Clinical significance of 8q24/*c-MYC* translocation in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) has heterogeneous clinical, histological, and molecular features. We evaluated the clinical characteristics and prognoses of patients with DLBCL carrying 8q24 translocations. A total of 1864 consecutive patients with non-Hodgkin's lymphoma were treated in the Adult Lymphoma Treatment Study Group from 1998 to 2005. Of the 252 patients with DLBCL with abnormal karyotypes, 28 patients with DLBCL with the 8q24 translocation were identified. There were 14 men and 14 women, with a median age of 61 years. The 8q24 translocation was observed significantly more frequently among patients with poor performance status, among patients with high lactate dehydrogenase level, and among patients with bone marrow involvement. The 5-year overall survival was 43.9% among the patients with 8q24 translocation, and 67% among the patients with other chromosomal abnormalities. The 8q24 translocation group showed significantly poorer prognosis than the group with other translocations. In addition, patients with t(14;18) and 8q24 translocation showed significantly poorer prognosis than those with 8q24 translocation alone. It will be necessary to study whether more aggressive chemotherapy or rituximab combination chemotherapy is effective in 8q24 translocation cases. (*Cancer Sci* 2009; 100: 233–237)

Diffuse large B-cell lymphoma (DLBCL) accounts for approximately 50% of non-Hodgkin's lymphomas (NHL) and has heterogeneous clinical, histological, immunophenotypic, cytogenetic, and molecular features. Currently, the international prognostic index (IPI) is widely used as a prognostic factor.[1] However, it has been established that clinical features and treatment responses are also dependent on genetic and molecular features that modify disease aggressiveness. The *c-MYC* gene at 8q24 is involved in three translocations, most commonly t(8;14) (q24;q32), and less often t(2;8) (p12;q24) and t(8;22) (q24;q11).[2] Overexpression of *c-MYC* drives cell proliferation and the expression of other genes involved in cell growth.[3] *C-MYC* translocation is characteristic of Burkitt lymphoma; t(8;14) occurs in 80–90% of Burkitt lymphoma cases, and in up to 5–10% of DLBCL cases.[4] *C-MYC* activates genes that promote cell-cycle progression and suppresses inhibitory proteins like p21CIP1 and p27KIP1.[5] Paradoxically, *c-MYC* also promotes apoptosis via the p19ARF–p53 apoptotic pathway.[5] *c-MYC* appears to be more commonly found in DLBCL with extranodal involvement and associated with a more aggressive clinical course.[3]

The relationship between 8q24/*c-MYC* translocation and the clinical outcome of DLBCL has been the subject of controversy. We therefore studied the clinical characteristics of DLBCL with 8q24/*c-MYC* translocation and correlated the findings with clinical outcome.

Patients and Methods

Patients. A total of 1543 consecutive patients with NHL were treated in the Adult Lymphoma Treatment Study Group (ALTSG) in Japan from 1998 to 2005. Chromosomal data were available for 489 (58%) of the 842 patients with DLBCL. Among them, 252 (52%) patients who had abnormal karyotypes were evaluated. Pathological evaluation of the materials from each patient was carried out at several central review meetings by six hematopathologists in the ALTSG pathology review board. A consensus diagnosis on each patient was obtained using the third World Health Organization classification. All patients were newly diagnosed, were previously untreated, and received anthracycline-containing combination chemotherapy. Briefly, the CyclOBEAP (cyclophosphamide [CPA], vincristine [VCR], bleomycine, etoposide, doxorubicin [DXR], prednisone [PDN]) regimen[4] was administered primarily to younger patients (≤65 years old) with DLBCL ($n = 78$), and the CHOP (CPA, DXR, VCR, PDN) regimen was administered primarily to older patients (full-dose CHOP [65–79 years old] [$n = 168$], and two-thirds dose CHOP [$n = 6$]). The DLBCL patient who received rituximab was not included in this study. The median follow-up period was 62 months (range 24–86 months). Approval of the study protocol was obtained from the institutional review board at each participating institute. Informed consent was obtained from patients according to the Declaration of Helsinki.

