The Distinction between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma with c-*myc* Rearrangement

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To compare immunophenotypic and molecular features between Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) with c-myc rearrangements (c-mvc^R DLBCL), we analyzed 18 cases of B-cell non-Hodgkin's lymphoma with c-myc^R that were confirmed by chromosomal and/or Southern blotting analyses. The cases were histologically classified into 10 BLs and five DLBCLs. The remaining three cases could not be classified because of suboptimal quality of the surgical materials. BLs were from five adults and five children, whereas all DLBCLs were from adults. BLs were positive for CD20 (10/10 cases examined), CD10 (9/ 10), Bcl-2 (1/9), and Bcl-6 (10/10), whereas they were negative for CD3 (0/10) and EBV (0/8), by Epstein-Barr virus (EBV) EBER-1 RNA in situ hybridization. c-*Myc*^R DLBCLs were positive for CD20 (5/5), CD10 (2/5), Bcl-2 (3/4), and Bcl-6 (4/4), whereas none of them were positive for CD3 and EBV. A mean of MIB-1 index (MIB-1⁺ cells/neoplastic cells, %) of BLs (98.1%) was higher than that of c-myc^R DLBCLs (66.3%; P < .0001). Somatic mutation of immunoglobulin heavy-chain gene variable region (VH gene) in BLs (four cases) ranged from 0.7 to 4.9% with an average value of 2.3%, whereas those in DLBCLs (three cases) from 8.2 to 32.0% with an average value of 17.0%. It is, therefore, concluded that a growth fraction of nearly 100%, as well as a monotonous proliferation of medium-sized cells and c-*myc*^R, should be of value in the diagnosis

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of BL, which is probably different from $c-myc^{R}$ DL-BCL. In addition, CD10⁺, Bcl-2⁻, and low frequency of mutation of the VH gene could be helpful for the histologic distinction of BL from $(c-myc^{R})$ DLBCL.

KEY WORDS: Burkitt lymphoma, c-*myc* rearrangement, Diffuse large B-cell lymphoma, Immunoglobulin heavy chain gene, Immunohistochemistry, MIB-1, Somatic mutation.

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Chromosomal translocations of 8g24, encoding an oncogene of c-myc, are considered to be associated with oncogenesis of non-Hodgkin's lymphoma (NHL) (1-4). These chromosomal translocations dysregulate the expression of c-myc, a gene encoding a basic helix-loop-helix (bHLH) transcription factor that binds to DNA in a sequence-specific fashion. c-*Myc* normally plays a central role in the transcriptional regulation of an emerging set of downstream genes that control diverse cellular processes, including cell cycle progression and programmed cell death (apoptosis). Translocation of c-*myc* and immunoglobulin gene, t(8;14)(q24;q32), t(2;8)(p13;q24), and t(8;22)(q24;q11) are observed in almost all Burkitt lymphomas (BLs), highly aggressive B-cell lymphomas. The histologic hallmark of BL is a monomorphic proliferation of mediumsized lymphoma cells with several small nucleoli and a starry-sky appearance (5, 6). The neoplastic cells usually express CD19, CD20, and CD10 (5, 6).

Although identification of c-*myc* rearrangement is prerequisite to a diagnosis of BL, it is occasionally seen in other B-cell neoplasms, such as diffuse large B-cell lymphoma (DLBCL), lymphoblastic lymphoma (LBL), follicular lymphoma (FL), and multiple myeloma (MM) (7–14). Most cases of LBL, while showing a starry-sky appearance, can be easily distinguished from BL by their convoluted nuclei with

