Primary mediastinal B-cell lymphoma: detection of \textit{BCL2} gene rearrangements by PCR analysis and FISH

Cherie H. Dunphy · Dennis P. O’Malley · Liang Cheng ·
Tina Y. Fodrie · Sherrie L. Perkins ·
Kathleen Kaiser-Rogers

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Abstract Primary mediastinal large B-cell lymphoma (PMBCL) has a characteristic clinical presentation, morphology, and immunophenotype, representing a clinically favorable subgroup of diffuse large B-cell lymphoma (DLBCL). By gene expression profiling (GEP), PMBCL shares features with classical Hodgkin lymphoma (cHL). Of further interest, \textit{BCL6} gene mutations and \textit{BCL6} and/or \textit{MUM1} expression in a number of PMBCLs have supported an activated B-cell (ABC) origin. Several studies, including GEP, have failed to detect \textit{BCL2} gene rearrangements (GRs) in PMBCL. An index case of \textit{t}(14;18)+ PMBCL prompted our study of the incidence of \textit{BCL2} GRs in PMBCL by polymerase chain reaction (PCR)/fluorescence in situ hybridization (FISH) analyses and its possible clinical impact. Twenty-five retrospectively identified, well-defined PMBCLs (five with cytogenetics) from three institutions were analyzed for a \textit{BCL2} GR by PCR/FISH analyses. The formalin-fixed, paraffin-embedded tissue blocks of 24 available cases were also analyzed by BCL2 immunohistochemistry (IHC). Of the five with cytogenetics, two had a \textit{t}(14;18) (q32; q21). Of the 25 analyzed by PCR, 2 had no amplifiable DNA (aDNA), including 1 \textit{t}(14;18)+ case. Of those with aDNA, two showed a \textit{BCL2} GR; by FISH analysis, three demonstrated a \textit{BCL2} GR. \textit{BCL2} protein expression by IHC analysis was variably detected in 21 out of 24 (strongly, uniformly expressed: 6, including all with a \textit{t}(14;18) or a \textit{BCL2} gene rearrangement; moderately weakly expressed in a subset of the malignant cells: 15). Available clinical follow-up of this \textit{BCL2}+ subset showed a similar course to the other PMBCL cases. Our results imply that a subset of PMBCL [(4 out of 24 analyzed) in our series] may be of GC origin. A larger study is necessary to determine any clinical significance.

Keywords Primary mediastinal B-cell lymphoma · \textit{BCL2} gene · PCR and FISH analysis

Introduction

Primary mediastinal B-cell lymphoma (PMBCL) is a diffuse large B-cell lymphoma (DLBCL) first described in 1981 and postulated to arise from noncirculating thymic B lymphocytes [1, 2]. It is recognized as a distinct entity within the World Health Organization (WHO) classification of lymphomas and represents a clinically favorable subgroup of DLBCL [i.e., better 5-year survival rate (64%), than all DLBCLs after therapy (46%)] [3, 4]. It is characterized by a distinctive clinical presentation, morphology, and immunophenotype. Interestingly, by gene-
expression profiling, PMBCL shares features with classical Hodgkin lymphoma [4, 5]. Of further interest, **BCL6** gene mutations and **BCL2** and/or **MUM1** expression in a number of PMBCLs have supported an activated (or post-germinal center) B-cell (ABC) origin [6]. In addition, several studies, including gene-expression profiling, have failed to detect **BCL2** gene rearrangements in PMBCL [7–9]. An index case of PMBCL associated with a t(14; 18) prompted our study of the incidence of **BCL2** gene rearrangements in PMBCL by polymerase chain reaction (PCR) analysis and fluorescent in situ hybridization (FISH) and its possible clinical impact.

