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Review Article

BCL2 and MYC Dual-Hit Lymphoma/Leukemia

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Translocation of the *BCL2* gene on the chromosome band 18q21.3 results in consistent expression of the Bcl2 protein, an apoptosis inhibitor. *BCL2* usually translocates to *the immunoglobulin* (*IG*) *heavy chain* (*IGH*) gene as t(14;18)(q32;q21.3) and rarely to IG light chain (*IGK*, *IGL*) loci as t(2;18)(p11;q21.3) or t(18;22)(q21.3;q11). The t(14;18) translocation is observed in 70-95% of follicular lymphoma cases and 20-30% of diffuse large B-cell lymphoma (DLBCL) cases. The *MYC* gene on chromosome band 8q24 acts as an accelerator of cell proliferation. *MYC* translocates to 14q32/*IGH* as t(8;14)(q24;q32) or less commonly to 2p11/*IGK* as t(2;8)(p11;q24) or 22q11/*IGL* as t(8;22)(q24;q11). The 8q24/*MYC* translocation is detected in nearly all Burkitt lymphoma (BL) and up to 10% of DLBCL cases. Both translocations rarely occur in an identical cell and this lymphoid malignancy is termed *BCL2* and *MYC* dual-hit lymphoma/ leukemia (DHL). The pathological diagnosis in most cases of DHL with *BCL2-IG* and *MYC-IG* translocation is B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL, although DLBCL is most common in DHL with *BCL2*-IG and *MYC-nonIG* translocation. The frequency of DHL with *BCL2* and *MYC* translocation is estimated at around 2% of all B-cell malignancies. The condition is characterized by elevated serum lactate dehydrogenase levels, the presence of B symptoms, bone marrow involvement, advanced disease stage, extranodal involvement, and central nervous system (CNS) involvement at presentation or disease progression. Despite treatment strategies including CNS-targeted therapy, the prognosis for DHL is extremely poor. In this review, the current knowledge of the clinicopathological status of DHL is summarized and discussed. 〔*J Clin Exp Hematopathol 51(1) : 7-12, 2011*〕

Keywords: *BCL2*, *MYC*, dual-hit, double-hit

INTRODUCTION

There are several characteristic chromosomal abnormalities in B-cell malignancies, most of which take the form of reciprocal translocation involving the immunoglobulin (*IG*) genes. Typical chromosomal abnormalities in follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), and Burkitt lymphoma (BL) are shown in Table 1. Due to the number of possible abnormalities, a specific translocation does not necessarily imply a specific histology. In this review, the *BCL2* gene and *MYC* gene, which are major examples of translocation partners of *IG* genes, are described. Also, a rare type of lymphoid neoplasm with *BCL2-IG* and *MYC* translocation as dual-hit lymphoma/ leukemia (DHL) is expounded.

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BCL2 GENE

The 18q21.3/*BCL2* gene was initially observed in the mid-1980s by cloning of the breakpoint in cases with t(14;18) translocation,[1] which is presumed to result from an error during VDJ rearrangement of the *IG* gene in the bone marrow. Neoplastic B-lymphocytes with this translocation constantly express the Bcl2 protein, an apoptosis inhibitor, whereas, in normal B-cell differentiation, the Bcl2 protein is not expressed in the germinal center (GC). Nearly all normal B-cells undergo apoptotic cell death in the GC, with only a small population of B-cells with high affinity escaping apoptosis and expressing the Bcl2 protein again. These B-cells migrate out of the secondary follicle and are located in the marginal zone as memory B-cells. In contrast, neoplastic B-lymphocytes with Bcl2 overexpression by t(14;18) translocation become apoptosis-resistant and proliferate in the GC. Thus, the pathological status of t(14;18) translocation is the overexpression of the Bcl2 protein in the GC, resulting from an error in transcriptional control.[2] However, even in the abnormal transcriptional state, the Bcl2 protein itself is the same as that in the normal transcriptional state. It is noteworthy that Bcl2 protein expression in lymphoma cells occurs quite frequently, even in cases without chromosomal translocation. The *BCL2* gene often translocates to the *IG* heavy

