Amplified and Rearranged \textit{bcl-2} Gene in Two Lymphoma Cell Lines, FL-218 and FL-318, Carrying a 14;18 Translocation$^1$

Ryuichi Amakawa, Shirou Fukuhara,$^2$ Hitoshi Ohno, Satoru Tanabe, Mitsuru Horii, Fumio Matsuyama, Isoroku Kato, Tokio Kakita, and Osamu Nagauchi

The First Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto 606 [R. A., S. F., H. O., S. T., M. H., F. M., I. K., T. K.], and Department of Clinical Laboratory, Kyoto City Hospital, Kyoto 604 [O. N.]

ABSTRACT

Two human B-cell lines carrying a 14;18 chromosome translocation [t(14;18)(q32;q21)], designated FL-218 and FL-318, were established from effusion cells of two Japanese patients manifesting the transformed histology of follicular lymphoma. The FL-218 and FL-318 cell lines were composed of cells in the hyperdiploid range, which had two and three or four 18q− chromosomes, respectively. These 18q− chromosomes were not distinguishable from an 18q− chromosome derived from t(14;18) since they exhibited the same banding pattern. Southern blot analysis revealed that in both cell lines, breakage of the \textit{bcl-2} gene occurred within the major breakpoint cluster region and the truncated gene juxtaposed to an immunoglobulin heavy chain gene locus. The autoradiographic intensity of the retained fragment each on 18q− chromosome was more enhanced than that of the translocated fragment on 14q+ chromosome. These findings suggest that the extra 18q− chromosome found in t(14;18)-positive cancer does not arise from de novo independent t(14;18) but from duplication of a preexisting 18q− chromosome.

INTRODUCTION

Translocations involving the long arm of chromosome 14 in B-cell cancer occur primarily at the 14q32 band, the site of the IgH$^1$ gene locus, which has been identified as being a 14q+ chromosomal marker. We proposed earlier that the 14q involvement in translocations may be important for distinguishing morphologically different but functionally comparable subgroups of lymphoid cancer (1, 2) and that a 14q+ marker-positive lymphoid cancer should be phylogenetically and clinically divided into subclasses according to the individual translocations (3, 4). Our proposal is strongly supported by the recent findings in molecular genetics (5) that genes or unknown subgroups of lymphoid cancer (1,2) and that a 14q+ marker-positive lymphoid cancer should be phylogenetically and clinically divided into subclasses according to the individual translocations (6-13).

Karyotype evolution of 14q+ marker-positive lymphoid cancer has been well studied in the two subclasses, t(8;14)(q24;q32) and t(14;18)(q32;q21). In a group of t(8;14)-positive cancers, a partial trisomy for 1q (+1q) constitutes a “major route” of the karyotype evolution (14), which is absent in endemic t(8;14)-positive Burkitt’s lymphoma affecting African children. A group of t(14;18)-positive cancers is prevalent in the United States (15-19), a subclass which is not as common in Japan (6-13).

Case Report and Cell Culture. A 73-year-old Japanese female noted generalized lymph node swelling and an abdominal tumor in August 1986. The diagnosis of inguinal lymph node biopsy in September 1986 (Ref. 22, case 7) was established as follicular small cleaved cell lymphoma, having diffuse areas composed of mixed small and large cells, according to the New Histologic Formulation (23). Peritoneal effusion developed, and her condition rapidly deteriorated despite combination chemotherapy. In January 1987, she died of interstitial pneumonitis. The peritoneal effusion cells collected in September 1986 were cultured in RPMI 1640 supplemented with 20% fetal calf serum.

The second patient (Ref. 22, case 5) was a 67-year-old Japanese male who had previously been diagnosed as having follicular small cleaved cell lymphoma. After the indolent course of 90 months, he manifested a rapidly growing abdominal mass accompanied by the development of ascites, and he died in February 1987. The autopsied abdominal tumor revealed diffuse large cell lymphoma. Culture of the effusion cells was established in January 1987.