**Materials and methods**

Retrieval of case and clinical follow-ups

Twenty-five consecutive cases, meeting the WHO criteria (definition and description) for a diagnosis of PMBCL (provided below) [3], were retrospectively identified from the three participating academic institutions. The WHO definition and description for PMBCL is stated as follows “a subtype of DLBCL arising in the mediastinum of putative B-cell origin with distinctive clinical, immunophenotypic, and genotypic features. Patients present with localized disease and signs and symptoms relating to large anterior mediastinal masses, sometimes with impending superior vena cava syndrome. When disseminated, other extranodal sites are often involved, such as kidney, liver, skin and brain. The neoplastic cells vary in size and shape. In most cases, the cells have abundant cytoplasm.” There is often associated fibrosis in the background. The cells typically express **CD19** and **CD20**, and often lack surface light chain expression by flow cytometry. They may weakly express **CD30**, either focally or extensively. **CD10** and **CD5** are absent. The available clinical data regarding presentation, bone marrow staging, and therapeutic follow-up, as well as histologic sections, flow cytometric immunophenotypic data, immunohistochemical immunophenotypic data, and conventional cytogenetic results (available in five cases) were reviewed, and the results supported a diagnosis of PMBCL in each of these cases.

Retrospective analysis of **BCL2** rearrangement by polymerase chain reaction

Tissue samples from 25 patients were qualitatively analyzed for a **BCL2** rearrangement, using a nested PCR assay and gel electrophoresis. Formalin-fixed paraffin-embedded tissue was digested in a proteinase K digestion buffer for 24 h at 56°C and then purified using the QIAamp DNA Mini Kit according to manufacturer’s instructions (QUIGEN, Valencia, CA, USA).

A nested PCR assay was performed using a **BCL2** rearrangement assay kit manufactured by InVivoScribe Technologies (San Diego, CA, USA). It involves two nested PCRs, using four sets of primers that target the joining region of the immunoglobulin heavy chain gene and distinct regions of the **BCL2** gene. Two sets of primers were used to identify **BCL2** rearrangements, involving the major breakpoint (Mbr). The second two sets of primers target the minor cluster region (Mcr). The limit of detection, using nested amplifications, is less than one **BCL2**-rearrangement positive cell in ten thousand normal cells (limit of detection <10<sup>−4</sup>). An additional set of primers targeted a HLA class II gene to ensure that the quality and quantity of DNA present was sufficient to generate a valid result. A genomic sequence (Factor V) was also amplified and detected to serve as an additional amplification control for this assay. All PCR reactions were amplified on the GeneAmp PCR System 9700 (Applied Biosystems).

Gel electrophoresis was performed on a 2% agarose gel for 180 min at 70 volts. A DNA size marker (HAE 20 cut p1598, Sigma-Aldrich, St. Louis, MO, USA) was used to determine the presence of the 215-bp product formed when the **BCL2** rearrangement was detected in the major breakpoint region. A 1,000-bp product formation indicated the presence of the **BCL2** rearrangement in the minor cluster region. The absence of the 215- or 1,000-bp product indicated the absence of the **BCL2** rearrangement in the sample. Both positive and polyclonal controls for **BCL2** were analyzed in conjunction with the tissue samples. A “no DNA” control was included as well to ensure the sterility of PCR reagents. Positive and polyclonal controls were included in the **BCL2** rearrangement assay kit provided by InVivoscribe Technologies. Because by a nested PCR assay, rare cells may be detected that carry **BCL2** translocations, all cases positive for a **BCL2** translocation were confirmed, as described below, by FISH analysis.

Retrospective analysis of **BCL2** rearrangement by fluorescent in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on 24 paraffin embedded samples using Vysis’ **BCL2** dual color, break apart probe (Abbott Molecular/Vysis Inc., Des Plaines, IL, USA) according to the manufacturer's protocol. For each paraffin-embedded sample, an adjacent hematoxylin- and eosin-stained section was evaluated by an experienced pathologist, and the tumor was marked before FISH analysis. Each FISH slide was scored in a blinded fashion by two independent individuals. For those samples demonstrating a clearly abnormal fluorescence pattern (>20% abnormal cells), consistent with rearrangement of the **BCL2** gene, at least 100 cells were examined. For those
samples demonstrating a fluorescence pattern consistent with the presence of an intact BCL2 gene (<10% abnormal cells), at least 200 cells were examined. The cutoff values used for the BCL2 assay were based on our databases for multiple break apart probes, as well as our experience with paraffin samples. In addition, the BCL2 break apart assay was performed on three known abnormal [three follicular lymphomas with a t(14; 18) by routine cytogenetic studies] and six normal paraffin-embedded control samples (all, reactive follicular hyperplasia) in conjunction with this study.

