Case report

Transformation of double-hit follicular lymphoma to plasmablastic lymphoma: a partial role of MYC gene rearrangement

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Follicular lymphoma (FL) is genetically characterized by BCL2/IGH translocation. Some FL cases histologically transform to high-grade lymphoma, and the majority of cases transform to diffuse large B-cell lymphoma. We report herein an unusual FL case that transformed to plasmablastic lymphoma (PBL) with MYC gene rearrangement as early as 12 months after FL diagnosis. IGH/MYC translocation, the most common cytogenetic abnormality seen in de novo PBL, was also detected in the transformed tumor (double-hit lymphoma). The patient became resistant to chemotherapy and died 4 months after transformation. We speculate that the "second hit" of MYC rearrangement played a crucial role in PBL transformation (PBL-T) in this case. Highly specific three-color FISH analysis demonstrated the presence of BCL2/IGH/MYC triple fusion signals on a single chromosome as we expected, but BCL2/IGH and IGH/MYC fusion signals also coexisted in a single nucleus. The PBL-T tumor was genetically heterogeneous, despite being histologically quite homogeneous PBL. Surprisingly, three-color FISH analysis revealed that the preceding FL tumor was also genetically heterogeneous, simultaneously harboring BCL2/IGH, IGH/MYC and BCL2/IGH/MYC fusion signals (i.e. double-hit lymphoma), despite being histologically quite homogeneous FL. This suggests that MYC rearrangement played a partial role in PBL-T. Genetic instability including MYC rearrangement in the preceding FL tumor would contribute to PBL-T and poor outcome in this case. This study will broaden our understanding of the pathogenesis of high-grade transformation of FL and help improve patient outcome.

Keywords: Follicular lymphoma, Histological transformation, Plasmablastic lymphoma, Double-hit lymphoma, Three-color FISH

INTRODUCTION

Follicular lymphoma (FL) is genetically characterized in most cases by the presence of the reciprocal chromosomal translocation t(14;18)(q32;q21), which results in the deregulated expression of the anti-apoptotic BCL2 oncogene under the control of the IGH enhancer region. Histological transformation of FL to high-grade lymphoma occurs in 2% of cases each year in the rituximab era.1) The majority of FLs transform to diffuse large B-cell lymphoma (DLBCL).

Herein we describe an unusual FL case that transformed to plasmablastic lymphoma (PBL) with MYC gene rearrangement. To investigate the pathogenesis of PBL transformation (PBL-T) in this case, especially the role of additional MYC rearrangement, we performed three-color FISH analysis. We revealed the genetic heterogeneity of the transformed PBL tumor and, surprisingly, of the preceding FL tumor.

CASE REPORT

A 59-year-old man with no history of immunodeficiency developed a fever and night sweats in January 2013. A computed tomography (CT) scan revealed an abdominal-pelvic mass and a submandibular lymphadenopathy. Laboratory evaluation showed levels of hemoglobin at 9.3 g/dl (normal range 14-18 g/dl), serum lactate dehydrogenase (LDH) at 562 IU/ml (normal range 80–200 IU/ml) and soluble interleukin-2 receptor (sIL-2R) at 12200 U/ml (normal range 145–519 U/ml). The tumors showed abnormal uptake of fluorodeoxyglucose (FDG) on positron emission tomography...
MYC’s role in FL transformation to PBL

PET scan [standard uptake value (SUV) max: 9.54] (Fig. 1A). An abdominal mass needle biopsy showed a partial infiltration of medium-sized lymphoid cells within necrotic tissue. The cells were positive for CD20, CD79a, CD10 and BCL2, suggesting B-cell lymphoma of follicular center origin. A submandibular lymph node excision biopsy performed under general anesthesia demonstrated architectural effacement by abnormal lymphoid cells predominantly composed of centrocytes and a few centroblasts displaying a nodular pattern (Fig. 2A, B). The tumor cells were positive for CD20, CD79a, CD10 and BCL2 (Fig. 2C–F), and negative for CD3 and CD5 (data not shown). Cytogenetic analysis of the lymph node showed 47, XY, del(6)(q?), t(14;18)(q32;q21), +der(18)t(14;18)(q32;q21). Interphase fluorescence in situ

