27 abnormalities. SH/EB is an EBV-immortalized lymphoblastoid cell line established in our laboratory from the peripheral blood of a normal individual.

DNA probes. A 0.7-kb BglII-EcoRI genomic fragment, pc33,15 containing human Ig λ gene of constant region C33, and a 3-kb EcoRI-BglII genomic fragment, J1,16 containing the joining region of the Ig heavy chain gene were also obtained from JCRB.

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Southern blotting. DNA from 50 normal individuals was kindly provided by the Centre d’Etude du Polymorphisme Humain in Paris, France. High molecular-weight DNA was extracted by the standard sodium dodecyl sulfate (SDS)/proteinase K/phenol method. A 5-μg sample of DNA digested with restriction endonucleases purchased from Takara (Kyoto, Japan) was electrophoresed through a 0.8% agarose gel, transferred to a nylon membrane (Hybond N'; Amersham, Buckinghamshire, UK), and then hybridized with 32P-labeled probes (labeled by a Prime-it Random Primer Labeling Kit; Stratagene, La Jolla, CA). The conditions of hybridization and washing were according to the manufacturer’s recommendations.

Northern blotting. Cytoplasmic RNA was extracted from each of the cell lines using NP-40 and vanadylribonucleoside complexes, and poly(A)+RNA was selected by an oligo(dT)cellulose spin column (Pharmacia, Uppsala, Sweden). Samples of 2-μg poly(A)+RNA were electrophoresed through a 0.8% agarose gel containing formaldehyde. Transfer, hybridization, and washing were essentially the same as those for Southern blotting. Multiple Tissue Northern Blots (a nylon membrane on which 2 μg of poly(A)+RNA from human pancreas, kidney, skeletal muscle, liver, lung, placenta, brain or heart have been electrophoresed and blotted; Clontech, Catalog No. 7760-1, San Diego, CA) were used for the Northern blot analysis for normal tissues. A 0.24- to 9.5-kb RNA Ladder (GIBCO-BRL, Gaithersburg, MD) was used as an RNA size marker. A 0.4-kb HindIII genomic fragment containing exon 4 of the human β-actin gene (Nippon Gene, Tokyo, Japan) was used as a probe to estimate amounts of blotted RNA. Densitometric analysis of autoradiograms were performed with a DMU-33C (Advantage Toyo, Tokyo, Japan).

Construction of genomic library and cloning. The DNA of MD901 cells was completely digested with EcoRI and electrophoresed through a 0.6% agarose gel. A fraction of the 9- to 11-kb fragments corresponding to the rearranged band on Southern blot analysis was collected, ligated into EcoRI-digested λDASH II arms (Stratagene), and packed with the Gigapack Gold System (Stratagene). The constructed library was screened with a pC3 probe. DNA of positive clones was extracted, digested with several restriction enzymes, and subcloned into plasmid pUC vectors.

To obtain a normal clone spanning the 3q27 locus, we screened a cosmid genomic library of human placenta (Clontech Laboratories) and a human-placenta genomic library that was constructed by partial digestion with Alul-HindIII followed by ligation into Charon 4A arms (Amersham).

Fluorescence in situ hybridization. Preparations of the human chromosome specimens and biotin-labeled probes, hybridization, and washing were performed as described. Amplification of the fluorescent signals were performed by fluorescein isothiocyanate-conjugated antiavidin antibody. The chromosome specimens were finally counterstained with propidium iodide, and photgraphs were taken using Nikon B-2A and UV filters (Nikon, Tokyo, Japan).

DNA sequencing. DNA fragments of interest were subcloned into plasmid pUC18 or 19. Both strands of the cloned DNA were sequenced by an Applied Biosystems 373A automatic DNA Sequencer (Foster City, CA) or by the dideoxynucleotide method for double-stranded DNAs, using a Sequenase version 2 kit (United States Biochemical, Cleveland, OH).

RESULTS

Southern blot analysis of the C3 locus of MD901 cells. As the established cell line MD901 was shown to have translocations t(3;22) and t(8;22) by cytogenetic analysis, we examined the cells for the rearrangements of Ig λ light chain gene by Southern blotting with pC3 as a probe. Rearranged bands of 10 kb (upon EcoRI digestion) and 5 kb (upon HindIII digestion) in addition to germline bands were detected (Fig 1).