Table 1. Major chromosomal translocation in B-cell lymphoma

Histology	Chromosomal translocation	frequency (%)	Related gene
Follicular lymphoma	t(14;18) (q32;q21)	70-95	*BCL2*
	t(3;14) (q27;q32)	10-15	*BCL6*
Diffuse large B-cell lymphoma	t(3;14) (q27;q32)	30-35	*BCL6*
	t(14;18) (q32;q21)	20-30	*BCL2*
	t(8;14) (q24;q32)	10	*MYC*
Burkitt lymphoma	t(8;14) (q24;q32)	70	*MYC*
	t(2;8) (q12;q24)	8	*MYC*
	t(8;22) (q24;q11)	22	*MYC*

chain (*IGH*) gene as t(14;18)(q32;q21.3) and rarely to *IG* light chain (*IGK*, *IGL*) loci as t(2;18)(p11;q21.3) or t(18;22)(q21.3;q11).[3] The t(14;18) translocation is observed in 70-95% of FL cases[4,5] and 20-30% of DLBCL cases.[6,7]

MYC GENE

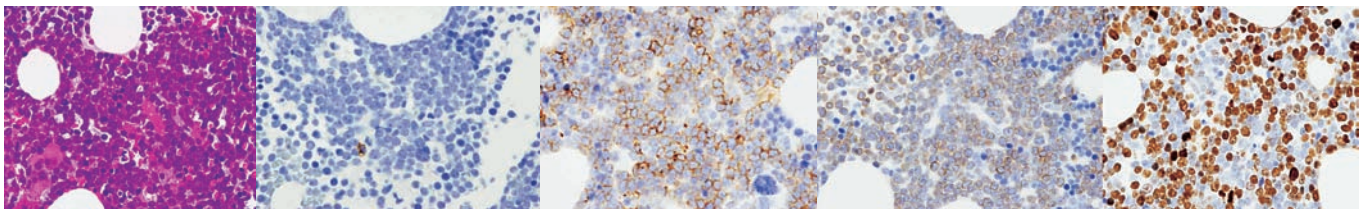
The 8q24/*MYC* gene was discovered in the analysis of the chromosomal breakpoint in cases with BL. This gene was found to resemble the myelocytomatosis viral oncogene (*v*-Myc) in chickens and was denominated as *c-MYC* (*MYC*). The Myc protein is a transcription factor that controls gene expression by binding to nuclear DNA. The *MYC* gene is reported to be amplified in various types of cancer and is thought to be an oncogene that encodes the Myc protein, which is involved in the regulation of apoptosis. The *MYC* gene is expressed in almost all normal tissues and is highly expressed in tissues with high proliferation rates. The *MYC* gene is expressed in a rapid manner under the proliferative stimulation, with a half-life within 30 minutes. The half-life of the Myc protein also is within 30 minutes. In cases with translocation between the *MYC* gene and *IG* gene, the Myc protein is constantly expressed in all stages of cell turnover. Thus, *MYC* translocation is not an abnormality in cell differentiation, but in cell turnover. In B-cell lymphoma, reciprocal translocation between the *MYC* gene and *IGH* gene as t(8;14)(q24;q32) is often observed, whereas translocation between the *MYC* gene and *IGK* or *IGL* gene as t(2;8)(p11;q24) or t(8;22)(q24;q11) is rarely observed.[3] *MYC* translocation is detected in almost all cases of BL and about 10% of cases of DLBCL.[8] It is not always true that the *MYC* translocation is the primary event of genomic abnormality in oncogenesis. The *MYC* translocation in FL and DLBCL might be the secondary genomic event.