Fig. 1. Whole body PET/CT imaging. A. In January 2013, abdominal FDG uptakes (SUV max: 9.54) were detected in the right abdominal-pelvic mass and left submandibular lymph node. B. In September 2013, after completion of R-CHOP therapy, the abdominal-pelvic mass was significantly decreased in size and FDG uptake was markedly reduced. C. In January 2014, the abdominal-pelvic bulky tumor regrew and a right pleural lesion appeared. FDG uptake was increased (SUV max: 23.26).

Fig. 2. Histological and immunophenotypic findings of follicular lymphoma. Sections of submandibular lymph node in February 2013 showed neoplastic follicles with attenuated mantle zones (A, hematoxylin and eosin, ×40). Neoplastic follicles were composed of centrocytes and centroblasts (B, hematoxylin and eosin, ×400). The tumor cells expressed CD20 (C, ×40), CD79a (D, ×40), CD10 (E, ×40) and BCL2 (F, ×40).
hybridization (FISH) analysis demonstrated $BCL2/IGH$ fusion signals in 54.0% of analyzed cells (Table 1). A diagnosis of FL grade 2 was made. Bone marrow involvement was also demonstrated (stage IVB, FLIPI: high). The FL was with high tumor burden meeting the GELF criteria: a diameter of abdominal tumor mass was over 7 cm, and the patient presented B symptoms (a fever and night sweats). In September 2013, after eight cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone), the tumor was significantly decreased (partial remission) (Fig. 1B). Serum sIL-2R levels were decreased to 500 U/ml. Rituximab maintenance was not performed because it was not covered by health insurance.