Molecular cloning. To elucidate the origin of the rearranged band identified by the pC3 probe on Southern blotting, a fraction of the 9- to 11-kb EcoRI fragments was enriched by gel electrophoresis, cloned into λDASH II arms, and screened by pC3 as a probe. Two independent λ- phage clones were obtained (Asho-1 and Asho-2) that had the same restriction enzyme maps. Compared with the reported map of the C3 locus, the clone contained the region spanning C32 (Ke’ Oz’), J3, and C33 (Ke’ Oz’). However, the map of the 5′ part of the clone was different from that of the J3 gene (Fig 2).

Using the 1.2-kb HindIII-XhoI fragment of Asho-1 as a probe (ST1 in Fig 2), we also obtained germline fragments
The locations of probes are indicated by horizontal thick bars. \( \lambda \)-sho-A, -E, and -M are the \( \lambda \)-phage clones, and cosBS-1 and -2 are the cosmid clones, containing normal 3q27 covering 60 kb of the region encompassing the breakpoint with overlapping \( \lambda \)-phage or cosmid clones (Fig 2).

**In situ hybridization.** To locate the origin of the breakpoint region, fluorescence in situ hybridizations were performed using probe ST5 on metaphases from normal cells (data not shown). The observed hybridization signals were identified to be at 3q27, indicating that the \( \lg \) light chain gene was fused to chromosome 3 in MD901 and that cloned genomic fragment \( \lambda \)sho-1 (Fig 2) contained the der(3) breakpoint of 3q22.

**Sequences of the breakpoint region.** To clarify the mechanisms involved in the translocation, we determined the nucleotide sequences around the breakpoint junction and the corresponding region on normal chromosome 3 (Fig 3). The 3' portion of the sequence of the breakpoint junction showed extended homology to that of the coding region of J\( _{\beta2} \), which was previously reported (Vasicek TJ. GenBank database, NCBI, Bethesda, MD; accession no. X51755), and the 5' portion was identical to part of normal chromosome 3. There are five nucleotide mismatches between J\( _{\beta2} \) and \( \lambda \)-sho-1. Similar mutations around the breakpoint of chromosomal translocations have been described. Between the two joined chromosomes, there are 29-bp nucleotides that do not originate from either the normal chromosome 3 or chromosome 22.

A heptamer of CAGAGAG and a nonamer of GCAGAAAGA separated by 24 bp were present in the region on chromosome 3 corresponding to the breakpoint junction. These sequences have a homology to the consensus recombinational signal of Ig genes and T-cell receptor genes (identical to 5/7 nucleotides of CAC(A/T)GTG and 5/9 nucleotides of ACACAAAACC, respectively).

**Southern blot analysis for cases with t(3;14) by the 3q27-specific probe.** We performed Southern blot analysis with ST1 as a probe on 50 DNA samples from the peripheral blood of normal individuals and could not find any restriction fragment length polymorphisms upon EcoRI digestion (data not shown). However, upon analyzing three cases of B-cell malignancy (one case of Burkitt-type leukemia and two cases of B-NHL) with t(3;14)(q27;q32), in addition to
MD901, we detected DNA rearrangements in all three cases and the cell line MD901 (Fig 4). The same filter membrane was regenerated and subsequently hybridized with the JH probe. The results indicated that at least one rearranged band detected by the ST1 probe in all the cases appeared to be the same band detected by the JH probe (Fig 5). Thus, the 3q27 locus and JH are considered to become fused in these cases. The data of Southern blot analysis in Fig 4 indicate that the breakpoints of the three cases and MD901 were within a limited 3-kb region in 3q27, that is, 3-kb HindIII-EcoRI fragment containing the region corresponding to ST1 probe (Fig 2). The region of 3q27 involved in the translocation was designated as the BCL5 locus, as the rearrangements were observed specifically in B-cell lymphomas and leukemias.

