

Case Study

Double-Hit Lymphoma Demonstrating t(6;14;18)(p25;q32;q21), Suggesting Two Independent Dual-Hit Translocations, MYC/BCL-2 and IRF4/BCL-2

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Here, we report a rare case of double-hit lymphoma, demonstrating t(6;14;18)(p25;q32;q21), suggesting two independent dual-translocations, c-MYC/BCL-2 and IRF4/BCL-2. The present case had a rare abnormal chromosome, t(6;14;18)(p25;q32;q21), independently, in addition to known dual-hit chromosomal abnormalities, t(14;18)(q32;q21) and t(8;22)(q24;q11.2). Lymph node was characterized by a follicular and diffuse growth pattern with variously sized neoplastic follicles. The intrafollicular area was composed of centrocytes with a few centroblasts and the interfollicular area was occupied by uniformly spread medium- to large-sized lymphocytes. CD23 immunostaining demonstrated a disrupted follicular dendritic cell meshwork. The intrafollicular tumor cells had a germinal center phenotype with the expression of surface IgM, CD10, Bcl-2, Bcl-6, and MUM1/IRF4. However, the interfollicular larger cells showed plasmacytic differentiation with diminished CD20, Bcl-2, Bcl-6, and positive intracytoplasmic IgM, and co-expression of MUM1/IRF4 and CD138 with increased Ki-67-positive cells (> 90%). MUM1/IRF4 has been found to induce c-MYC expression, and in turn, MYC transactivates MUM1/IRF4, creating a positive autoregulatory feedback loop. On the other hand, MUM1/IRF4 functions as a tumor suppressor in c-MYC-induced B-cell leukemia. The present rare case arouses interest in view of the possible “dual” activation of both c-MYC and MUM1/IRF4 through two independent dual-translocations, c-MYC/BCL-2 and IRF4/BCL-2. [J Clin Exp Hematop 53(2) : 141-150, 2013]

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INTRODUCTION

Translocation t(14;18)(q32;q21) is a hallmark of follicular lymphoma (FL), which results in the juxtaposition of BCL2 with enhancer elements of the immunoglobulin heavy chain (IGH). On the other hand, translocations involving c-MYC at 8q24 and IGH, k or l (IGL) light chain, are characteristic of Burkitt’s lymphoma (BL). B-cell lymphomas with simultaneous IGH-BCL2 and c-MYC rearrangements, also known as “double-hit” lymphomas (DHL), are rare, representing < 1% of all lymphomas and approximately 4% of high-grade B-cell lymphomas.1,2

BCL2 was first described in the early 1980s by its involvement in t(14;18) in FL.3 It has potent anti-apoptotic functions without mediating proliferative signals. c-MYC is a transcription factor controlling the expression of many target genes involved in cell cycle regulation, metabolism, DNA repair, stress response, and protein synthesis.4 Their concurrent translocations are thought to lead to increased proliferation (c-MYC) and reduced apoptosis (BCL2), thereby driving more aggressive tumor growth.5 Among DHL cases, the c-MYC partner is more frequently IGL [t(8;22)(q24;q11)] than IGH [t(8;14)(q24;q32)], while in classic BL cases, the c-MYC partner is usually IGH and rarely IGL.6,9 The pathogenetic significance of t(8;22) in DHL is still unknown; however, its presence implies that t(14;18) precedes t(8;22).2,6,9

DHL with IGH-BCL2 and c-MYC rearrangements is characterized by a highly aggressive clinical course, complex karyotypes, and demonstrates pathologic features overlapping with BL, diffuse large B-cell lymphoma (DLBCL) and B-lymphoblastic lymphoma/leukemia (B-LBL).2,6,8,10 DHL plays an important part in the 2008 World Health Organization classification, as cases of aggressive B-cell lymphoma showing monomorphous proliferation of blasts with a very high proliferation rate, often of a germinal center (GC)
phenotype (CD10+, Bcl-6+, MUM1/IRF4-), although the other part is heterogeneous.\textsuperscript{1,2}

Besides t(14;18) and t(8q24), there is no cytogenetic abnormality commonly observed in DHL cases, although certain numerical and structural aberrations are seen frequently, including trisomy 12 and trisomy 7.\textsuperscript{6}

Here, we report a unique case of double-hit lymphoma, demonstrating a rare abnormal chromosome, t(6;14;18)(p25; q32;q21), suggesting an independent clone with dual-translocations of IRF4/BCL-2 in addition to c-MYC/BCL-2, and showing plasmacytic differentiation.