In January 2014, at 12 months after the initial FL diagnosis,

Table 1. Cytogenetic studies: G-banding and interphase FISH analysis

<table>
<thead>
<tr>
<th></th>
<th>January 2013, Lymph node</th>
<th>January 2014, Pleural effusion</th>
<th>May 2014, Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological diagnosis</td>
<td>Follicular lymphoma, Grade 2</td>
<td>Plasmablastic lymphoma</td>
<td>Plasmablastic lymphoma</td>
</tr>
<tr>
<td>Cyogenetic studies</td>
<td>47,XY,del(6)(q2?),t(14;18)(q32;q21),+der(18)(t(14;18)(q32;q21))[1]/47,idem,−Y,+mar[2]/46,XY[16]</td>
<td>48,X,Y;+add(1)(p11),ins(1;?)(q21;?),add(4)(q31),add(6)(p23),+7,+9,+12,add(14)(q32),del(17)(p?),−18,der(18),t(14;18)(q32;q21),+21,add(22)(q11.2)[8]</td>
<td>50,XY,+add(1)(p11),add(6)(p23),+7,+8,+9,+10,add(10)(q22),+12,add(14)(q32),del(17)(p?),+18,der(18)(t(14;18)(q32;1)q21)x2,+add(22)(q11.2),+der(?)t(1;?)q21(1;?)1/51,idem,add(4)(p11),+7/11/46,XY[6]</td>
</tr>
<tr>
<td>FISH analysis</td>
<td>$BCL2/IGH$: 54.0%</td>
<td>$BCL2/IGH$: 98.0%</td>
<td>$BCL2/IGH$: 10.0%</td>
</tr>
<tr>
<td></td>
<td>$IGH/MYC$: not examined</td>
<td>$IGH/MYC$: not examined</td>
<td>$IGH/MYC$: 12.0%</td>
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Fig. 3. A. Cytology of pleural effusion in January 2014 showed large neoplastic plasmacytoid cells with abundant cytoplasm and eccentrically located round nuclei with prominent nucleoli (Wright-Giemsa, ×600). B–J. Histological and immunophenotypic findings of plasmablastic lymphoma. Sections of abdominal lymph node in January 2014 showed a diffuse proliferation of immunoblastic and plasmablastic cells (hematoxylin and eosin) (B, ×40 and C, ×400). The tumor cells lacked CD20 (D, ×400) and CD10 (E, ×400), and expressed BCL2 (F, ×400), CD38 (G, ×400) and CD138 (H, ×400). The tumor cells lacked kappa (I, ×400) and lambda (J, ×400). The Ki-67 proliferation index was 90% (K, ×400). L&M. Conventional FISH analysis of bone marrow cells in May 2014 showed $BCL2/IGH$ fusion signals in 10.0% (L) and $IGH/MYC$ fusion signals in 12.0% (M) of analyzed cells (i.e. double-hit lymphoma).
the patient again developed night sweats, abdominal distention and lower back pain. A CT scan revealed an abdominal-pelvic bulky mass, pleural effusion and ascites. Laboratory evaluation showed levels of serum LDH at 867 IU/ml and sIL-2R at 2830 U/ml. Tumors showed increased abnormal uptake of FDG on PET scan [SUV max: 23.26] (Fig. 1C). Pleural effusion cytology showed large abnormal lymphoid cells with plasmablastic features: round eccentric nuclei, prominent nucleoli and abundant basophilic cytoplasm (Fig. 3A). An abdominal mass needle biopsy also revealed a diffuse proliferation of immunoblastic and plasmablastic cells (Fig. 3B, C). The tumor cells were negative for CD20 (Fig. 3D), CD79a, PAX5 and TdT (data not shown), weakly positive for CD10 (Fig. 3E) and positive for BCL-2 (Fig. 3F), CD38 (Fig. 3G) and CD138 (Fig. 3H). Monoclonal immunoglobulin was not detected by serum and urine electrophoresis. Cytogenetic analysis of the pleural effusion cells showed a complex karyotype: 48, X, Y, +add(1)(p11), ins(1;7)(q21;?), add(4)(q31), add(6)(p23), +7, +9, +12, add(14)(q32), del(17)(p?), del(18)(p?), del(18)(q21)x2, -21, add(22)(q11.2). FISH analysis demonstrated BCL2/IGH fusion signals in 98.0% of analyzed cells (Table 1). Based on these profiles, a diagnosis of PBL transformed from FL was made (stage IVB, IPI: high). Tumor cells were negative for kappa (Fig. 3I), lambda (Fig. 3J) and EBER in situ hybridization. The Ki-67 proliferation index was 90% (Fig. 3K). The patient was started with CHASE (cyclophosphamide, cytarabine, etoposide, prednisolone). Although he initially responded to treatment, the lymphoma soon became resistant. Other multiple intensive chemotherapy regimens were tried without success. Bone marrow aspiration in May 2014 demonstrated 8.4% abnormal plasmablastic cells. Cytogenetic analysis showed 50, XY, +add(1)(p11), add(6)(p23), +7, -8, +9, -10, add(10)(q22), +12, add(14)(q32), del(17)(p?), +18, del(18)(q14;18)(q32;q21)x2, -21, add(22)(q11.2), +der(7)(t;7)(q21). FISH analysis demonstrated BCL2/IGH fusion signals in 10.0% of analyzed cells (Fig. 13).

Based on these profiles, a diagnosis of PBL transformed from FL was made (stage IVB, IPI: high). Tumor cells were negative for kappa (Fig. 3I), lambda (Fig. 3J) and EBER in situ hybridization. The Ki-67 proliferation index was 90% (Fig. 3K). The patient was started with CHASE (cyclophosphamide, cytarabine, etoposide, prednisolone). Although he initially responded to treatment, the lymphoma soon became resistant. Other multiple intensive chemotherapy regimens were tried without success. Bone marrow aspiration in May 2014 demonstrated 8.4% abnormal plasmablastic cells. Cytogenetic analysis showed 50, XY, +add(1)(p11), add(6)(p23), +7, -8, +9, -10, add(10)(q22), +12, add(14)(q32), del(17)(p?), +18, del(18)(q14;18)(q32;q21)x2, -21, add(22)(q11.2), +der(7)(t;7)(q21). FISH analysis demonstrated BCL2/IGH fusion signals in 10.0% of analyzed cells (Fig. 3I). IGH/MYC fusion signals, the most common translocation detected in de novo PBL, was also demonstrated in 12.0% of the cells (i.e. double-hit lymphoma) (Table 1). The patient died of the disease 16 months after FL diagnosis and 4 months after PBL-T. In autopsy, the PBL tumor replaced mesenterium, retroperitoneal and pelvic space and involved the pancreas, adrenal glands, right kidney and duodenum. Histological and immunophenotypic findings were the same as that of abdominal mass in January 2014. No residual FL population was identified.