Cell Surface Marker Analysis. Cells were examined by the rosette assay for sheep erythrocyte receptors and receptors for the Fc portion of IgG (Fcγ receptor). Surface immunoglobulin were assayed by direct immunofluorescence using fluorescein isothiocyanate-labeled anti-human immunoglobulin antibodies. Reactivities with monoclonal antibodies were tested by the indirect immunofluorescence method. Monoclonal antibodies used in this study were OKIa-1 for the HLA-DR antigens, B1 (Coulter, Hialeah, FL), B2 (Coulter), B4 (Coulter), J5 for common acute lymphoblastic leukemia antigens (24), WT-1 (25), and Leu-1 (Becton-Dickinson, Mountain View, CA). Epstein-Barr virus nuclear antigen was tested according to the method of Reedman and Klein (26).

Cytogenetic Analysis. Metaphase preparations obtained after exposure of cells to 0.05 μg/ml of Colcemid for 1 h were treated with 0.075 M KCl for 20 min and then fixed with methanol:acetic acid (1:3) and stained with 4′,6-diamidino-2-phenylindole (DAPI) and 40% ethanol. All preparations were examined by phase-contrast microscopy.

Southern Blot Analysis. High molecular weight genomic DNA extracted from cells was digested with appropriate restriction endonucleases, separated in 0.7% agarose gel, denatured in alkaline, and transferred to a nylon membrane (Biodyne, Glen Cove, NY). The filter was hybridized with a nick-translated probe in a solution of 50% formamide, 4× Denhardt’s solution, 5× SSC, 0.1% SDS, 50 mM Tris-HCl (pH 6.5), and heat-denatured salmon sperm DNA (250 μg/ml) at 42°C for 24 h. The filter was washed in 2x SSC-0.1% SDS at room temperature and then in 0.1x SSC-0.1% SDS at 50°C. Autoradiography was done at −70°C with intensifying screens.

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2 To whom requests for reprints should be addressed, at The First Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, 54 Shogoin-Kawaramachi, Sakyo-ku, Kyoto 606, Japan.

3 The abbreviations used are: IgH, immunoglobulin heavy chain; E-rosette, erythrocyte rosette; SDS, sodium dodecyl sulfate; SSC, standard saline-citrate; CML, chronic myelogenous leukemia.
DNA Probes. Two DNA probes, named probe b and pFL-2, were used for detecting rearrangements of chromosome 18-specific DNA (bcl-2 gene). Probe b is a 2.8-kilobase SstI-HindIII DNA fragment from chromosome 18 (Fig. 1) (28, 29), provided by Dr. C. M. Croce (Wistar Institute, Philadelphia, PA) that spans the major breakpoint cluster region, where the majority of detectable (14;18) breakpoints occur. pFL-2 is a DNA fragment from chromosome 18, provided by Dr. J. Sklar (Stanford University, Stanford, CA) (30), and the probe can detect most t(14;18) translocations which do not fall within the major breakpoint cluster region (18). The JH gene probe used was a 3.4-kilobase EcoRl-HindIII DNA fragment of the joining region of the IgH gene, provided by Dr. T. Honjo (Kyoto University, Kyoto, Japan) (31).

RESULTS

Establishment of the FL-218 and the FL-318 Cell Lines. After 4 weeks of primary cell culture the effusion cells from the first patient with follicular lymphoma and the second with diffuse lymphoma showed definite proliferation, and the established cell lines were designated FL-218 and FL-318, respectively. FL-218 cells were small to medium and grew in suspension forming loose clumps. FL-318 cells were medium to large and grew in a single cell suspension. Cells of both cell lines had a doubling time of 36-48 h. Surface marker profile of the FL-218 and the FL-318 cells is similar to the corresponding parental cells (Table 1). The FL-218 and FL-318 cells were positive for HLA-DR, B1, and B4, and they were negative for erythrocyte rosette formation, Leu-1, and WT-1. The FL-218 cells had monoclonal immunoglobulin (L, G) on the cell surface and expressed the J5 antigen only to a limited degree. The FL-318 cyte rosette formation, Leu-1, and WT-1. The FL-218 cells had a doubling time of 36-48 h. Surface marker profile of the FL-cell lines were designated FL-218 and FL-318, respectively.