Methods

Cytogenetic studies. We used a standard technique for chromosome analysis.[6] Biopsied lymph nodes or other tumors were immediately disaggregated with scalpels in RPMI-1640 medium, and single cells were suspended in RPMI-1640 medium supplemented with 20% fetal bovine serum at a concentration of 10^6 cells/mL. For the short-term unstimulated culture method, metaphase cells were arrested by exposing them to colcemid at a final concentration of 0.02 g/mL for the last 2 h before harvesting. After hypotonic treatment with 0.075 mol/L KCl at room temperature for 15 min, the cells were fixed with ethanol and acetic acid (3 : 1). Metaphase preparations were made by the steam-dry method. After the slides had been aged at 37°C for 4–7 days, the trypsin G-banding technique was used for analysis of karyotypes. Chromosome identification, karyotypic designation, and determination of clonality were carried out in accordance with the International System for Human Cytogenetic Nomenclature.[7]

Immunohistochemistry. Paraffin-embedded sections of each sample were immunostained with monoclonal antibodies against CD5 (Novocastra, Newcastle, UK), CD10 (Novocastra), CD20 (Novocastra), BCL2 (Dako, Glostrup, Denmark), BCL6 (Novocastra), MUM-1 (DAKO), and Mib-1 (Novocastra). The following categories were defined: negative, <30% positively stained tumor cells; and positive, ≥30% positively stained tumor cells.

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Table 1. Diffuse large B-cell lymphoma with 8q24 translocation

Type of 8q24/*MYC* translocation	Translocation	Frequency
Immunoglobulin type	t(8;14)(q24;q32)	19 (67.9%)
	t(8;22)(q24;q11)	1 (3.6%)
Non-immunoglobulin type	der(8)(q24)	8 (28.6%)
Additive chromosomal abnormality		
18q21.3/*Bcl-2*	t(14;18)(q32;q21)	8 (28.6%)
3q27/*Bcl-6*	t(3;14)(q27;q32)	1
	t(1;3)(q25;q27)	1
	der(3)(q27)	5
17p11/TP53	der(17)(p11)	2

Response. Tumor response was assessed after 12 weeks of chemotherapy or at the end of treatment, and was classified as complete response (CR), unconfirmed complete response (CRu), partial response (PR), stable disease, or progressive disease according to the International Workshop criteria.[8]

Statistical analysis. All statistical analyses were carried out with SAS software (version 9; SAS Institute, Cary, NC, USA). Progression-free survival (PFS) was calculated from the date of beginning chemotherapy to the date of progression or relapse or to the date of the last contact. Overall survival (OS) was calculated from the date of beginning chemotherapy to the date of death from any cause or to the date of the last contact. OS and PFS were used as parameters, and analysis was carried out according to the Kaplan–Meier method. The statistical significance of differences in survival was assessed by the log-rank test. Differences between groups were evaluated by the Mann–Whitney *U*-test.

Results

Clinical characteristics and immunohistochemical analysis of DLBCL with 8q24 translocation. The 8q24 translocation was seen in 28 (11%) of the 252 patients with DLBCL with abnormal karyotypes. Among the 28 patients with DLBCL with 8q24 translocation, immunoglobulin (IG)-type translocation was seen in 20 patients, including 19 (67.9%) with t(8;14)(q24;q32) and one (3.6%) with t(8;22)(q24;q11). Eight patients with der(8)(q24) had a non-IG-type translocation. In addition to 8q24 translocation, eight patients had t(14;18)(q32;q21), one had t(3;14)(q27;q32), one had t(1;3)(q25;q27), five had der(3)(q27), and two had der(17)(p11) (Table 1). The DLBCL patients were divided into the following two groups and their clinical characteristics were compared: patients with 8q24 translocation ($n = 28$), and those with other chromosomal abnormality ($n = 224$) (Table 2). There were no significant differences in age, sex, stage, or presence or absence of bulky mass between the two groups. Poor performance status (PS), high serum lactate dehydrogenase (LDH) level, bone marrow involvement, and poor IPI were significantly more prevalent among patients with 8q24 translocation than among patients with other translocations. The CR rate was 67% among patients with 8q24 translocation and 83% among patients with other translocations ($P = 0.02$). The CRu and PR rates were 0 and 21%, respectively, among patients with 8q24 translocation, and 1 and 13%, respectively, among patients with other translocations.