BCL2 AND *MYC* DUAL HIT LYMPHOMA/LEUKEMIA

An Example Case

Two chromosomal translocations characteristic for B-cell malignancies are rarely detected in identical tumor cells. Such cases are called "dual-hit lymphoma/leukemia" or "double-hit lymphoma." Although these cases are typically diagnosed by chromosomal analysis (G-banding), diagnosis by fluorescence *in situ* hybridization (FISH) analysis is thought to be of equal value. However, in cases diagnosed only by FISH, the translocation partner of the *MYC* gene may not be identified, depending on the FISH probe. A representative example case is shown in Fig. 1. Immunohistochemical analysis of the lymphoma cells in Fig. 1 showed partial positivity of CD20 and positivity of CD10 and *BCL2*. The MIB-1 labeling index was 64.7%, which is clearly lower than that in usual BL. In this case, both of t(14;18)(q32;q21) as *BCL2-IGH* translocation and t(8;22)(q24;q11) as *MYC-IGL* translocation were detected in 18 of 20 cells analyzed. Hematoxylin-eosin staining revealed the histology of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (IL). IL has been included as a new category of B-cell lymphoma in the 4th edition of the WHO classification,[3] with morphological and genetic features of both DLBCL and BL. Starry sky macrophages are typically present in IL, as well as many mitotic figures and prominent apoptosis, causing a resemblance to BL. The cellular morphology is variable. In some cases, the cells resemble those in BL, but with more variation in nuclear size and contour than is considered acceptable for BL. Some cases are consistent with BL morphologically, but have an atypical immunophenotype and/or genetic features.[3] Some IL cases were previously classified as Burkitt-like lymphoma or atypical BL. Most cases of DHL are thought to be categorized as IL.

Immunohistochemistry



Chromosomal analysis

A: 46,XX,t(8;22)(q24;q11),t(14;18)(q32;q21) 18/20 cells

B: 46,XX 2/20 cells

Fig. 1. A representative case of dual-hit lymphoma/leukemia. Immunohistochemical examination of a bone marrow specimen showed partial positivity of CD20 and positivity of CD10 and Bcl2. The MIB-1 labeling index (Ki-67) was lower than that for typical Burkitt lymphoma. Chromosomal analysis in the bone marrow showed *BCL2-IGH* and *MYC-IGL* translocations.

Case Series

We reported a clinicopathological analysis of 27 cases of DHL with *BCL2-IG* and *MYC-IG* translocations.[9] All cases were diagnosed as DHL by chromosomal analysis. Twenty-two cases were diagnosed at presentation and 5 cases at relapse or disease progression. The median age at the diagnosis of DHL was 51 years. The clinical entity was B-cell lymphoma in 23 cases and B-cell leukemia in 4 cases. Extranodal involvement sites were observed in 25 cases (93%), and bone marrow involvement was observed in 15 cases of the 23 lymphoma cases (65%). Central nervous system (CNS) involvement was observed in 15 cases (56%) at presentation or during the clinical course. The median overall survival (OS) period after the diagnosis of DHL was only 6 months, and 1-year survival rate was 22% (Fig. 2A). Seven of the 27 cases showed *BCL6* translocation in addition to *BCL2-IG* and *MYC-IG* translocations, indicative of triple-hit lymphoma/leukemia (THL). The median survival period of the THL cases was only 4 months, and this survival period was shorter than that in the other 20 cases without *BCL6* translocation (Fig. 2B). Chromosomal analysis of the 27 cases revealed 11 cases with t(14;18) and t(8;14) translocation, 9 cases with t(14;18) and t(8;22) translocation, 4 cases with t(14;18) and t(2;8) translocation, 1 case with t(2;8) and t(8;14) translocation, and 2 cases with t(8;14;18) translocation. The translocation partner of *MYC* was *IGH* in 14 cases and *IGK/L* in 13 cases. The proportion of the cases with *MYC-IGH/L* translocation was clearly higher than that in usual BL. The MIB-1 labeling index was measured in 14 cases, and it was significantly higher in the 7 cases with *BCL2-IG* and *MYC-IGH* translocation compared with that in the other 7 cases with *BCL2-IG* and *MYC-IGK/L* translocation. This finding might be due to the distinction of the breakpoint of the *MYC* gene between the cases with *MYC-IGH* and those with *MYC-IGK/L* translocation.[10] However, the survival duration was not significantly different between the 14 cases with *BCL-IG* and *MYC-IGH* translocation and the other 13 cases with *BCL2-IG* and *MYC-IGK/L* translocation. In the pathological review of 20 of the 27 DHL cases, there were 15 cases of IL, 2 cases of FL, grade 3, and 3 cases of composite lymphoma ; there were no cases of typical BL or typical DLBCL. Regarding the pathogenesis of DHL, it has yet to be defined whether the 2 translocations of *BCL2-IG* and *MYC-IG* occur simultaneously or separately. In at least 7 of the 27 cases, it was clearly shown by the time-sequence of chromosomal analysis that *BCL2-IG* translocation preceded *MYC-IG* translocation. It is possible that *BCL2-IG* translocation arises from the error in VDJ rearrangement in the bone marrow and *MYC-IG* translocation from the error in somatic hypermutation and class switch in the GC, which would result in *BCL2-IG* translocation preceding *MYC-IG* translocation in all cases of DHL. In fact, about half of the 27 DHL cases in our study showed *MYC-IGK/L* chromosomal translocation. This finding might be due to the fact that only 1 *IGH* gene exists in the germline under the condition of *BCL2-IG* translocation.