Identification of the transcriptionally active locus at 3q27. In Northern blotting using a 5.7-kb Xbal fragment located 15 kb apart from the breakpoint as a probe (ST46 in Fig 2), the transcript of 3.8 kb was detected ubiquitously in normal tissues (pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, and heart). The level of expression in these tissues was not high except for transcript observed in skeletal muscle (Fig 6). The level in skeletal muscle was estimated to be 5- to 17-fold as high as those in the other tissues by densitometric analysis using β-actin as a standard. Only a faint band was observed in an EBV-immortalized lymphoblastoid cell line, whereas a band of aberrant size, slightly but evidently larger than the normal size of 3.8 kb, was observed with amplifications in MD901 (Fig 7). All the tested Burkitt’s lymphoma cell lines (Raji, Ramos, Daudi, and EB3) showed increased expression of transcripts whose sizes were apparently normal (only Raji is shown in Fig 7).

DISCUSSION

We have isolated a breakpoint junction of chromosome region 3q27 in B-cell lymphoma with the reciprocal translocation t(3;22)(q27;q11). The joining region of the λ light chain gene, Jj, was fused to the region of 3q27, which we have designated as the BCL5 locus. Southern blot analysis with a specific probe for the BCL5 locus showed that the breakpoints were clustered within a limited region of 3 kb in all four cases with 3q27 translocation.
The sequencing analysis of the breakpoint region showed the existence of the heptamer-like and nonamer-like sequences, separated from each other by 24 bp. These sequences showed homology to the consensus recombination signal found in the Ig and T-cell receptor genes. The joining point on chromosome 22 was exactly the 5' end of the joining point. This suggests that recombinase recognized heptamer-like and nonamer-like sequences at the translocation junction.

The insertion of 29-bp nucleotides observed between the joined chromosomes is reminiscent of the extra nucleotides added by terminal deoxynucleotidyl transferase, when the Ig gene segments are being rearranged. Physiologically, rearrangement of the Ig heavy chain gene is often associated with the presence of the N region, whereas rearrangements of Ig light chain genes are not. This suggests that the translocation between the BCL5 and Ig λ light chain genes in MD901 occurred before the physiologic rearrangement of the Ig light chain gene.

The cell line MD901 carrying t(8;22) and t(3;22) expressed Ig κ on its surface membrane, which is not concordant with the common associations between the type of translocation and expression pattern of Ig light chains; t(2;8) expresses the κ chain, whereas t(8;22) and t(3;22) express the λ chain. Similar cases with such nonconcordance have been reported. Although it cannot be ruled out that λ chain rearrangement occurred before or after productive κ chain gene rearrangement, which is a rare event for B cells, this nonconcordance might suggest that the translocation t(3;22) in the MD901 cell line does not depend on the Ig light chain gene rearrangement. This may be supported by the presence of extra nucleotides at the joining site.

The clustering of breakpoints of cases with 3q27 translocation and aberrant expression from the BCL5 locus in the cell line MD901 indicate that the gene in the BCL5 locus may play an important role in tumorgenesis of B-cell lymphomas and leukemias. Two mechanisms of activation of a gene juxtaposed to the Ig genes in consequence of chromosomal translocation are possible: transcriptional deregulation of a normal gene and production of a chimeric gene. On Northern blot analysis, an increased and aberrant transcript was detected in MD901 cells. This aberrant transcript was not seen in other cell lines and normal tissues, suggesting that it originates from the translocated allele. This band was not hybridized with the pC13 probe (data not shown), and we could not confirm that it is a fusion message of a BCL5/Ig λ gene as seen in follicular lymphoma bearing an amplified BCL2/Ig heavy chain fusion message.

Unexpectedly, overexpression of the BCL5 gene of normal size was observed in other Burkitt's lymphoma cell lines. These cell lines have not been reported to have abnormalities of 3q27, and we could not find any rearrangements or amplifications on Southern blot analysis using fragments near the breakpoint cluster region of 3q27 locus (data not shown). Possibilities of other genetic alterations including deletions or mutations in regulatory regions as seen in c-myc deregulation in some cases of Burkitt's lymphoma are suggested. Characterization of the BCL5 gene is necessary to clarify the mechanisms of these overexpressions of BCL5 transcripts in Burkitt's lymphoma cell lines, including MD901.

Proteins encoded by genes that were cloned from the breakpoint junction of chromosomal translocation have been reported to have very crucial function in cell growth, proliferation, and tumorgenesis. The cDNA cloning and the characterization of the BCL5 gene may also provide important information.

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