Immunohistochemically, CD5 was positive in 14.3% of the patients with 8q24 translocation and in 11.6% of the patients with other chromosomal abnormalities. The CD10-positive rate was higher in the 8q24 translocation group (35.7%) than in the other chromosomal abnormalities group (12.5%) ($P = 0.001$). BCL-2 was positive in 36% of the patients with 8q24 translocation and in 45% of the patients with other chromosomal abnormalities ($P = 0.24$). BCL-6 was positive in 53.6% of the patients with 8q24 translocation and in 54% of the patients with other chromosomal abnormalities ($P = 0.46$). The MUM-1-positive rate was lower in the 8q24 translocation group than in the other chromosomal abnormalities group (14.3 vs 42%, $P = 0.005$).

The 28 cases with DLBCL with 8q24 translocation were assigned to the germinal center B-cell like (GCB) group or the non-GCB group, according to the classification method of Hans *et al*.[9] When the 8q24 translocation patients were divided into the GCB group and the non-GCB group, the GCB group consisted of 20 cases (71.4%) and the non-GCB group consisted of eight cases. Among the patients in the other chromosomal abnormalities group, 55.4% were in the GCB group.

Comparison of OS and PFS between the 8q24 translocation group and the other chromosomal abnormalities group among the patients with DLBCL. The survival curves of the 8q24 translocation group ($n = 28$) and the other chromosomal abnormalities group ($n = 224$) are shown in Fig. 1. The 5-year OS rate was 43.9% among the patients with 8q24 translocation and 67% among the patients with other chromosomal abnormalities ($P = 0.001$) (Fig. 1a). The 8q24 translocation group showed significantly poorer prognosis than the other chromosomal abnormalities group in terms of PFS ($P = 0.001$) (Fig. 1b).

Comparison of OS and PFS between the patients with DLBCL with 8q24 translocation and patients with Burkitt lymphoma with 8q24 translocation. Ten patients with Burkitt lymphoma with 8q24 translocation were treated in the ALTSG during the study period. The median age of the 10 patients with Burkitt lymphoma was 46 years (range 18–60 years). There were seven men and three women. An elevated LDH level was observed in 100%, and a bulky mass larger than 8 cm was present in 50% of the patients. All patients received anthracycline-containing combination chemotherapy (CyclOBEAP or hyper CVAD).

The 5-year OS was 43.9% among the 28 patients with 8q24 translocation-positive DLBCL and 48.6% among the 10 patients with 8q24 translocation-positive Burkitt lymphoma, showing no significant difference (Fig. 2a). The 5-year PFS was 28.9%

Table 2. Characteristics of patients with diffuse large B-cell lymphoma with or without 8q24 translocation

Characteristic	8q24 translocation ($n = 28$)	Other abnormality ($n = 224$)	*P*-value
Median age (years)	61 (range 29–79)	64 (range 40–87)	Not significant
Sex (M/F)	14 (50%)/14	117 (52%)/107	Not significant
Performance status (0, 1/2–4)	11 (39%)/17	142 (63%)/82	0.01
Stage (I, II/III, IV)	6 (21%)/22	80 (36%)/144	Not significant
Lactate dehydrogenase (N/>N)	5 (18%)/23	82 (37%)/142	0.02
Bulky mass present	5 (18%)	51 (23%)	Not significant
Bone marrow involvement present	16 (57%)	77 (34%)	0.03
International prognostic index (L, L–I/H–I, H)	7 (25%)/19	90 (40%)/134	0.02
Complete response	18 (67%)	186 (83%)	0.02