There are about 200 reported cases of DHL with *BCL2-IG* and *MYC-IG* translocation, which involve 7 case series dealing with over 10 DHL cases, including our report discussed above (Table 2). These reports uniformly describe the extremely poor prognosis of DHL.[9,11-16] In most cases, the survival period was less than 1 year from the diagnosis of DHL. Macpherson *et al*. analyzed 39 patients with Burkitt-like, small noncleaved non-Burkitt's lymphoma. Of those cases, 13 patients had the karyotype of DHL. Eleven patients showed *BCL2-IG* and *MYC-IG* translocation, and 1 patient showed *MYC*-nonIG translocation detected by G-banding.[11] Chromosomal data was not described in the remaining case. In the 13 DHL patients, 4 were receiving palliative therapy, 6 were being treated with standard chemotherapy, and 3 were being treated with high-dose chemotherapy (1 with bone mar-

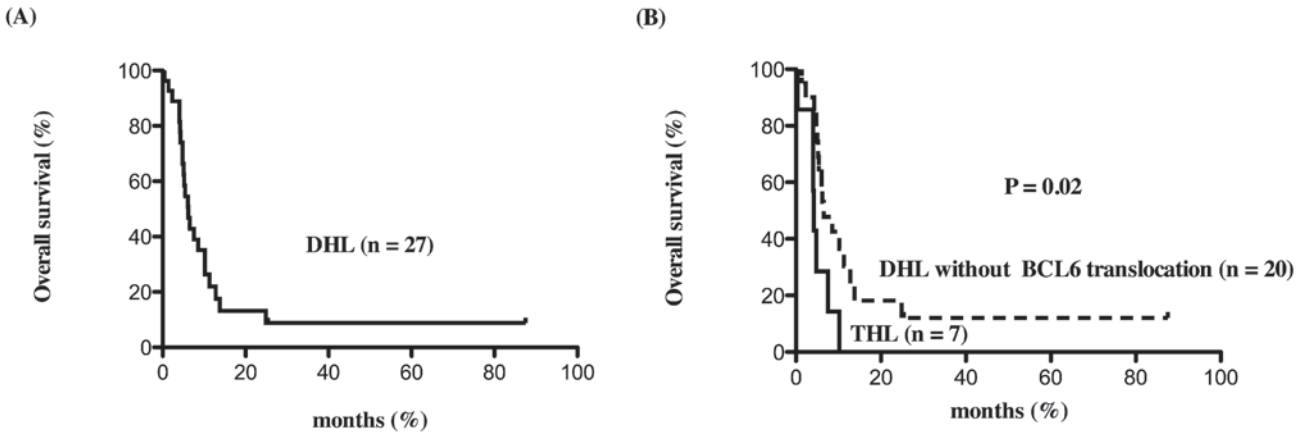


Fig. 2. Case series of dual-hit lymphoma/leukemia (DHL). Overall survival in 27 cases with DHL carrying both *BCL2-IG* and *MYC-IG* translocations (ref. 9). (***2A***) The median survival period for patients with DHL was 6 months. (***2B***) The median survival period of the triple-hit cases (*BCL2*, *MYC*, and *BCL6* translocations) was only 4 months.