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<th>Immunological markers of FL-218 and FL-318 cells</th>
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<td>CD7 (WT-1)</td>
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<td>Epstein-Barr virus nuclear antigen</td>
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* NT, not tested.

Cytogenetic Studies. The FL-218 and FL-318 cell lines were composed of cells in the hyperdiploid range, with a modal number of 47 and 51 chromosomes, respectively. Banded karyotypes of the FL-218 and the FL-318 cells were similar to those of the parental cells but with minor differences. Twenty cells from the FL-218 cell line were analyzed. Each of these cells showed a gain of one chromosome 12 and loss of one chromosome 18, and they had four common marker chromosomes (Fig. 2). 10q-, 14q+, and 2x18q-. In the FL-318 cell line, all of the 20 cells analyzed showed a gain of one of each of chromosomes 7, 13, and 15, and 12 of these cells had 6 common marker chromosomes (Fig. 3): 2p-, 14q+, 15q-, 2x18q-, and i(18q-). The remaining 8 cells lacked one of the two 18q- chromosomes, as well as the parental cells. In both cell lines, a 14q+ marker chromosome with an additional band at the end of a normal chromosome 14 was the result of a reciprocal translocation between the long arms of chromosome 14 and 18 [(14;18)(q32.3;q21.3)]; however, the resulting 18q- chromosome was not discriminated from other 18q- chromosome, including an 18q- isochromosome found in the FL-318 cells, since they displayed the same banding pattern.

Southern Blot Analysis. Fig. 4 shows representative results of Southern blot analysis of DNAs from the FL-218 and the FL-318 cells. When the DNAs were digested each with BamHI and HindIII, probe b detected two rearranged fragments of a bcl-2 gene. The FL-218 cells did not display the germ line form, which was found in the FL-318 cells. The pFL-2 probe did not detect any rearranged bcl-2 fragments in both cell lines (Fig. 5). These findings were verified by the hybridization after digestion by other restriction enzymes such as EcoRI and SstI, and after long exposure of the autoradiograms, no additional rearranged fragments were found under these conditions. This indicates that breakage of the bcl-2 gene in these two cell lines occurred within the major breakpoint cluster region and suggests that the intact bcl-2 gene locus was eliminated from the FL-218 cells, in which a normal chromosome 18 was missing. The JH probe detected two rearranged fragments in the FL-218 and the FL-318 cell lines, and one of the rearranged fragments hybridized with probe b, indicating that the truncated part of a bcl-2 gene on chromosome 18 juxtaposed to the IgH gene locus on chromosome 14. The size of all the rearranged fragments of these genes in both cell lines was identical to that of the corresponding parental cells (Fig. 4A).

Amplification of Rearranged bcl-2 Fragment. In all digests with BamHI, HindIII, EcoRI, and SstI of the FL-218 and FL-318 cells, the rearranged bcl-2-containing fragment that did not comigrate with a rearranged JH-containing fragment was autoradiographically more intense than the comigrating fragment of the 14q+ chromosome (Fig. 4). The preferential transfer of the smaller rearranged fragment is possible in some blots; however, the enhanced hybridization of a longer fragment is observed in the SstI digest of FL-218 and FL-318, as well as in the HindIII and EcoRI digests of FL-318. On a blot of the HindIII digest in the FL-218, having two rearranged fragments in the FL-218 and the FL-318 cell lines, and one of the rearranged fragments hybridized with probe b, indicating that the truncated part of a bcl-2 gene on chromosome 18 juxtaposed to the IgH gene locus on chromosome 14. The size of all the rearranged fragments of these genes in both cell lines was identical to that of the corresponding parental cells (Fig. 4A).