N: Normal; L: Low; L-I: Low-intermediate; H-I: High-intermediate; H: High

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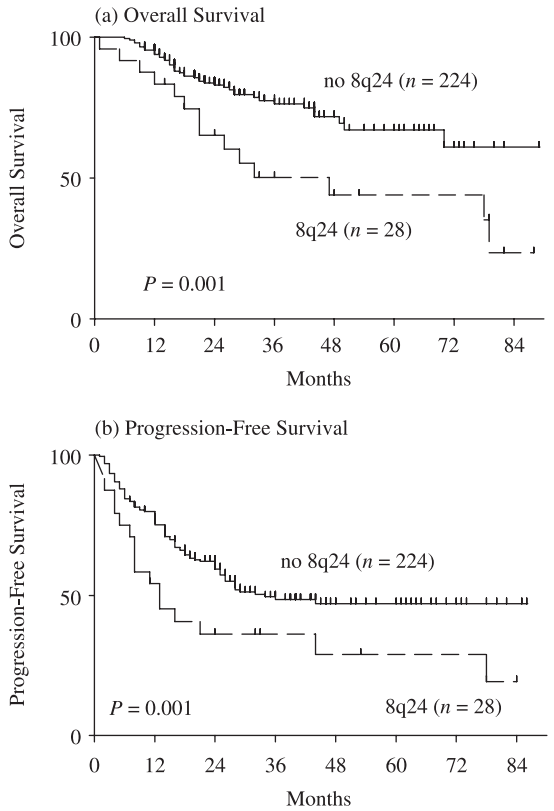

Fig. 1. (a) Overall and (b) progression-free survival curves of patients with diffuse large B-cell lymphoma according to the presence or absence of 8q24 translocation.

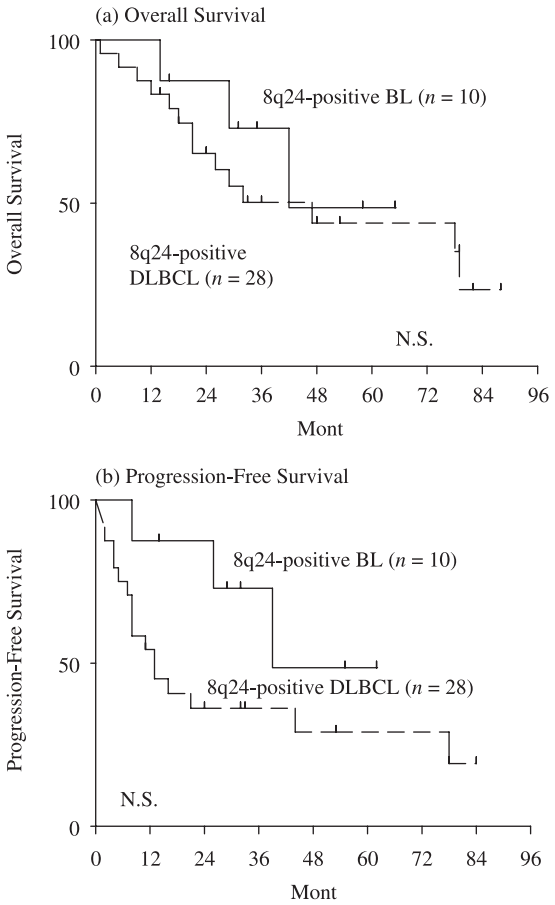

Fig. 2. (a) Overall and (b) progression-free survival curves of patients with diffuse large B-cell lymphoma and Burkitt lymphoma (BL) with 8q24 translocation. N.S., not significant.

among the patients with 8q24 translocation-positive DLBCL and 47% among the patients with 8q24 translocation-positive Burkitt lymphoma, also showing no significant difference (Fig. 2b).

Comparison of OS and PFS between the patients with DLBCL with 8q24 translocation with or without t(14;18)(q32;q21). Among the patients with DLBCL with 8q24 translocation, the 5-year OS was 0% in the eight patients with t(14;18)(q32;q21) and 74.9% in the 20 patients without t(14;18)(q32;q21), showing a statistically significant difference ($P < 0.0001$) (Fig. 3a). The 5-year PFS was 0% among those with t(14;18)(q32;q21) and 46.5% among those without t(14;18)(q32;q21), also showing a significant difference ($P < 0.0001$) (Fig. 3b).

Comparison of OS and PFS between the patients with DLBCL with 8q24 translocation with or without 3q27 translocation. The 5-year OS was 50% among the patients with 8q24 and 3q27 translocation, and 44% among the patients with 8q24 but not 3q27 translocation, showing no significant difference. The 5-year PFS was 33.3% among the patients with 8q24 and 3q27 translocation and 27.5% among the patients with 8q24 but not 3q27 translocation, also showing no significant difference.