Table 2. Case series of DHL

Authors	Reference	Total	MYC partner IG	MYC partner nonIG	MYC partner unknown (FISH only)	Median OS (mon)
Tomita, *et al*.	9	27	27	0	0	6
Macpherson, *et al*.	11	13[*1]	11	1	0	2.5
Kanungo, *et al*.	12	14	12	0	2	9
Le Gouill, *et al*.	13	16	7	1	8	5
Niitsu, *et al*.	14	19	11	8	0	14
Johnson, *et al*.	15	54	30	24	0	4[*2]
Snuderl, *et al*.	16	20	9	2	9	4.5

[*1] karyotype of 1 case is not described ; [*2] by personal communication

row transplantation). The median survival period in the 13 patients with DHL was only 2.5 months, which was significantly shorter than that in the other 26 patients without DHL. Kanungo *et al*. reported 14 DHL cases, 12 with concurrent t(14;18) and *MYC*-IG translocation detected by G-banding and 2 diagnosed only by FISH.[12] None of these patients had a history of FL. The pathological diagnosis included 9 cases of BL/atypical BL and 3 cases of DLBCL. The remaining 2 cases were plasmablastic myeloma and low-grade B-cell lymphoma. The MIB-1 labeling index of the low-grade B-cell lymphoma was only 5%. All cases expressed the Bcl2 protein. The presence of Bcl2 expression in a case resembling BL or atypical BL might be a clue for the coexistence of t(14;18) translocation. *BCL2-IG* and *MYC* translocations might have the form of indolent lymphoma in a small minority of DHL cases.

Gouill *et al*. reported 16 DLBCL cases with t(14;18) and *MYC* rearrangement.[13] Seven cases were diagnosed as DHL with t(14;18) and *MYC-IG* translocation and 1 case as DHL with t(14;18) and *MYC-nonIG* translocation by G-banding. The other 8 cases were diagnosed as DHL only by FISH. Regarding the clinical features, high frequencies of ECOG performance status＞2 (81%), elevated lactate dehydrogenase (LDH) levels (100%), stage IV disease (100%), and age-adjusted international prognostic index (81%) were reported. Immunohistochemical analysis showed a GC profile[17] in all cases. Niitsu *et al*. found 19 DLBCL cases with t(14;18) translocation and *MYC* rearrangement detected by G-banding in 394 DLBCL patients with abnormal karyotypes (4.8%).[14] Among these 19 cases, *MYC-IG* type translocation was seen in 11 cases, and *MYC-nonIG* type translocation was seen in 8 patients as der (8)(q24). DHL was observed most frequently among patients with high LDH levels, B symptoms, bone marrow involvement, and advanced disease stage. Immunohistochemical analysis showed that 16 of the 19 cases belonged to the GC profile. Nine patients underwent intensive chemotherapy with CyclOBEAP[18] with/without rituximab, and 3 of those 9 patients received autologous stem cell transplantation (SCT). The median OS period and 2-year OS rate in the 19 DHL patients were 14 months and 23%, respec-

tively. Even if patients had a complete response to chemotherapy, they subsequently suffered early relapse, with a 2-year progression-free survival rate of 0%. The researchers indicated that only a few patients received rituximab and that its usefulness should be assessed in a further study. Thus intensive chemotherapy might improve prognosis to a certain extent.