Retrospective immunohistochemical analysis

The formalin-fixed, paraffin-embedded tissue blocks of 24 available cases were also analyzed by BCL2 immunohistochemistry (LSAB2 kit, Dako Corporation, Carpinteria, CA, USA; 1:10 dilution; antigen retrieval: 6.0 pH citrate buffer–steam for 30 min) to determine correlation of a BCL2 rearrangement with BCL2 protein expression. These blocks were also analyzed by immunohistochemistry for expressions of CD10 (clone 56C6, predilute, Neomarkers, Fremont, CA, USA; antigen retrieval: 10.0 pH citrate buffer–steam for 30 min), BCL6 (clone 4242, Cell Signalling, Danvers, MA, USA; 1:20 dilution; antigen retrieval: 6.0 pH citrate buffer–steam for 30 min), MUM1 (clone MUM1p, Dakocytomation, Carpinteria, CA, USA; 1:50 dilution; antigen retrieval: 6.0 pH citrate buffer–steam for 30 min) and CD138 (CD138/Syndecan-1, Cell Marque, Hot Springs, AR, USA). All of these markers were analyzed by an automated staining and detection system (BenchMark XT, Ventana Medical Systems, Phoenix, AZ, USA), if not performed at the time of initial diagnosis. Cases were classified into three expression patterns: (1) a germinal center (GC) B-cell pattern (expressing CD10 and/or BCL6 but not activation markers: CD138 and MUM1); (2) an activated GC B-cell pattern (expressing at least one of the GC B-cell markers and one of the activation markers); and (3) an activated (post-germinal center) B-cell (ABC) pattern (expressing MUM1 and/or CD138 but not germinal center B-cell markers) [9].

Results

Retrieval of cases

Twenty-five cases retrospectively reviewed from the three perspective academic institutions met the stated general WHO criteria for a diagnosis of PMBCL (Table 1). The patients ranged from 15 to 83 years of age with a female to male ratio of 1.4:1.0. All patients presented with a primary, large anterior mediastinal mass, and 21 out of 25 initial diagnoses were based on a mediastinal biopsy. The other four diagnostic biopsies originated from supraclavicular lymph nodes (two cases), thoracic lymph nodes (one case), or a lung biopsy (one case). No other sites of lymphomatous involvement were identified, and all staging bone marrows were negative for lymphomatous involvement. All had the typical morphology of PMBCL, characterized by a massive, diffuse proliferation of variably sized cells associated with abundant, pale cytoplasm and variably dense compartmentalizing fibrosis (Fig. 1). Of the 11 cases with available flow cytometric immunophenotypic data, 4 demonstrated lack of any surface light chain expression, which frequently occurs in PMBCL. Of the 23 cases with available immunohistochemical immunophenotypic data at the time of original diagnosis, all were CD45- and CD20-positive. CD30 was expressed in 13 out of 16 analyzed cases, as is also frequently observed in PMBCL. CD30 was expressed in one of the cases with a BCL2 rearrangement, was negative in one of the cases with a BCL2 rearrangement, and was not performed in the two remaining cases with a BCL2 rearrangement. CD10 was not expressed in the majority of cases (18 out of 24); it was weakly to moderately expressed in the remaining cases, only 2 of which had a BCL2 rearrangement. Although CD10 is stated as being absent in the WHO description of PMBCL, CD10 expression does not exclude a diagnosis of PMBCL. In addition, the two cases with a t(14; 18) by conventional cytogenetic studies were CD10-negative. CD23 was expressed in five of six cases tested. The CD23-negative case was not associated with a BCL2 rearrangement. Two of the 5 CD23+ cases were associated with a BCL2 rearrangement. Of the five cases with conventional cytogenetic results, two revealed a t(14; 18) (q32; q21). Both of these cases were associated with additional abnormalities. The other three cases showed abnormalities as depicted in Table 1. Two of these three cases revealed abnormalities that have been frequently described in PMBCL (i.e., abnormalities of 9p and 6p).

Retrospective analysis of BCL2 rearrangement by PCR

Of 25 cases studied, 2 did not yield amplifiable DNA [including 1 case with a t(14; 18) by conventional cytogenetic studies]. Of the 23 cases with amplifiable DNA, a BCL2 gene rearrangement was detected in 2 cases (Fig. 2). These two cases had not had conventional cytogenetic studies performed.