POLYMERASE CHAIN REACTION, SEQUENCING, AND FISH ANALYSIS

Molecular studies and interphase FISH analysis were performed on FL and PBL specimens. For FL, the submandibular lymph node biopsy specimen from February 2013 was used. For PBL, because only few numbers of cells were available in the abdominal mass needle biopsy and pleural effusion cell block specimens from January 2014, the abdominal mass specimen from May 2014 autopsy was used.

Polymerase chain reaction analysis for BCL1/IGH was performed on DNA extracted from frozen sections using established BIOMED-2 consensus primers.3 The result yielded clonal rearrangements at the major breakpoint region with identical migration patterns in the FL and PBL, suggesting that these two neoplasms were clonally related (Fig. 4A). Sequencing of the amplicons showed an identical junction sequence consisting of the BCL2 sequence from the 3' untranslated region in exon 3 and the JH6 sequence (Fig. 4B), confirming that the two tumors are the identical neoplastic clone.

To investigate the pathogenesis of PBL-T in this case, especially the role of additional MYC gene rearrangement, we performed three-color FISH analysis on the PBL stamp preparations. A cocktail of chemically synthesized single-color dual-fusion FISH probes (SureFISH; Agilent Technologies, TX, USA) were used: red-labeled BCL2, green-labeled IGH and aqua-labeled MYC. We identified the presence of BCL2/IGH/MYC triple fusion signals on a single chromosome (Fig. 5A; white arrows), but BCL2/IGH (Fig. 5A, B; orange arrows) and IGH/MYC (Fig. 5A, C; blue arrows) fusion signals also coexisted in a single nucleus. Therefore, we retrospectively performed FISH analysis on the FL stamp preparations. Surprisingly, aberrant BCL2/IGH/MYC (26% of the interphase nuclei evaluated) (Fig. 6A; white arrow) and IGH/MYC fusion signals (45%) (Fig. 6A, C; blue arrows) were detected, concurrent with typical BCL2/IGH fusion signals (94%) (Fig. 6A, B; orange arrows) (i.e. double-hit lymphoma).

DISCUSSION

Although FL is a clinically indolent disease, transformed FL is usually characterized by an aggressive course, poor response to chemotheraphy and short survival.1,4 The median overall survival following transformation in patients with transformation within 18 months of FL diagnosis was inferior compared with patients with a later transformation.1 The present case transformed to PBL as early as 12 months after FL diagnosis and died of the disease 4 months after transformation.

FL transformation to high-grade lymphoma occurs by divergent rather than direct clonal evolution. Most genetic alterations typical of transformed FL can be found at a lower prevalence in indolent FL, suggesting that a single alteration may not be sufficient to drive transformation.4 Detailed genetic analyses have revealed several discrete mechanisms driving transformation.5 Alteration of genes deregulating cell cycle progression and the DNA damage response (CDKN2A/B, MYC and TP53) have been frequently observed in transformed FL, suggesting that the loss of genetic stability and deregulated proliferation are critical steps in transformation.5 MYC deregulation is implicated in 20% of all human malignancies and is often associated with poor prognosis.6 Double-hit lymphoma (DHL) is a high-grade B-cell
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Fig. 4. A. Polymerase chain reaction analysis for BCL2/IGH rearrangements showed similar rearranged bands between the FL and the PBL. M, size markers; a, submandibular lymph node in February 2013; b, abdominal mass in the May 2014 autopsy; MBR, major breakpoint region. B. Sequencing of the BCL2/IGH rearrangement from the FL and the PBL demonstrated identical junction sequences, confirming that the two tumors are clonally related.

