Diffuse Large B-Cell Lymphoma in Chinese Patients

Immunophenotypic and Cytogenetic Analyses of 124 Cases

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Abstract

*In diffuse large B-cell lymphoma (DLBCL), BCL2 expression usually correlates with the t(14;18)(q32;q21) in germinal center B-cell (GCB) subtype and with gain/amplification of chromosome 18q21 in the activated B cell–like subtype. Studies have suggested that the GCB subtype is less common in Chinese than in Western populations. We studied 124 Chinese DLBCL cases using immunohistochemical, conventional cytogenetics, and interphase fluorescence in situ hybridization analyses. A cohort of 114 well-characterized DLBCL cases from Western populations was also analyzed for comparison. Lower incidences of the GCB subtype (*P *= .0001) and the t(14;18) translocation (*P *= .0001) were present in Chinese cases. However, BCL2 overexpression was more frequent in Chinese compared with Western cases (*P *= .0054). BCL2 expression was associated with gain of chromosome 18/18q in the Chinese and Western cohorts. More interestingly, BCL2 expression was associated with gain of chromosome 3/3q in Chinese DLBCL cases, whereas this association was less significant in Western cases.*

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, which accounts for approximately 30% to 40% of non-Hodgkin lymphomas diagnosed in Western countries and 60% of B-cell lymphomas in Eastern Asia.[1,2] DLBCL displays heterogeneous clinical, histologic, immunophenotypic, cytogenetic, and molecular features, suggesting that it may, in fact, comprise several different disease entities. Gene expression profiling (GEP) analysis showed that DLBCL consists of at least 2 subtypes (ie, germinal center B-cell–like [GCB] and activated B cell–like [ABC] subtypes) with marked differences in prognosis.[3,4] There are also a small number of DLBCL cases that do not express distinctive GCB or ABC signatures and cannot be subclassified based on GEP analysis.[3,4] Hans et al[5] subsequently reported that the expression pattern of CD10, BCL6, and MUM1 defined immunohistochemically can be used to subclassify DLBCL into GCB (including the GCB subtype and primary mediastinal large B-cell lymphoma) and non-GCB (including the ABC subtype and unclassified cases) subtypes with predicted clinical outcome similar to that predicted by complementary DNA microarray analysis. Choi et al[6] recently developed a new immunostain algorithm with higher accuracy in classifying DLBCL into GCB and non-GCB subgroups in which two additional biomarkers, GCET1[7] and FOXP1,[8] were evaluated as well.

BCL2 protein is located in the inner membrane of mitochondria and functions as an antiapoptotic protein.[9] Studies of DLBCL in Western populations show that BCL2 expression is highly associated with the translocation t(14;18)(q32;q21) in GCB DLBCL,[10] whereas amplification of the *BCL2* gene and other mechanisms may be responsible for up-regulation of BCL2 expression in the ABC DLBCL.[11-13]

Several studies suggest that the GCB subtype is significantly less common in Eastern Asian patients with DLBCL than in Caucasian patients.[14-16] A similar lower incidence of GCB DLBCL is also reported in Chinese patients. However, BCL2 expression and its possible mechanism remain poorly understood in Chinese patients with DLBCL.

We investigated the frequency of different subtypes of DLBCL in Chinese patients by using both the Hans et al[5] and Choi et al[6] subclassification algorithms. We studied the frequency of BCL2 overexpression in these cases and explored the possible mechanisms of BCL2 overexpression in Chinese DLBCL cases by analyzing the cytogenetic alterations in patients with BCL2+ and BCL2– DLBCL. A well-characterized cohort of DLBCL cases from Western populations was used for comparative analysis.

Materials and Methods

Cases

We studied 124 de novo DLBCL cases in adult patients admitted to hospitals in Shanghai, China, between August 2003 and May 2007.[17] All of the cases were reviewed by expert hematopathologists (X.Z. and K.F.), and the diagnoses were confirmed based on the World Health Organization criteria. All cases had sufficient cytogenetics data. Approximately 72% of the cases were from patients who designated Shanghai as their formal residence, and 28% had residences in other provinces of China.

We also analyzed 224 untreated Western de novo DLBCL cases previously characterized by GEP using Lymphochip complementary DNA microarrays and comparative genomic hybridization (CGH).[18] Of the 224 cases, 114 cases were also studied by immunohistochemical analysis for BCL2, CD10, BCL6, multiple myeloma oncogene 1 (MUM1), germinal center B-cell expressed transcript 1 (GCET1), and Forkhead box-P1 (FOXP1); and by fluorescence in situ hybridization (FISH) analysis for the *BCL2* translocation. The immunophenotypic results for these 114 cases have been previously reported.[6] These 114 cases were from various institutions in the United States and Western Europe, including 26 from Vancouver, Canada; 38 from Nebraska; 7 from the Southwest Oncology Group in the United States; 21 from Oslo, Norway; and 22 from Würzburg, Germany. This study was approved by the institutional review boards at the University of Colorado Health Sciences Center, Denver; Fudan University School of Medicine, Shanghai, China; and the University of Nebraska Medical Center, Omaha.