Retrospective analysis of BCL2 rearrangement by FISH

Nineteen of the 24 analyzed paraffin-embedded samples demonstrated no evidence of a BCL2 rearrangement; the mean number of cells with a relevant abnormal signal
pattern was 0.9% (range 0–2.5%) in these specimens. These data did not differ significantly from the six normal control specimens assayed with the \(BCL2\) break-apart probe. The three remaining samples did reveal evidence of a \(BCL2\) rearrangement. In one abnormal sample (case no. 16), 66% of the approximate 100 interphase cells examined demonstrated a fluorescence pattern consistent with a rearrangement involving \(BCL2\); in the second abnormal sample (case no. 18), 89% of the cells examined demonstrated a \(BCL2\) rearrangement; and in the third abnormal sample (case no. 25), 86% of the cells examined demonstrated a \(BCL2\) rearrangement (Fig. 3). Of note, this third abnormal sample did not reveal a \(BCL2\) gene rearrangement by PCR analysis. Despite several attempts, sufficient probe hybridization for analysis was not able to be obtained from two samples [including the case with a \(t(14; 18)\) by conventional

### Table 1 PMBCL cases with cytogenetic, molecular, and immunohistochemical data

| Case no. | Age | Sex | Flow | Conv cytogen | PCR bcl-2 | FISH bcl-2 | IHC bcl-2 | IP (GC, AGC, ABC)\(^a\) |
|---------|-----|-----|------|--------------|------------|------------|------------|------------------------|
| 1       | 42  | M   | NP   | 83–90 tetra , Y, +X, +X dup1q, −1 der/re9p&11p | Neg        | Neg        | bcl-2 −    | ABC (MUM1+)            |
| 2       | 53  | F   | NP   | NP            | Neg        | Neg        | bcl-2 +++  | ABC (MUM1+)            |
| 3       | 83  | F   | MBC  | 90–91 tetra num abn incl 2 copies of \(t(14; 18)\) | NADNA      | NR         | bcl-2 +++  | GC (BCL6+)             |
| 4       | 31  | F   | NP   | NP            | Neg        | Neg        | bcl-2 ++   | AGC (MUM1+, BCL6+)      |
| 5       | 41  | M   | NP   | NP            | Neg        | Neg        | bcl-2++ (+) | AGC (MUM1+, BCL6+)      |
| 6       | 56  | F   | MBC  | \(t(1; 4), (5; 8)\) add 6p, ring | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+)      |
| 7       | 24  | F   | BC with loss of lt ch exp | del 1p, −Y, −15 \(t(2; 5)\) (q21; q13.3) | NADNA      | NR         | bcl-2 v+ (s) | NA                     |
| 8       | 35  | F   | NR   | NR            | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+, CD10+) |
| 9       | 44  | M   | NR   | NR            | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+)      |
| 10      | 24  | M   | NP   | NP            | Neg        | Neg        | bcl-2 v+ (s) | NR                     |
| 11      | 23  | M   | NP   | NP            | Neg        | NA         | NA         | AGC (MUM1+, BCL6+)      |
| 12      | 30  | F   | NP   | NP            | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+)      |
| 13      | 25  | F   | MBC  | NP            | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+, CD10+) |
| 14      | 23  | M   | MBC with wk CD10 (s) | NP            | Neg        | Neg        | bcl-2 −    | AGC (MUM1+, BCL6+)      |
| 15      | 41  | M   | BC with loss of lt ch exp | NP            | Neg        | Neg        | bcl-2 v+ (s) | ABC (MUM1+)            |
| 16      | 65  | F   | MBC with CD10 + | NP            | Pos        | Pos        | bcl-2 +++  | AGC (MUM1+, BCL6+)      |
| 17      | 37  | M   | NR   | NP            | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+)      |
| 18      | 76  | F   | BC with loss of lt ch exp | NP            | Pos        | Pos        | bcl-2 v+   | AGC (MUM1+, BCL6+, CD10+) |
| 19      | 20  | M   | MBC CD22+ CD11c+ MBC CD22+ | NP            | Neg        | Neg        | bcl-2 v+ (s) | ABC (MUM1+)            |
| 20      | 24  | M   | NP   | Multiple abns with \(t(14; 18)\) | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+, CD10+) |
| 21      | 15  | F   | NR   | NP            | Neg        | Neg        | bcl-2 v+ (s) | AGC (MUM1+, BCL6+)      |
| 22      | 39  | F   | NR   | NP            | Neg        | Neg        | bcl-2 −    | ABC (MUM1+)            |
| 23      | 34  | M   | NR   | NP            | Neg        | Neg        | bcl-2 v+ (s) | ABC (MUM1+)            |
| 24      | 28  | F   | NP   | NP            | Neg        | Neg        | bcl-2 v+ (s) | ABC (MUM1+)            |
| 25      | 65  | F   | BC with loss of lt ch exp CD10- | Multiple abns with \(t(14; 18)\) | Neg        | Pos        | BCI-2++    | GC (BCL6+)             |