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TABLE 1	1. C	linical,	Histologic,	Immunohistochemical,	and	Molecular	Data
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Casa	1 ~~~		Site of Material	Karyotype	Southern c- <i>myc</i>	Immunohistochemistry						EDED 1	VH Somatic Mutation		Duo an o sia
No. (y)	(y)	Gender				20	3	10	Bcl-6	Bcl-2	MIB-1	RNA ISH	Frequency (%)	m./t. (Germline)	(mo)
Histo	ogic	BL group													
3	42	F	LN	NT	R	+	-	+	+	_	99.3	-	4.9	7 /144 (DP48)	Alive (72)
4	4	F	Ovary	t(8;14)	R	+	-	+	+	+	95.9	-	2.7	4 /147 (DP 7)	Alive (59)
5	5	М	LN	NT	R	+	-	+	+	_	98.9	-	0.7	1 /144 (DP63)	Dead (8)
7	71	Μ	Colon	t(8;14)	NT	+	_	$^+$	+	_	98.9	NT			Dead (4)
8	32	Μ	Jejunum	t(8;14)	NT	+	_	$^+$	+	_	98.3	-			Dead (2)
9	4	М	Omentum	t(8;14)	NT	+	-	+	+	-	98.8	-	2.8	4 /144 (DP63)	Dead (2)
10	7	М	LN	t(8;14)	NT	+	-	$^+$	+	-	98.6	-			Dead (24)
11	42	F	LN	t(8;14)	R	+	_	-	+	-	98.9	-			Unknown
15	7	Μ	Ileum	t(8;14)	NT	+	_	+	+	-	98.9	-			Unknown
16	9	Μ	Intestine	NT	R	+	_	+	+	NT	94.7	-			Alive (105)
					Subtotal	10/10	0/10	9/10	10/10	1/9	98.1	0/9	2.3		
											(average)		(average)		
Histologic DLBCL group															
2	50	F	Soft tissue	NT	R	+	-	+	+	NT	69.9	-	8.2	12 /147 (DP35)	Unknown
6	71	F	BM	t(8;22)	NT	+	-	+	+	-	61.3	-			Alive (32)
12	58	М	LN	t(8;14)	R	+	-		+	+	89.7	-	32.0	47 /147 (DP54)	Unknown
13	74	F	LN	t(8;14)	R	+			NT	Ŧ	48.0	-	10.9	16 /147 (DP54)	Dead (31)
18	66	М	LN	t(8;14)	NT	+	-	-1	+	+	62.6	-			Dead (45)
					Subtotal	5/5	0/5	2/5	4/4	3/4	66.3	0/5	17.0		
								-			(average)		(average)		
Histogically undetermined group															
1	81	М	Skin	NT	R	+]	+	+		98.4	+			Unknown
14	66	Μ	Stomach	t(8;14)	NT	+	-	+	+		99.5	-			Alive (39)
17	28	Μ	Stomach	t(8;14)	NT	+	-		+		99.4	-			Dead (3)
					Subtotal	3/3	0/3	2/3	3/3			1/3			

DLBCL, diffuse large B-cell lymphoma; LN, lymph node; BM, bone marrow; NT, not tested; R, rearrangement; +, positive; -, negative; BL, Burkitt's lymphoma; EBER, Esptein-Barr versus ER-1; m./t., mutation number/total nucleotide number; VH, immunoglobulin heavy chain gene variable region; ISH, in situ hybridization. Positive means that >20%-positive cells were immunostained by each antibody except MIB-1. (The percentages of cells positive for MIB-1 were estimated. The numbers of MIB-1–positive cells and all tumor cells were determined by an actual count in areas (2.5 mm²) counted at high-power field (×400). Background small lymphocytes and starry sky macrophages were excluded. A mean of three areas in each case was taken as the MIB-1 index).

fine chromatin and detection of antigens such as TdT. FL and MM are different from BL in the cytologic features. However, DLBCL occasionally mimic BL. The neoplasms composed of medium-sized to large cells with a starry sky appearance and classified as small non-cleaved cell lymphoma, non-Burkitt in the Working Formulation (15) or as highgrade B-cell lymphoma, Burkitt-like (provisional entity) in the REAL (5) are difficult to differentiate from BL. In the new World Health Organization (WHO) classification, the gold standard for the diagnosis of BL should be the presence of t(8;14)(q24; q32) and its variants or c-myc rearrangement (6). If cytogenetic or Southern blot analysis is not available, BL should not be diagnosed without a Ki-67 fraction of close to 100% (6). DLBCLs with c-mvc rearrangement, however, are known to account for 5-7% of DLBCL (10-12). What are differences between BL and DLBCL with c-myc rearrangement (c-myc^R DLBCL)?