Immunohistochemical Stains

Immunohistochemical stains for 124 Chinese DLBCL cases were performed on formalin-fixed, paraffin-embedded tissues using antibodies against the following proteins: BCL6, CD10, MUM1, GCET1, FOXP1, and BCL2 **Table 1**. Semiquantitative evaluation of protein expression was performed by 2 hematopathologists (Y.C. and K.F.). The algorithm by Hans et al[5] and the new algorithm developed by Choi et al[6] were used in subclassification. Cases were considered BCL6+, CD10+, and MUM1+ if 30% or more of the tumor cells were stained when the algorithm by Hans et al[5] was applied. The cutoff value for GCET1, FOXP1, and MUM1 was 80% and for BCL6 and CD10, 30%, when the new algorithm developed by Choi et al[6] was applied. Cases were considered BCL2+ when 30% or more of the tumor cells expressed BCL2 protein.

Conventional Cytogenetic Analysis

Standard G-banded analysis was performed on unstimulated (1-2 day) and/or B-cell mitogen-stimulated (3-day) (lipopolysaccharide, Sigma, St Louis, MO) suspension cultures from minced tissue. A minimum of 20 metaphases were analyzed in each case. The clone was defined, and karyotypes were described according to the recommendations of the 2005 International System for Human Cytogenetic Nomenclature.[19]

Table 1
Antibodies and Methods of Antigen Retrieval and Detection Used*

Antibody	Clone	Source	Dilution	Antigen Retrieval	Detection
BCL6	PG-B6p	DAKO, Carpinteria, CA	1:10	WB, high pH	EnVision
CD10	56C6	DAKO	1:80	WB; 0.01 mol/L citrate, pH 6.0	EnVision
MUM1/IRF4	MUM1p	DAKO	1:50	WB, high pH	EnVision
BCL2	124	DAKO	1:50	WB, high pH	EnVision
GCET1	RAM341b/c1/c2	Montes-Moreno et al[7]	1:1	WB; EDTA, pH 8.0	ABC
FOXP1	JC12	Banham et al[8]	1:80	WB; EDTA, pH 8.0	ABC

ABC, avidin-biotin complex; FOXP1, Forkhead box-P1; GCET1, germinal center B-cell expressed transcript 1; MUM1, multiple myeloma oncogene 1; WB, 95°C-99°C water bath.
* High pH, DAKO high pH retrieval solution; EnVision, DAKO EnVision+ mouse peroxidase.

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FISH Analysis

Interphase FISH for the t(14;18) translocation was performed on 118 Chinese DLBCL cases using an LSI *IgH* SpectrumGreen/LSI *BCL2* SpectrumOrange probe (Abbott-Vysis, Downers Grove, IL). The gain of chromosome 18q21 was also simultaneously evaluated. For the present study, 94 analyses were performed on cell pellets prepared for concurrent cytogenetic study, and 50 analyses were performed on touch slides made from fresh tumor tissues. Sample preparations and hybridizations were conducted following the manufacturer's recommendations. Whenever possible, at least 500 interphase cells were scored for each probe. A clonal aberration was defined as percentage of cells with any given aberration more than the normal cutoff limits that were determined from 10 cytogenetically healthy people. The cutoff for the t(14;18) translocation was 1% and for gain and amplification, 2%. FISH results were compared with the conventional G-banding results in all cases, and concordant results were observed in each case (data not shown). Interphase nuclei of normal cells showed 2 red signals for *BCL2* and 2 green signals for *IgH*. The presence of the t(14;18) produced 2 yellow fusion signals (red + green) on 14q32 and 18q21 **Image 1A**. The presence of 3 or more red signals of 18q21 was classified as a gain or amplification in cases with diploidy. The split red signals due to translocation were considered as 1 red signal for copy number calculation **Image 1B**.

Statistical Analysis

Differences in the distribution of individual parameters among patient subsets were analyzed using the χ^2 test or Fisher exact test. Mean age was compared with the 2-tailed *t* test. A value of *P* of less than .05 was considered significant. SPSS version 11.0 statistical software was used for the data analysis (SPSS, Chicago, IL).