\section*{CASE REPORT}

\section*{Clinical history}

A 71-year-old man was admitted to hospital because of severe bilateral leg edema and appetite loss. Upon admission, a huge abdominal mass and left inguinal swollen lymph nodes (LNs) were observed. Laboratory findings revealed a highly elevated white blood cell count (41.36 × 10\textsuperscript{9}/L) with 45.0\% abnormal cells, with reduced red blood cell count (3.49 × 10\textsuperscript{12}/L), hemoglobin (10.7 g/dl), and platelet count (97 × 10\textsuperscript{9}/L). The abnormal cells contained many small cells (43.0\%), with a few medium- to large-sized ones (2.0\%) (Fig. 3d). Serum chemistry revealed elevated levels of aspartate aminotransferase (154 IU/L; normal, 8-38), lactate dehydrogenase (5,316 IU/L; normal, 106-211), soluble interleukin-2 receptor (3,813 U/mL, normal, 145-519), and ferritin (916 ng/mL; normal, 2.3-121). Serum levels of IgG, IgA and IgM were normal and C-reactive protein was slightly elevated (1.0 mg/dL; normal, < 0.3). Serum M protein and Bence-Jones protein were not detected.

\textsuperscript{18}F-Fluorodeoxyglucose positron emission tomography demonstrated multiple swollen LNs and soft tissue with strong \textsuperscript{18}F-Fluorodeoxyglucose accumulation (SUVmax = 10.5) in bilateral cervical, supraclavicular, axillary, mediastinal, paraaortic, mesenteric, retroperitoneal, and inguinal regions. Involvement of the spleen and multiple bone lesions was also observed, such as in femur, tibia, rib, ilium, and vertebra. Left inguinal LN biopsy and bone marrow (BM) aspiration were performed.

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\section*{Histologic findings of lymph node}

In a low-power field, the lesion was characterized by a follicular and diffuse growth pattern with variously sized follicles (Fig. 1a). The intrafollicular area was composed of numerous centrocytes with a few centroblasts (Fig. 1b). The interfollicular area was occupied by uniformly spread medium- to large-sized lymphocytes with round or slightly indented nuclei. A portion of the large lymphoid cells had one or two prominent nucleoli. In this lesion, relatively large numbers of cells undergoing mitosis and apoptosis were observed (Fig. 1c).

On flow cytometric analysis, the small cells (Fig. 4a) were positive for CD10, CD19, CD20, CD38, surface IgM (sIgM), and \textlambda-light chain and negative for CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD30, CD34, CD56, and \textx-light chain. On the other hand, medium- to large-sized cells (Fig. 4b) demonstrated positive reactivity for CD10 and CD19, and diminished or negative expression of CD20, sIgM, and \textlambda, whereas there was increased expression of CD38.

Immunohistochemical study demonstrated that both small and larger tumor cells were negative for CD3, CD10, \textx-light chain, and terminal deoxynucleotidyl transferase. CD20 (Fig. 1d), surface \textlambda-light chain (Fig. 1e), and Bcl-2 demonstrated strong positivity for the intrafollicular area, whereas in the interfollicular area, a few tumor cells were CD20-positive (Fig. 1d). Bcl-2 was weakly positive for interfollicular tumor cells. Lambda-light chain was negative for interfollicular tumor cells (Fig. 1e). A portion of the intrafollicular tumor cells demonstrated Bcl-6 expression (Fig. 1f). sIgM was positive for intrafollicular tumor cells (Fig. 2a), whereas cytoplasmic IgM was positive for interfollicular tumor cells (Fig. 2b). MUM1/IRF4 was positive for both intrafollicular and interfollicular tumor cells (Fig. 2c). CD38 and CD138 were positive in a small number of larger cells. Many Ki-67-positive cells (> 90\%) were observed in the interfollicular area, whereas positivity for Ki-67 of small cells was low (< 20\%) (Fig. 2d). CD23 immunostaining demonstrated a disrupted follicular dendritic cell (FDC) meshwork (Fig. 2e). There were no Epstein-Barr virus (EBV)-encoded small RNA (EBER)-positive tumor cells in the lesion, as determined by \textit{in situ} hybridization.

\section*{Histologic findings of bone marrow}

BM aspiration demonstrated a nuclear cell count of 26.3 × 10\textsuperscript{9}/µL with 71.3\% of two types of abnormal cell (Fig. 3a). Cells of one of the types (18.4\%) were small in size, with a limited cytoplasm and irregularly shaped nuclei (Fig. 3b), and the others (52.9\%) were medium to large in size, with small basophilic cytoplasm including abundant vacuoles and round to oval nuclei with indistinct nucleoli (Fig. 3c).