Amplification of Rearranged bcl-2 Fragment. In all digests with BamHI, HindIII, EcoRI, and SstI of the FL-218 and FL-318 cells, the rearranged bcl-2-containing fragment that did not comigrate with a rearranged JH-containing fragment was autoradiographically more intense than the comigrating fragment of the 14q+ chromosome (Fig. 4). The preferential transfer of the smaller rearranged fragment is possible in some blots; however, the enhanced hybridization of a longer fragment is observed in the SstI digest of FL-218 and FL-318, as well as in the HindIII and EcoRI digests of FL-318. On a blot of the HindIII digest in the FL-218, having two rearranged fragments of a bcl-2 gene which are close in size, the densitometric measurement demonstrated that the intensity ratio of the rearranged fragment between the 18q- and 14q+ chromosomes was almost 2:1. These findings indicate that the rearranged fragment of the bcl-2 gene which was retained on 18q- chromosome is amplified in both cell lines.

DISCUSSION

Two B-lymphoma cell lines carrying a t(14;18)(q32;q21) chromosome translocation, designated FL-218 and FL-318,
AMPLIFIED AND REARRANGED bcl-2 IN t(14;18)

Fig. 2. G-banded karyotype of the FL-218 cell line. The modal karyotype is 47,XX,+12,-18,del(10)(q23.2q24.3),t(14;18)-(q32.3;q21.3),+18q-. Arrow, 14q+ chromosome; arrowheads, 18q- chromosomes.

Fig. 3. G-banded karyotype of the FL-318 cell line. The modal karyotype is 51, XY,+7,+13,+15,del(2)(p21p23),t(14;18)-(q32.3;q21.3),del(15)(q13q15),+18q-, +i(18q-). Arrow, 14q+ chromosome; arrowheads, 18q- chromosomes.
Fig. 4. Southern blot analysis of genomic DNAs extracted from the FL-218, the FL-318, and each of the parental cells. Human placenta DNA was used as control. Approximately 2 μg of DNAs were digested with BamHI (A), SstI (B), HindIII (C), and EcoRI (D), respectively, and serial hybridization with “probe b” and JH probe was performed. A, Lane 1, human placenta; Lane 2, parental FL-218 cells; Lane 3, FL-218 cells; Lane 4, parental FL-318 cells; Lane 5, FL-318 cells. B–D, Lane 1, human placenta; Lane 2, FL-218 cells; Lane 3, FL-318 cells. All rearranged fragments of the bcl-2 and JH genes in both cell lines were in the same size as those of each the parental cells, such as shown in A. Arrowheads, comigration of a rearranged bcl-2 fragment with a rearranged JH fragment, representing the breakpoint on the 14q+ chromosome. Asterisks, rearranged bcl-2 fragments which were retained on the 18q− chromosomes. Both in the FL-218 and the FL-318 cells, the rearranged bcl-2 fragments on 18q− chromosomes are more intense as compared with the rearranged fragments on 14q+ chromosomes. kb, kilobases.
were newly established each from two Japanese patients, who were categorized as having transformed histology of follicular lymphoma (32). In both cell lines, the clonal origin of each cell line was demonstrated by the cytogenetic findings and the surface marker profiles and confirmed by the identical rear

In this study, combining the cytogenetic and the molecular analysis on the two established lymphoma cell lines, we were able to address the nature of the origin of the extra 18q− chromosome that constitutes the peculiar karyotype evolution in Japanese t(14;18)-positive lymphoma (22). Southern blot analysis using two chromosome 18− specific DNA probes and a JH probe indicated that in the FL-218 and FL-318 cells having multiple 18q− chromosomes, rearrangement of the bcl-2 gene on chromosome 18 occurred within the major breakpoint location, which has been recognized to play a crucial role in the pathogenesis of CML. In this regard, duplication of 18q− chromosome is in contrast with that of Ph1 chromosome.

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AMPLIFIED AND REARRANGED bcl-2 IN t(14;18)


Amplified and Rearranged *bcl-2* Gene in Two Lymphoma Cell Lines, FL-218 and FL-318, Carrying a 14;18 Translocation

Ryuichi Amakawa, Shirou Fukuhara, Hitoshi Ohno, et al.


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