In this article, we analyzed clinical, histologic, immunohistochemical, and molecular features of 18 cases of B-cell NHL with c-*myc* rearrangement,

to examine 1) the difference between BL and c-*myc*^R DLBCL and 2) whether MIB-1 fraction of almost 100% is feature specific to BL. We found that c-*myc*^R DLBCL were clearly different from BL in term of immunoreactivity with CD10, Bcl-2, and MIB-1, as well as the frequency of somatic mutation of the immunoglobulin heavy chain (IgH) gene variable region (VH gene).

MATERIALS AND METHODS

Case Selection

Eighteen cases of B-cell NHL with c-*myc* rearrangement detected by a cytogenetic analysis or Southern blotting were retrieved from the files of malignant lymphoma in six pathology departments. No cases associated with AIDS were included in this study.

Histologic and Immunohistochemical Studies

A part of resected tissue was fixed in 20% buffered formalin and embedded in paraffin for the routine



FIGURE 1. A, Burkitt lymphoma (Case 9). Hematoxylin-eosin stain. A medullary, cohesive and monotonous proliferation of medium-sized neoplastic cells is the histologic hallmark of Burkitt lymphoma. **B**, an immunoperoxidase stain for MIB-1. Almost all of the neoplastic cells are positive for MIB-1 (see Table 1). **C**, c-*myc*^R DLBCL (Case 12); hematoxylin-eosin stain. A medullary proliferation of the large neoplastic cells with coarsely reticulated nuclear chromatin and centrally located prominent nucleoli are features of DLBCL, immunoblastic variant. **D**, an immunoperoxidase staining for MIB-1, MIB-1⁺ tumor cells accounts for about 90% (see Table 1). Negatively stained large nuclei are occasionally seen.

histologic and immunohistochemical studies. All cases were re-evaluated by all authors, and the histologic diagnosis was established according to the new WHO classification (6). Immunohistochemistry was performed on paraffin-embedded sections using the streptavidin-biotin complex technique (16). Monoclonal and polyclonal antibodies used in this study were as follows: CD3 (CD3 ϵ , DAKO-PATTS[DA], DAKO, Glostrup, Denmark), CD10 (CD10, Novocastra Laboratories Ltd. [NC], Newcastle Upon Tyne, UK), CD20 (L26, DA), Bcl-6 (Santa Cruz, CA), Bcl-2 (Bcl-2, DA) and MIB-1 (MIB-1, DA). A given lymphoma was judged positive when >20%-positive cells were immunostained by each antibody except MIB-1. The percentages of positive cells for MIB-1 were estimated. The numbers of MIB-1 positive cells and all tumor cells were determined by an actual count in areas (2.5 mm^2) counted at high-power field $(400\times)$. Background small lymphocytes and starry-sky macrophages were excluded. A mean of three areas in each case was taken as the MIB-1 index.

Molecular Studies

Epstein-Barr virus RNA in situ *hybridization* Epstein-Barr (EB) virus RNA *in situ* hybridization (EBER-1 RNA-ISH) was performed as described elsewhere for detection of the genome of EBV (17).

Somatic mutation of the IgH gene variable region

The IgH gene was amplified by semi-nested PCR, according to a method described elsewhere (18), and variable region (CDR2 and FW3) was analyzed.

RESULTS

Clinical, histologic, immunohistochemical, and molecular data are shown in Table 1. The patients were distributed in age from 4 to 81 years old, with a male to female ratio of 12:6. Eleven cases presented with extranodal lymphoma, whereas seven had nodal lymphoma. Cytogenetic data was available in 13 cases. Twelve cases carried t(8;14)(q24; q32) and one case t(8;22)(q24;q11), and karyotypes