On flow cytometric analysis, the small cells (Fig. 4c) were positive for CD10, CD19, CD20, CD23, sIgM, and \textlambda, and negative for CD3, CD4, CD5, CD8, CD25, CD43, CD56, CD138, and \textx. CD38 was partially positive. On the other hand, larger cells (Fig. 4d) demonstrated positive reactivity for CD10 and CD19, diminished or negative expression of CD20, CD23, sIgM, and \textlambda, whereas they showed increased expression of CD38, CD138, and CD43.

On a clot section, numerous medium- to large-sized clusters composed of centrocytes and medium- to large-sized blastic tumor cells were observed (Fig. 3e). Immunohistochemical
Fig. 1. Histological and immunohistochemical findings of the resected lymph node. (1a) In a low-power field, the lesion was characterized by a follicular and diffuse growth pattern with variously sized follicles. H&E stain, ×4. (1b) In a high-power field, intrafollicular tumor cells were composed of numerous centrocytes with a few centroblasts. H&E stain, ×40. (1c) In a high-power field, uniformly spread medium- to large-sized blastic cells with round or slightly indented nuclei were observed in the interfollicular area. A portion of large lymphoid cells had one or two prominent nucleoli. Note relatively large numbers of scattered mitotic figures and apoptotic bodies. H&E stain, ×40. (1d) CD20 immunostaining demonstrated numerous CD20-positive tumor cells in the follicular area, whereas only a few CD20-positive cells were present in the interfollicular area. ×10. (1e) Surface λ-light chain was positive for the follicular area, but negative for the interfollicular area. ×20. (1f) A portion of intrafollicular tumor cells (*) showed bcl-6 expression. ×20.
study demonstrated staining resembling that of LN cells.

**Chromosomal analysis and fluorescence in situ hybridization (FISH) analysis**

Chromosomal analysis demonstrated two types of abnormal karyotype from the resected LN. One clone (9 out of 20 cells) demonstrated complex abnormalities including t(14;18) (q32;q21) and t(8;22)(q24;q11.2), resulting in IGH-BCL2 and c-MYC-IGL fusions, respectively (Fig. 5a). The other clone (8 out of 20 cells) demonstrated t(6;14;18)(p25;q32;q21) (Fig. 5b). Only the former clone was also observed in 9 out of 20 BM cells. The fusion of IGH-BCL2 occurred in 97.0% of BM cells by FISH analysis (Fig. 5e). Breakpoints (splits) in the c-MYC locus were also observed in BM cells by FISH (Fig. 5f).

**Southern blot analysis**

The monoclonal expansion of B cells was confirmed by an IgH rearrangement band demonstrated by Southern blot hybridization obtained from BM (Fig. 5c). Another clone was also suggested in LN cells in addition to the same clone shown in BM (Fig. 5d).
Clinical course

The patient was diagnosed with stage IV DHL. He showed none of the B symptoms, such as fever, body weight loss, or night sweats. We performed 3 courses of chemotherapy with rituximab (500 mg of rituximab on day 1, 70 mg of doxorubicin hydrochloride on day 3, 1.0 mg of vincristine sulfate on day 3, 1,000 mg of cyclophosphamide on day 3, and 40 mg of prednisolone on days 3 to 7), which induced prompt resolution of the symptoms and ameliorated imaging and laboratory findings. Two weeks after the 3rd chemotherapy, however, medium- to large-sized lymphoma cells were again observed in the peripheral blood with rapid elevation of serum lactate dehydrogenase level.

DISCUSSION

According to the 2008 World Health Organization classification, DHL cases are classified into various types. Many of them are classified as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (BCLU), and DLBCL not otherwise specified (DLBCL-NOS), but to a lesser extent also including FL and B-LBL.6,7,9,11

In the present case, the LN was characterized by a follicular growth pattern with variously sized follicles. By CD23 immunostaining, however, the FDC meshwork was not preserved. The diagnosis of FL requires an exclusively follicular growth pattern, verified by the presence of CD21+ or CD23+ FDC meshworks. Loss of the FDC meshworks and subsequent dissolution of follicles is considered a sign of early transformation of FL.12 The most common histology transformed from FL is DLBCL. The second most common histology encountered is BCLU. These cases show diffuse architecture with a mixture of medium- to large-sized transformed cells and may reveal a starry-sky pattern and moderate numbers of mitotic figures,9,13 which is compatible