\(Conv\) \(cytogen\) Conventional cytogenetics, \(PCR\) polymerase chain reaction, \(FISH\) fluorescent in situ hybridization, \(IHC\) immunohistochemical, \(IP\) immunophenotype, \(GC\) germinal center, \(AGC\) activated germinal center, \(ABC\) activated B- (post-germinal center) cell, \(NP\) not performed, \(MBC\) monoclonal B cells, \(NADNA\) no amplifiable DNA, \(NR\) no results, \(BC\) B cells, \(lt\) \(ch\) \(exp\) light chain expression, \(NA\) not available, \(abns\) abnormalities, \(v+\) \(v+\) \(subset\) \(\)\(indicates\) only positive markers are listed

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\(^{a}\) Indicates only positive markers are listed
cytogenetic studies and no amplifiable DNA by PCR analysis]. Of note, a number of the samples demonstrated one to three extra intact BCL2 signals in a significant portion of the examined cells, suggesting the presence of polysomy 18 and/or a partial aneuploidy involving this locus.

Retrospective IHC analysis

Of the 24 cases analyzed by BCL2 immunohistochemistry, BCL2 protein expression was variably detected in 21 cases (strongly and uniformly expressed in 6 cases, including the 4 with a t(14; 18) or a BCL2 gene rearrangement, and moderately to weakly expressed in a subset of the malignant cells in the remaining 15 cases). The immunohistochemical analysis of the 23 available cases with CD10, BCL6, CD138, and MUM1 revealed a germinal center (GC) immunophenotype in 2 cases [1 with t(14; 18) by conventional cytogenetics and 1 with t(14; 18) by conventional cytogenetics and FISH alone], an activated GC immunophenotype in 14 cases (the remaining 2 cases with BCL2 gene rearrangements detected by PCR and FISH) and an activated B-cell (ABC) immunophenotype in the remaining 7 cases, as defined by Chang et al. [10].
Clinical follow-up

The treatment and clinical follow-up of all available cases (21 of 25 cases) are provided in Table 2. One case with a BCL2 gene rearrangement by PCR and/or FISH and an activated GC immunophenotype (case no. 18) had a good response to conventional chemotherapy with or without radiotherapy and was in complete remission at last follow-up (2 years after initial diagnosis and subsequent therapy). The case with a t(14; 18) by conventional cytogenetic studies and a GC immunophenotype (and no amplifiable DNA by PCR analysis and insufficient probe hybridization by FISH analysis) experienced two recurrences within 2.5 years of initial diagnosis, despite CHOP-Rituxan chemotherapy and consolidative radiotherapy. The case with a BCL2 gene rearrangement detected by PCR and an AGC immunophenotype (case no. 16) experienced a late relapse in the mediastinum, supraclavicular lymph nodes, and lung and died of disease within 1.5 years. The fourth patient with a t(14; 18) by conventional cytogenetic studies and a BCL2 gene rearrangement by FISH (case #25) had no available clinical follow-up. In comparison to the PMBCL cases without a t(14; 18) or a BCL2 gene rearrangement, there does not appear to be any significant difference in outcome, as there were also relapses and one patient died of disease within 2 years in this group.