TABLE 2 Chromosomal Abnormalities

Case 1	No. Karyotype	
Histol	ogic BL group	
4	46, XX, t(8;14) (q24;q32), add (11) (q23), add (13) (q34)	[7/8]
	46, XX, del (6) (q13q23), t(8;14) (q24;q32), add (11) q23), add (13) (q34)	[1/8]
7	46, XY, ins (1;?) (q21;?), t(8;14) (q24;q32), add (11) (q23), add (17) (p11)	[8/13]
8	46, XY, -13, t(8;14) (q24;q32), +der(13) t(13;?) (q26;?)	
9	46, XY, 13q+, 17p-, t(8;14) (q24;q32)	
10	46, XY, t(8,14) (q24;q32), dup(1) (q21q32)	
15	46, XY, t(8;14) (q24;q32)	
	46, XY, t(8;14) (q24;q32) +1q	
	46, XY, t(8;14) (q24;q32) +1q 15q-	
Histol	ogic DLBCL group	
6	50. XX. add (3) (al2), del(6) (a2), t(8:22) (a24:a11), add (11) (a223) +12 del(13) (a12a14), t(14:18) (a32:a21),	
0	+20. add (21) (a21) +2mar	[20/20]
12	48. XY, +X, add (2) (p ² ₂), add (3) (p ² ₂), add (3) (q ² ₁), add (6) (q ² ₁), +add (7) (p ¹ ₁), -8 , add (11) (q ² ₃)	[]
	-13 , der(14) t(8:14) (α 24: α 32), add (15) (α 23), add (16) (α 13), -21 , $+4mar$	[6/18]
13	51, XX, add (1) (q?4), +3; +3, +7, t(8;14) (q24;q32), +10, +18	[5/10]
Histog	gically undetermined group	
14	46, XY, t(8;14) (q24;q32)	[35/36]
Number (f metanbase with abnormal karvotyme/number of metanbase analyzed	

.0793).

of 10 cases are shown in Table 2. c-Myc rearrangements were found in nine cases by Southern blotting analysis.

A consensus diagnosis by re-evaluation of routine HE section of all 18 cases among all authors is shown in Table 1. Ten cases were diagnosed as BL, whereas five cases were diagnosed as DLBCL. Because consensus diagnoses could not be obtained because of suboptimal quality of materials available, the remaining three cases were put into an undetermined group. The BL group consisted of children and adults, whereas the DLBCL group consisted of adults only.

Results of immunohistochemical study and EBER-1 RNA-ISH are shown in Table 1. BLs represented 10 positive cases/10 examined cases for CD20, 0/10 for CD3, 9/10 for CD10, 10/10 for Bcl-6, 1/9 for Bcl-2, and 0/8 for EBV. DLBCLs represented 5/5 for CD20, 0/5 for CD3, 2/5 for CD10, 4/4 for Bcl-6, 3/4 for Bcl-2, and 0/5 for EBV. The undetermined group represented 3/3 for CD20, 0/3 for CD3, 2/3 for CD10, 3/3 for Bcl-6, 0/3 for Bcl-2, and 1/3 for EBV. The Bcl-2 expression differed between BLs (1/9) and DLBCLs (3/4). There was no statistically significant difference between them (χ^2 -test). The MIB-1 index of BLs and DLBCLs were distributed from 94.7 to 99.3%, with a mean of 98.1% and from 48.0 to 89.7% with a mean of 66.3%, respectively. There was a statistically significant difference between them (t test, P < .0001). [Fig. 1]

Frequency of somatic mutation of the VH gene in the BL group (four cases) ranged from 0.7 to 4.9%, with an average of 2.3%, whereas that in the DLBCL group (three cases) ranged from 8.2 to 32.0%, with an average of 17.0%. (Table 1) The average of somatic mutation frequency in the DLBCL group was higher than that in the BL group, but there was no

DISCUSSION To compare clinicopathologic features of BL with

those of $c-myc^{R}$ DLBCL, we analyzed 18 cases of B-cell lymphoma with c-myc rearrangements. Except for three cases with suboptimal specimens, we reached a consensus diagnosis of either BL or DL-BCL in all 15 cases. Because we failed to obtain a consensus diagnosis in several cases using the REAL classification, the description in the new WHO classification can give more reproducible pathologic criteria for the differentiation between BL and DLBCL.

statistical difference between them (t test, P =

Although the translocation of c-*myc* and Ig genes is the gold standard for BL, we demonstrated some differences between BLs and c-*myc*^R DLBCL. $CD10^+$, Bcl-2⁻, and extremely high rate of MIB-1⁺ cells were characteristic for BL. The mean MIB-1 index in BLs was 98.1%, and the indices generally were >98%, except in two cases. A growth fraction of nearly 100% can be expected for BLs. In $c-myc^{R}$ DLBCLs, on the contrary, MIB-1 index was distributed from 48.0 to 89.7%, and the mean (66.3%) was lower than that of BLs. More than 95% of MIB-1 expression was not seen in any of the five cases of c-myc^R DLBCL. Degree of the MIB-1 expression varied from case to case and were not clearly different from DLBCL without c-myc rearrangement (data not shown). As Bcl-2 was previously reported to be negative for BL (19), Bcl-2 did not react with all cases of BL but one. An absence of Bcl-2 protein, as well as extremely high rate of the MIB-1 index is a good marker for BL.