Fig. 3. Histological findings of bone marrow. (3a-3c) Bone marrow aspiration smear demonstrated normocellular marrow with 71.3% of two types of abnormal cell (3a, May-Giemsa stain, ×40), including ongoing mitosis (arrow). Cells of one type (18.4%) were small in size, with limited cytoplasm and irregularly shaped nuclei (3b, May-Giemsa stain, ×100), and the others (52.9%) were medium to large in size, with small basophilic cytoplasm including abundant vacuoles and round to oval nuclei with indistinct nucleoli (3c, May-Giemsa stain, ×100). (3d) In the peripheral blood, many small lymphoma cells (43.0%) and a few medium- to large-sized cells (2.0%) were observed (May-Giemsa stain, ×40). (3e) On a clot section, numerous medium- to large-sized clusters composed of centrocytes and medium- to large-sized blastic tumor cells were observed (H&E stain, ×40).
with the interfollicular regions of our case.

Immunophenotypically, most DHL cases have a GC phenotype with the expression of CD10, Bcl-2, and Bcl-6, and lack MUM1/IRF4.6-10,14-16

In the present case, the small cells of the LN intrafollicular region and BM were positive for CD10, CD19, CD20, slgM, λ, Bcl-2, and Bcl-6, and negative for CD43, CD56, and CD138, which is compatible with a GC phenotype, except for positive reactivity for MUM1/IRF4. In contrast, the larger cells of the LN interfollicular region and BM showed a non-GC type: diminished or negative expression of CD20, Bcl-2, Bcl-6, slgM, and λ, whereas cytoplasmic IgM was positive and there was increased expression of CD38, CD138, MUM1/IRF4, and CD43, although CD10 and CD19 were positive.

The proliferation (Ki-67) rate in DHL is variable, ranging from 40 to 50%, up to > 99%.7 BLCU cases have a proliferation rate from 80 to virtually 100%, and most cases are > 95%.7 In the present case, many Ki-67-positive cells (> 90%) were observed among larger cells, which might have resulted in the exclusively rapid leukemic progression and resistance to chemotherapy in this case.

A marked decrease in the expression of CD20, decreased intensity or complete absence of IGL restriction, and increased expression of CD38, all of which were observed in our case, are reported in B-cell lymphomas with c-MYC translocations or DHL.5,17 However, plasmacytic differentiation with diminished Bcl-2, Bcl-6, and slgM, and co-expression of MUM1/IRF4 and CD138 are not common for DHL with IGH-BCL2 and c-MYC rearrangements. LBL was ruled out by negative terminal deoxynucleotidyl transferase expression.1,2 Classic BL was also excluded by characteristic FL translocation, the t(14;18), and the absence of EBV-EBER.6,9,18

Recently, FL grade 3B has been shown to be a distinct neoplasm from FL grade 1/2 and 3A, according to the cytogenetic and immunohistochemical profiles.11 Some cases of FL...
grade 3B have a break in the c-MYC gene locus and show positive reactivity for MUM1/IRF4, both of which are absent in FL grade 1/2 or 3A. However, the reported MUM1/IRF4-positive cases are CD10-negative. The simultaneous expression of CD10 and MUM1/IRF4 in our case represents a different phenotype from their cases. Moreover, none of the FL grade 3B cases with positive MUM1/IRF4 reactivity show MUM1/IRF4 translocations.

The most prominent observation of the present case was that a rare chromosomal translocation, t(6;14;18)(p25;q32;q21), was proven only in LN cells, whereas complex abnormalities including t(14;18)(q32;q21) and t(6;22)(q24;q11.2), resulting in IGH-BCL2 and c-MYC-IGL fusions, were seen in both LN and BM cells. FISH analysis for IGH-IRF4 could not be performed; however, the presence of t(6;14)(p25;q32) chromosomal translocation suggested the activation of MUM1/IRF4. Unfortunately, molecular analysis for the IgH-CDR3 gene, which is useful to detect B-cell clonality, was not available. Another B-cell clone was, however, suggested by Southern blot analysis for the IgH gene from LN cells, in addition to one lymphoma clone observed in both LN and BM.