| Case no. | Conv cytogen | PCR bcl-2 | FISH bcl-2 | Treatment and follow-up |
|----------|--------------|-----------|------------|-------------------------|
| 1        | 83–90 tetra, −Y, +X, +X dup1q, −1 der/re9p&11p | Neg | Neg | CHOP and XRT; CR; 6 years |
| 2        | NP | Neg | Neg | R-CHOP and XRT; CR; 5 years |
| 3        | 90–91 tetra num abn incl 2 copies of t(14; 18) | NADNA | NR | R-CHOP in CR at 1 year; recurred in right eye at 2 years |
| 4        | NP | Neg | Neg | CHOP and XRT; CR; 5 years |
| 5        | NP | Neg | Neg | R-CHOP; CR; 4 years |
| 6        | t(1; 4), t(5; 8) add 6p, ring | Neg | Neg | R-CHOP; CR at 1 year; relapsed within 3 years; treated with 2 cycles R-ICE, BEAM chemotx, auto SCT; CR 1 year out |
| 7        | del 1p, −Y, −15 t(2; 5) (q21; q13.3) | NADNA | NR | R-CHOP; CR; 4 years |
| 8        | NR | Neg | Neg | R-CHOP; CR; 3 years |
| 9        | NR | Neg | Neg | No follow-up available |
| 10       | NP | Neg | Neg | CHOP and XRT; CR; 7.5 years |
| 11       | NP | Neg | NA | CHOP and XRT; CR; 6 years |
| 12       | NP | Neg | Neg | R-CHOP and XRT; CR; Developed recurrence supraclavicular LN 9 months after tx; received ABMT; CR 3 years after transplant |
| 13       | NP | Neg | Neg | R-CHOP and XRT; CR; 4 years |
| 14       | NP | Neg | Neg | R-CHOP and XRT; CR; 3 years |
| 15       | NP | Neg | Neg | R-CHOP and XRT; CR; local mediastinal relapse at 6 months; treated with ABMT; CR; 2.5 years |
| 16       | NP | Pos | Pos | R-CHOP; CR; late relapsed in mediastinum, supraclav nodes, lung; DOD 1.5 years |
| 17       | NP | Neg | Neg | R-CHOP and XRT; CR; 2.5 years |
| 18       | NP | Pos | Pos | R-CHOP and XRT; CR; 2 years |
| 19       | NP | Neg | Neg | CHOP; brain mets treated with Decadron; MTX followed by Vincristine and then Leucovorin; Liver mets treated with ESHAP CR; 5 years |
| 20       | NP | Neg | Neg | CHOP and XRT; Rituxan DEPA; at 1 year last follow-up; pulmonary nodules causing at least 50% opacification of thorax; no additional follow-up |
| 21       | NP | Neg | Neg | COPADM1, M2, and CYM1; 5961C chemotx and XRT; Rituxan relapse and DOD within 2 years |
| 22       | NP | Neg | Neg | R-CHOP; CR at 5 years |
| 23       | NP | Neg | Neg | No follow-up available |
| 24       | NP | Neg | Neg | No follow-up available |
| 25       | Multiple abns with t(14; 18) | Neg | Pos | No follow-up available |

CR Complete remission, XRT radiation therapy, R Rituxan, ABMT autologous bone marrow transplant, DOD dead of disease
Discussion

Primary mediastinal B-cell lymphoma is defined by the WHO classification as a primary large anterior mediastinal mass diffusely infiltrated by variably sized lymphoid cells with abundant, pale cytoplasm associated with compartmentalizing fibrosis. The cells have a B-cell immunophenotype frequently associated with lack of surface light chain expression by flow cytometric immunophenotypic analysis and with variable expression of CD30 by immunohistochemical analysis. Our 25 retrospectively identified cases met the WHO criteria based on clinical presentation, morphology, and immunophenotyping.

As mentioned previously, PMBCL has been postulated to arise from thymic B cells. This postulation has been supported, based on expression of MAL. MAL mRNA was initially identified by a differential screening approach during the search for T-cell maturation-associated cDNAs and has been shown to be associated with the intermediate and later stages of intrathymic T-cell differentiation [11]. Subsequently and interestingly, MAL was shown to be a distinct molecular marker of PMBCL, being demonstrated in 70% of PMBCLs and in only 3% of nonmediastinal DLBCLs, in a study by Copie-Bergman, et al. [12].