Univariate and multivariate analyses. In patients with DLBCL, the OS was significantly worse for patients with the following characteristics: age of 60 years or older, performance status of 2–4, high serum LDH level, Ann Arbor stage III/IV, more than one extranodal site, B symptoms, and 8q24 translocation (Table 3). Multivariate analysis revealed that the seven prognostic factors were associated with OS. Among the prognostic factors, age, performance status, serum LDH level, and 8q24 translocation were significantly associated with survival.

Discussion

The relationship between 8q24/*c-MYC* translocation and the clinical features of DLBCL has been the subject of controversy.

Table 3. Univariate and multivariate analysis of prognostic factors in diffuse large B-cell lymphoma

Variable	Unfavorable factor	Univariate		Multivariate	
		Hazards ratio	*P*-value	Hazards ratio	*P*-value
Age	>60 years	2.42	0.001	2.21	0.006
Performance status	2–4	2.08	0.003	1.86	0.02
Lactate dehydrogenase	>Normal	2.61	0.001	2.37	0.002
Stage	III/IV	1.81	0.01	–	–
Extranodal site	>One site	1.61	0.01	–	–
B symptoms	Present	1.41	0.03	–	–
8q24 translocation	Positive	2.92	0.001	2.65	0.001

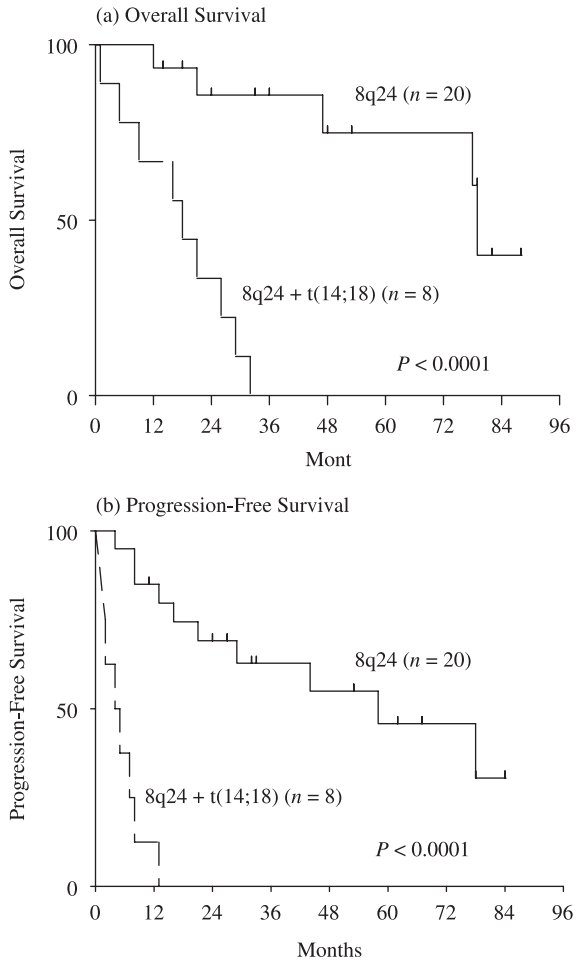

Fig. 3. (a) Overall and (b) progression-free survival curves of patients with diffuse large B-cell lymphoma with 8q24 translocation according to the presence or absence of t(14;18) translocation.

Akasaka *et al*. analyzed *c-MYC* translocation by inverse polymerase chain reaction, reporting that the 2-year OS rate was 37.2% among patients with c-MYC-positive DLBCL ($n = 12$) and 62.6% among patients with c-MYC-negative DLBCL ($n = 191$), and that c-MYC-positive DLBCL had a significantly poorer prognosis ($P = 0.04$).[10] However, the 5-year survival rate among patients with c-MYC-negative DLBCL or c-MYC-positive DLBCL was 37.2 or 41.7%, respectively ($P = 0.42$).[10] Offit *et al*. reported that there was no difference in survival between patients who had t(8;14) and patients who had other abnormal karyotypes.[11] On the other hand, Au *et al*. compared the 8q24 translocation group ($n = 9$) with the other chromosomal abnormalities group ($n = 61$), and reported that the 8q24 translocation group had significantly poorer prognosis ($P = 0.042$).[3] In our study, 252 patients with DLBCL, who were registered consecutively in the ALTSG and had abnormal karyotypes, were divided into two groups: those with 8q24 translocation and those with other chromosomal abnormalities. The patients with 8q24 translocation had a significantly poorer prognosis than those without 8q24 translocation. In addition, DLBCL patients with a normal karyotype were excluded from the current study.