Fig. 5. Triple fusion FISH analysis on stamp preparations of abdominal PBL mass from May 2014 autopsy. A cocktail of chemically synthesized oligonucleotide-based dual-fusion probes was used: red-labeled BCL2, green-labeled IGH and aqua-labeled MYC. Fluorescence microphotographs of the same visual field were taken through each of four color filters (DAPI, FITC, Texas-red and aqua) and overlaid in each combination with imaging software. A. BCL2/IGH/MYC triple fusion (white arrows) signals were demonstrated. B. BCL2/IGH fusion (orange arrows) and C. IGH/MYC fusion (blue arrows) signals were also found in a single nucleus.
Fig. 6. Triple fusion FISH analysis on stamp preparations of submandibular lymph node FL from the February 2013 biopsy. A. BCL2/IGH/MYC triple fusion (white arrows, 26% of the interphase nuclei evaluated) signals were identified. B. BCL2/IGH fusion (orange arrows, 94%) and C. IGH/MYC fusion (blue arrows, 45%) signals were also found in a single nucleus.

Table 2. Clinical features of cases that transformed from FL to PBL

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at FL diagnosis/Sex</th>
<th>Time from FL diagnosis to PBL transformation</th>
<th>Site of PBL at diagnosis</th>
<th>Treatment for PBL</th>
<th>Outcome</th>
<th>MYC gene rearrangement</th>
</tr>
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<tbody>
<tr>
<td>1(1)</td>
<td>69/Male</td>
<td>10 months</td>
<td>Bladder, LNs, testis</td>
<td>R-CHOP, DeVIC</td>
<td>DwD/6 months after PBL diagnosis</td>
<td>Yes</td>
</tr>
<tr>
<td>2(2)</td>
<td>67/Male</td>
<td>Simultaneously</td>
<td>LNs, bone</td>
<td>R-CHOP, ESHAP, ASCT</td>
<td>DwoD/20 months after FL diagnosis</td>
<td>No</td>
</tr>
<tr>
<td>3(3)</td>
<td>70/Male</td>
<td>Simultaneously</td>
<td>Jejunum, LNs</td>
<td>R-CHOP</td>
<td>AwoD/24 months from FL diagnosis</td>
<td>No</td>
</tr>
<tr>
<td>4(4)</td>
<td>72/Male</td>
<td>34 months</td>
<td>LNs, spleen</td>
<td>R-CHOP, GEMOX, radiotherapy</td>
<td>AwoD/10 months from PBL diagnosis</td>
<td>No</td>
</tr>
<tr>
<td>Present case</td>
<td>59/Male</td>
<td>12 months</td>
<td>Pleural effusion, LNs</td>
<td>CHASE, DeVIC, GDP, CPT-11 and DXR</td>
<td>DwD/5 months after PBL diagnosis</td>
<td>Yes</td>
</tr>
</tbody>
</table>

FL, follicular lymphoma; PBL, plasmablastic lymphoma; LN, lymph node; DeVIC, dexamethasone, carboplatin, etoposide, and ifosfamide; ESHAP, etoposide, methylprednisolone, cytarabine, and cisplatin; ASCT, autologous stem cell transplantation; GEMOX, gemcitabine and oxaliplatin; GDP, gemcitabine, dexamethasone, and cisplatin; CPT-11, irinotecan; DXR, doxorubicin; DwD, died with disease; DwoD, dead without disease; AwoD, alive without disease.
lymphoma with MYC and BCL2 and/or BCL6 rearrangements and was recently categorized as a distinct entity in the 2016 revision of the World Health Organization classification for lymphoid malignancies. DHL is characterized by a rapidly progressing clinical course that is refractory to aggressive treatment and has a short survival time. In addition to de novo disease, DHL occurs in the setting of transformation of indolent lymphoma, particularly FL, when the BCL2/IGH lymphoma acquires MYC translocation, as is the present case.