Johnson *et al*. analyzed 54 cases with concurrent *BCL2* and *MYC* translocations by G-banding, consisting of 30 cases of *MYC-IG* translocation and 24 cases of *MYC-nonIG* translocation.[15] The pathological diagnosis involved 36 cases of IL, 17 cases of DLBCL, and 1 case of FL. There was a strong association between DLBCL morphology and the presence of a *MYC*-nonIG translocation. The median OS period in the 54 patients was 4 months. When assessed according to the *MYC* translocation partner, patients with *MYC-nonIG* translocation showed a more favorable prognosis than those with *MYC-IG* translocation when treated with curative intent (median survival : 35 months vs. 4 months ; P = 0.0008). Snuderl *et al*. reported 20 DHL cases with concurrent *BCL2* and *MYC* rearrangements.[16] Nine cases were diagnosed as DHL with t(14;18) and t(8;22) translocation and 2 cases as DHL with t(14;18) translocation and add (8)(q24) by G-banding. The remaining 9 cases were diagnosed as DHL only by FISH. Six patients had a history of FL. Regarding the clinical characteristics, all of the 20 patients (100%) had elevated serum LDH levels, and 18 patients (90%) had advanced disease stage. Extranodal disease was present in 17 patients (85%). The pathological diagnosis involved 12 cases of IL, 7 cases of DLBCL, and 1 case of lymphoblastic lymphoma. The researchers also carried out case-control comparisons of DHL with BL and International Prognostic Index (IPI)-matched DLBCL. Although 18 DHL patients received rituximab-containing chemotherapy, the median OS in the 20 DHL patients was only 4.5 months, which was significantly inferior to both BL (P = 0.002) and IPI-matched DLBCL (P = 0.04) cases. The distinguishing features of DHL compared with BL included Bcl2 expression (P＜0.0001), Mum1/IRF4 (P = 0.006), MIB-1 index＜95% (P＜0.0001), and absence of EBV-EBER (P = 0.006). DHL commonly contained t(8;22) translocation compared with BL controls and exhibited a higher number of chromosomal aberrations (P = 0.0009).

Clinicopathological Characteristics and Treatment

The pathological diagnosis of most cases with *BCL2-IG* and *MYC-IG* translocations is IL, although it is DLBCL in the majority of cases with *BCL2-IG* and *MYC-nonIG* translocations, according to the new WHO classification.[3] DHL cases with *BCL2-IG* and *MYC-IG* translocation showing typical BL or typical DLBCL morphology are extremely rare. CD20 expression is positive in all cases of DHL, even if it is only weakly positive in a small population of cases.[19] Most DHL cases showed a molecular signature of IL[20] in gene expression profiling, although fewer cases more closely resemble BL.[21]

The frequency of DHL with *BCL2-IG* and *MYC* translocation has been estimated at around 2% of all B-cell malignancies in a comprehensive manner. DHL with *BCL2-IG* and *MYC* translocation is characterized by elevated serum LDH levels, the presence of B symptoms, bone marrow involvement, advanced disease stage, extranodal involvement, and CNS involvement at presentation or disease progression.[9,13,14,16]

Most patients die within months of diagnosis, regardless of chemotherapy with curative intent. Patients who reach complete remission tend to subsequently suffer early relapse. In the setting of autologous SCT, relapse or progression during chemotherapy often precludes it. The role of allogeneic SCT in DHL is also unclear, because of few reported cases. Also the role of additional efficacy of rituximab added to standard chemotherapy is unknown. Thus, a standard treatment strategy against DHL is yet to be elucidated.

FUTURE DIRECTION

Regardless of the extremely poor prognosis, the correct diagnosis of DHL is easy to achieve using G-banding and/or FISH. It is important to consider the possibility of DHL when treating aggressive B-cell lymphoma. Especially in cases without available G-banding and/or FISH at presentation showing relapse/progression within a short time of remission, typically in a rapid manner, re-biopsy with G-banding and FISH should be attempted to examine the presence of DHL. Also, in cases with a diagnosis of BL with unusual Bcl2 expression, the possibility of DHL should be examined. Intensive chemotherapy including at least CNS-targeted therapy should be started immediately at the confirmed diagnosis of DHL. DHL with *BCL2-IG* and *MYC* translocation is a noteworthy B-cell neoplasm with extremely poor prognosis. Prospective studies are urgently needed to establish a standard effective treatment strategy against DHL.

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