The patients with 8q24 translocation were compared with the patients who presented other chromosomal abnormalities, and many DLBCL patients with 8q24 translocation showed poor PS, high LDH level, and bone marrow involvement. In addition, among the patients with 8q24 translocation, an extranodal lesion was present in 32%, and 78% of the patients with an extranodal lesion had a lesion in the gastrointestinal tract. Kramer *et al*. reported that 7 of the 10 patients with DLBCL with *c-MYC* had an extranodal lesion, and five of them were gastrointestinal tract lesions.[12] It is thought that their results are similar to those of the present report.

Cytogenetically, there were hints that 8q24 translocation was the terminal event, including the lack of secondary cytogenetic events in the Burkitt lymphoma case with primary t(8;14), and the lack of further subclonal events after secondary 8q24 translocation in other cases. The occurrence of variant rather than classical 8q24 translocation as secondary progression events in follicular lymphoma and DLBCL is inevitable in most cases, due to the primary involvement of both 14q32 alleles in VDJ recombination and primary lymphoma translocations.[3] It is of interest that there were no significant differences in OS and PFS between the patients with DLBCL carrying 8q24 translocation and those with Burkitt lymphoma carrying 8q24 translocation. Therefore, it is possible that a B-cell lymphoma with 8q24 translocation is associated with poorer prognosis, regardless of whether it is DLBCL or Burkitt lymphoma. Using transcriptional and genomic profiling analysis, two reports highlighted the molecular differences that distinguish Burkitt lymphoma, atypical Burkitt lymphoma, and DLBCL.[13,14] In the study by Hummel *et al.*, 0% of the patients with molecular Burkitt lymphoma with *IG-MYC* had concurrent *immunoglobulin heavy chain (IGH)–BCL2* fusion and *BCL6* translocations.[13] On the other hand, 46% of the patients with DLBCL with *IG-MYC* had concurrent *IGH–BCL2* fusion and *BCL6* translocations. In that study, the 5-year OS rate of patients with molecular Burkitt lymphoma was 75%, and the 5-year OS rate of those with DLBCL was 39%. Dave *et al.* distinguished DLBCL from Burkitt lymphoma by gene-expression profiling and reported that among the nine patients with molecular Burkitt lymphoma, three patients had dual translocation of t(14;18) and t(8;14).[14] CHOP-like regimens are not adequate for these patients.

In recent years, patients with DLBCL with concurrent t(14;18)(q32;q21) and 8q24 translocation have been reported.[15,16] Le Gouill *et al*. reported 16 cases of DLBCL with t(14;18) and 8q24 translocation.[15] DLBCL with t(14;18) and 8q24 translocation showed a germinal center profile in all cases and a poor outcome. In the present study, the eight patients with t(14;18) and 8q24 translocation showed significantly poorer prognoses than the 20 patients with only 8q24 translocation. Therefore, DLBCL patients with concurrent t(14;18) and 8q24 translocation have very poor prognosis. The overall poor outcome of these patients appears to be independent of other clinical prognostic factors and is thought to be related to clonal evolution and the synergy of growth promotion by *c-MYC* and the antiapoptotic effect of *BCL-2* gene dysregulation.

In conclusion, the clinical, immunophenotypic, and prognostic findings suggest that DLBCL with 8q24 translocation has an aggressive clinical presentation. In particular, DLBCL with t(14;18) and 8q24 translocation is characterized by very poor prognosis. Therefore, studies on the effectiveness of more aggressive chemotherapy and rituximab combination chemotherapy for the treatment of DLBCL with 8q24 translocation are needed.

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