PBL is an uncommon subtype of DLBCL that shows diffuse proliferation of large neoplastic cells resembling immunoblasts and plasmablasts. PBL is clinically characterized by an aggressive course and high rates of relapse. The most common cytogenetic abnormality seen in PBL is IGH/MYC translocation. Most PBL cases are EBV-associated, with a high proliferation index. Our PBL-T case carried IGH/MYC translocation and a high Ki-67 proliferation index but was negative for EBV.

PBL-T of FL is extremely rare, and only four cases have been reported (Table 2). Only one case displayed MYC gene rearrangement. The other three cases without MYC rearrangement (cases 2, 3 and 4) achieved complete remission with R-CHOP and remained disease free for 10 to 24 months. One case with MYC rearrangement (case 1) was chemotherapy-resistant and died of disease less than 6 months after PBL-T. In that case, Ouansafi et al. identified MYC gene rearrangement in almost all nuclei of the PBL-T tumor using FISH analysis, although the partner gene was not shown. The authors also demonstrated that the PBL-T tumor exhibited a much lower intra-clonal diversity compared with the preceding FL tumor by somatic hypermutation analysis. No MYC rearrangement was detected in the preceding FL tumor.

The present case is the second case of PBL-T of FL with MYC gene rearrangement. We provide definitive evidence of histological transformation by demonstrating identical BCL2/IGH gene breakpoints in both PBL and FL. Conventional FISH analysis detected IGH/MYC fusion signals in addition to BCL2/IGH in the PBL-T tumor (Fig. 3L, M) (i.e. DHL). Taking the case of Ouansafi into consideration, we speculated that the “second hit” of MYC rearrangement played a crucial role in PBL-T in the current case. However, in contrast with previous case, the chromosome copy number increased in the current case and the karyotype became complex. Three-color FISH analysis demonstrated the presence of BCL2/IGH/MYC triple fusion signals on a single chromosome, as we expected (Fig. 5A; white arrows), but BCL2/IGH (Fig. 5A, B; orange arrows) and IGH/MYC (Fig. 5A, C; blue arrows) fusion signals coexisted in a single nucleus. Remarkably, the PBL-T tumor was genetically heterogeneous, despite being histologically quite homogeneous PBL. We also retrospectively performed triple fusion FISH analysis on the FL specimen. Surprisingly, the FL tumor was also genetically heterogeneous harboring aberrant BCL2/IGH/MYC (Fig. 6A; white arrow) and IGH/MYC fusion signals (Fig. 6A,C; blue arrows) in addition to typical BCL2/IGH fusion signals (Fig. 6A, B; orange arrows) (i.e. DHL), despite being histologically quite homogeneous FL. This indicates that the preceding FL tumor was already genetically heterogeneous and genomically instable. This would contribute to such an early transformation to high-grade lymphoma, PBL. This also suggests that the role of MYC rearrangement was partial in PBL-T in this case; MYC dysregulation might be a necessary condition for PBL-T but not sufficient to allow PBL-T. Other mechanisms such as multiple chemotherapeutic agents and other genetic alterations will contribute to PBL-T and poor outcome. In future studies, we will isolate genetically heterogeneous FLs at diagnosis using this simple three-color FISH technique and consider applying aggressive induction regimens to improve patient outcomes.

In summary, we reported an unusual case of FL transformed to PBL with MYC gene rearrangement. Using a highly specific three-color FISH technique, we demonstrated that the PBL-T tumor was genetically heterogeneous harboring BCL2/IGH, IGH/MYC and BCL2/IGH/MYC translocations simultaneously. Moreover, we revealed that the preceding FL tumor was also genetically heterogeneous, despite being histologically quite homogeneous FL. This suggests that the role of MYC dysregulation was partial in PBL-T. Genetic instability including MYC dysregulation in the preceding FL tumor would contribute to PBL-T and poor outcome in this case. This study will broaden our understanding of the pathogenesis of high-grade transformation of FL and help to improve patient outcome.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

4 Kridel R, Sehn LH, Gascoyne RD. Can histologic transformation of follicular lymphoma be predicted and prevented?