Cytogenetic abnormalities that have been described previously in PMBCLs have included frequent observations of genetic gains involving chromosomes 2, 5, 7, 9p, 12, and Xq as well as characteristic abnormalities of 9p in 50% of cases and alterations of chromosome 6q [13–15]. More recently, rare cases of PMBCL with t(14;18) have been described [16]. In our five cases that were analyzed by conventional cytogenetic studies, the three cases that were not associated with a t(14;18) did reveal a derivative and rearrangement involving chromosome 9p (1 case) and an add chromosome (6p; one case).

Although postulated to be of thymic B-cell origin, only recently has there been molecular characterization of PMBCL. In 1996, Tsang et al. analyzed molecular alterations involving BCL1, BCL2, BCL6, c-myc, H-ras, K-ras, N-ras, and p53 genes and for Epstein–Barr virus (EBV) infection in PMBCL [8]. Alterations of BCL1, BCL2, or ras genes and evidence of EBV infection were not observed; rearrangement of the BCL6 gene was detected in only one case (16% of cases analyzed).

However, a subsequent molecular study by Pileri et al. demonstrated that more than half of 40 PMBCLs studied displayed BCL6 gene mutations, usually occurring together with functioning somatic IgV(H) gene mutations, and BCL6 and/or MUM1/IRF4 expression [13]. These findings suggested derivation of PMBCL from either activated GC or activated (post-germinal center) B-cells, as CD10 and/or BCL6, but not activation markers (i.e., CD138 and MUM1), are expressed in a GC pattern, CD10 or BCL6 and CD138 or MUM1 are expressed in an activated GC pattern, and CD138 and/or MUM1, but not CD10 or BCL6, are expressed in the activated (or post-germinal center) B-cell (ABC) pattern [10]. However, as mentioned previously, PMBCL is associated with a better prognosis than nonmediastinal DLBCLs, and of interest, it has been demonstrated that DLBCLs with an activated GC or ABC immunophenotype have worst overall survival and event-free survival than DLBCLs with a GC immunophenotype [10, 17].

Gene-expression profiling studies of PMBCL have in fact supported a strong relationship between PMBCL and classical Hodgkin lymphoma [4, 5]. Over one third of the genes that were more highly expressed in PMBCL than in other DLBCLs were also characteristically expressed in classical Hodgkin lymphoma cells. These studies identified a molecular link between PMBCL and classical Hodgkin lymphoma and a shared survival pathway. Of interest, these gene expression profiling studies identified PMBCL as a clinically favorable subgroup, when compared with GC-like DLBCL and ABC-like DLBCL. Of interest, the PMBCL subgroup was somewhat more related to the GC-like subgroup of DLBCL, than to the ABC-like subgroup of DLBCL, even though PMBCL was clearly distinguishable from both subgroups of DLBCL. Interestingly, BCL2 gene rearrangements were not described in the gene expression profiling studies of PMBCL.

Our encounter with a case of PMBCL, defined by WHO criteria, associated with a t(14;18) prompted our study of the incidence of this rearrangement by PCR analysis and FISH analysis in this entity. Although our index case did not have amplifiable DNA by PCR analysis and did not have sufficient probe hybridization for FISH analysis, 3 of 23 additional cases of PMBCL with results by PCR and FISH analyses revealed a BCL2 gene rearrangement by PCR and FISH (2 cases) and by FISH alone (1 case, possibly due to sampling differences, or a different sensitivity by the PCR analysis).

Although our subset of cases with a BCL2 gene rearrangement may possibly represent non-PMBCL of follicular origin, closely mimicking the clinical and morphologic features of PMBCL, our results also may imply that a subset (4 out of 24) of PMBCL in our series may actually be of GC origin. In particular, two of our cases [those with t(14;18) by conventional cytogenetics and or FISH alone) revealed a GC immunophenotype by immunohistochemical staining. Available clinical follow-up of this subset showed a similar course to the other PMBCL cases. A larger study is necessary to determine a significant clinical impact in this subset. In addition, gene expression profiling of this subset would be interesting to compare to PMBCLs of ABC origin.
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