EBV was not detected by EBER-1 RNA-ISH in both BL and c-mvc^R DLBCL, but chromosomal abnormalities of c-*mvc*^R DLBCL were more complex, with many losses and gains involving chromosomes other than those in BL. Because breakpoints at c-*myc* and Ig genes have been reported to be similar in both BL and c-*myc*^R DLBCL by the long-distance PCR and cloning analyses (12), the hypothesis could be raised that both lymphomas are the same disease, showing different stages of tumor progression. This hypothesis, however, seems unlikely because of the following reasons. Both of BL and DLBCL are considered to be derived from germinal center (GC) B cells because of the expression of Bcl-6 and high frequency of somatic mutation of the rearranged VH genes (20-23). Somatic mutation frequencies of the VH gene of the endemic and sporadic BLs are reported to have ranges between 5-15% and 0-5%, respectively (24). In our series, the VH gene of BL was somatically mutated in a range of 0-5% with an average of 2.3%. On the other hand, two of three cases of c-mvc^R DLBCL examined revealed >10% of mutation frequency in rearranged VH gene. We previously reported an average of 11.9% of mutation frequency, with a broad range, in 60 cases of EBV⁻ DLBCL (25), and this pattern is similar to that of $c-mvc^{R}$ DLBCL. Moreover, using additional cases, we have found a statistical difference in somatic mutation rates between BLs and c-*myc*^R DLBCLs (unpublished data, in preparation).

 $c-Myc^{R}$ DLBCL is known to occasionally involve the coexistence of t(14;18) involving *bcl*-2 genes (26). Dual translocation of c-*myc* and *bcl*-2 is characterized by a rapid clinical course and extremely poor outcome, and some cases with dual translocation of c-*myc* and *bcl*-2 are regarded as instances of transformation of follicular lymphoma. There were no cases with dual translocation of *c*-*myc* and *bcl*-2 in our series.

BL accounts for 1–2% of total NHLs (27, 28), and most of them are of a sporadic type in non-African areas. Because c-*myc* rearrangement is found in 5–7% of DLBCL, which account for 30% of total NHLs, c-*myc*^R DLBCL is estimated to constitute 1.5– 2.1% of total NHL. The similar frequency of BL and c-*myc*^R DLBCL indicates that the rearrangement may occur in various GC stages in secondary B-cell differentiation. When the neoplastic proliferation is initiated by c-*myc*/Ig translocation at a given stage of the secondary B-cell differentiation in the GC, it is not likely that clinically and histologically distinct neoplasms, that is, BL and DLBCL, arise. It is, therefore, indicated that the normal counterparts of BL and c-*myc*^R DLBCL are different.

Although BL has aggressive clinical course and results in poor outcome, this tumor is highly sensitive to chemotherapeutic regimens, and the response is different from that of other aggressive B-cell lymphomas (3, 27). Five of eight patients with BL in this series had a poor prognosis, and a majority of them died of disease within 1 year. Although the clinical outcome of two patients was unknown in our series, Vitolo et al. (10) analyzed 71 cases with DLBCL including four cases of c-*myc*^R DLBCL. All cases were treated with an anthracycline-containing chemotherapy regimen. These four patients with $c-myc^R$ DLBCL had an aggressive disease with poor prognosis. Kramer et al. (11) reported that structural alterations of c-mvc were detected in 10 of 151 cases of DLBCL by Southern blot analysis (6.7%), and two of them had both bcl-6 and c-mvc rearrangements. In their series, c-mvc rearrangements were found in 16% of primary extranodal lymphomas, whereas only 2% of primary nodal cases had these abnormalities (P = .02). In particular, 5 of 18 cases (28%) of gastrointestinal lymphomas had the c-myc rearrangements. Seven of ten cases with c-myc rearrangements achieved a complete remission and six responders remained alive for >4 years, resulting in the trend of a better disease-free survival. Thus, our speculation that the normal counterparts of BL and c-mvc^R DLBCL are different can be clinically supported.

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