Almost all DHL are reported to have a complex karyotype with ≥ 3 numerical and/or structural aberrations. One clone in our case shown in both LN and BM demonstrated a complex karyotype, whereas another clone observed in only LN cells showed a simple translocation, t(6;14;18)(p25;q32;q21). Moreover, 2 out of 20 LN cells and 11 out of 20 BM cells demonstrated a normal karyotype. It has been reported that DHL may arise in two ways: one is that it arises from a preceding FL, and the other is that it arises directly from the much more prevalent B cells with t(14;18). Both of the two independent clones in our case exhibited t(14;18)(q32;q21), suggesting that they arose from common preceding FL.
MUM1/IRF4 is a myeloma-associated oncogene transcriptionally activated as a result of t(6;14)(p25;q32) chromosomal translocation of its juxtaposition to the IgH locus. MUM1/IRF4 is a key regulator of several steps of lymphoid, myeloid and dendritic cell differentiation. MUM1/IRF4 is induced by the activation of nuclear factor (NF)-κB pathway through mitogenic stimuli, including antigen receptor engagement, lipopolysaccharide, interleukin-4, and CD40 signaling.19,23 GC B cells have particularly low levels of MUM1/IRF4, possibly due to the absence of NF-κB.23 Although MUM1/IRF4 is expressed at varying levels throughout B-cell development, its expression peaks in plasma cells.24,25 Its potential development, its expression peaks in plasma cells.24,25 Its potential regulation of its own transcription has also been reported.16,25 Although the expression of MUM1/IRF4 mRNA is induced by human T-cell leukemia virus-1 (HTLV-1) infection26 or EBV-encoded latent membrane protein-1 activation,27 neither EBV nor HTLV-I was detected in our case.

The GC reaction and the differentiation of B cells into plasma cells and memory B cells is regulated by a network of transcription factors.28,29 BCL-6, which is strongly upregulated in GC B cells, suppresses apoptosis and promotes proliferation.22,28-31 MUM1/IRF4 functions as a positive regulator of transcription for many genes,24,32 and also suppresses other genes, such as BCL-6.22 Although most GC B cells lack MUM1/IRF4 expression, the few GC B cells located in the light zone of the GC with MUM1/IRF4 expression13 lost the expression of BCL6 and Ki-67, suggesting that these MUM1/IRF4+ GC cells are preparing to leave the GC and differentiate into plasma cells.25,34 The highly proliferating centroblasts of the GC dark zone fail to express the protein.34 Many MUM1/IRF4+ cells in the GC are negative for CD138/syndecan, suggesting that MUM1/IRF4 expression precedes CD138 expression.25,34 The plasmacytic differentiation with co-expression of MUM1/IRF4 and CD138 despite CD10 expression in our case might be related to MUM1/IRF4 translocation.

MUM1/IRF4 expression in both B and T lymphocytes is upregulated by mitogenic stimuli.33 Among B-cell lymphomas, the strongest expression of MUM1/IRF4 is observed in lymphoplasmacytoid lymphoma/immunocytoma and in multiple myeloma.34 In B-cell non-Hodgkin’s lymphoma, MUM1/IRF4 expression is observed in many DLBCL cases, and some marginal zone lymphoma and small lymphocytic lymphoma cases, whereas it is not seen in B-LBL, mantle cell lymphoma, or FL grade 1/2.33,34

Although a correlation between MUM1/IRF4 protein expression and translocation of the MUM1/IRF4 gene has recently been demonstrated in pediatric lymphomas,35 a translocation involving MUM1/IRF4 is hardly observed in adult cases, irrespective of MUM1/IRF4 protein expression.11 It has been reported that, among 20 proven Ig/IRF4-positive adult cases, none showed a BCL2 break and/or t(14;18),33 suggesting the rarity of the present case. Ig/IRF4 positivity is associated with young age and a favorable outcome; however, its clinical involvement in adult patients is still unknown.11

MUM1/IRF4 has been found to induce c-MYC expression in multiple myeloma cells.16,36 In turn, MYC transactivates IRF4, creating a positive autoregulatory feedback loop, suggesting that any therapy targeting MUM1/IRF4 transcription would have the benefit of decreasing c-MYC transcription.16,24 Moreover, as well as BCL2,23 both c-MYC and MUM1/IRF4 are induced by NF-κB activation,19,22,27 suggesting that NF-κB may be a therapeutic target38 in B-cell malignancies related to c-MYC and MUM1/IRF4. On the other hand, MUM1/IRF4 functions as a tumor suppressor in c-MYC-induced B-cell leukemia.36,41 The present rare case arouses interest in view of the possible “dual” activation of both c-MYC and MUM1/IRF4 through two independent dual-translocations, c-MYC/BCL-2 and IRF4/BCL-2.

In summary, we here report a unique case of double-hit lymphoma, demonstrating a rare abnormal chromosome, t(6;14)(p25;q32;q21), suggesting an independent clone with IRF4/BCL-2 in addition to c-MYC/BCL-2 dual-translocations, and showing plasmacytic differentiation. To the best of our knowledge, this is the first report describing the dual-hit translocations of IRF4/IGH and BCL-2/IGH as a result of t(6;14;18)(p25;q32;q21). The accumulation of similar cases and further examinations are desired.

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