Results

Patient Characteristics

There were samples from 124 Chinese patients included in the study. The median age of the patients was 57 years (range, 19-88 years), and there were 78 men (62.9%) and 46 women (37.1%). Because Shanghai is rather centrally located in China and almost a third of our cases were from other provinces, it is reasonable to suggest that our cases are representative of DLBCL in China.

Of the 124 cases, 122 were nodal disease and only 2 cases were extranodal disease at initial diagnosis. In China, patients with lymphadenopathy usually come to the cancer hospital for treatment, whereas patients who had extranodal disease may choose to go to other specialized hospitals. Because a large proportion of the cases in this study originated from the Shanghai Cancer Hospital, a high percentage of patients with nodal manifestations were included in our study.

There were 114 patients in the Western DLBCL cohort. The median age was 62 years (range, 14-88 years) and there were 62 male (54.4%) and 52 female (45.6%) patients. There were no significant differences in clinical features between the Chinese and Western DLBCL cases **Table 2**.

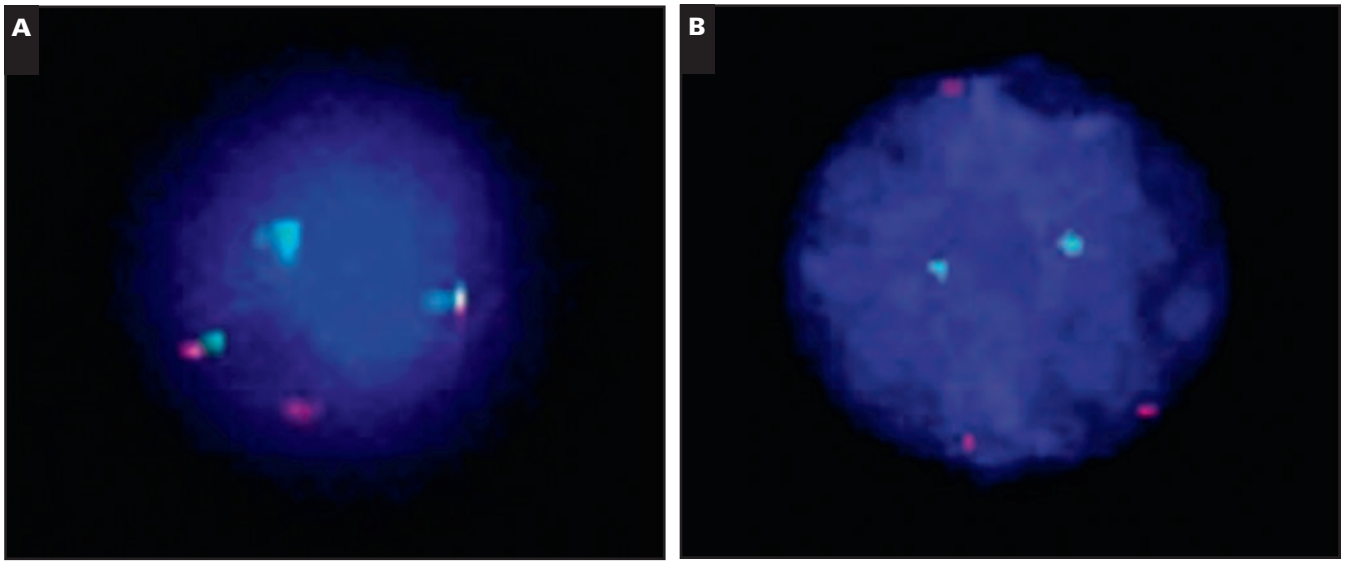

Image 1 Representative fluorescence in situ hybridization images using the *BCL2*/*IgH* probe in diffuse large B-cell lymphoma cases. **A**, The t(14;18)(q32;q21) translocation is illustrated in this cell showing 2 yellow fusion signals (*BCL2*/*IgH*) and residual red (*BCL2*) and green (*IgH*) signals. **B**, The presence of 3 (or more) red signals depicts a gain of 18q21.

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Table 2
Clinical Features of Patients With DLBCL in Chinese and Western Cohorts

Clinical Features	Chinese (n = 124)	Western (n = 114)*	*P*
Age (y)			
Median	57	62	.09
Range	19-88	14-88	
<60	71	53	.1257
≥60	53	61	
Sex			
Male	78	62	.3523
Female	46	52	
Nodal/extranodal	122/2	NA†	—

DLBCL, diffuse large B-cell lymphoma.
* Untreated Western de novo DLBCL cases previously characterized by gene expression profiling using Lymphochip complementary DNA microarrays.[18]
† No relevant data available in this cohort.

Low Frequency of the GCB Subtype of DLBCL in Chinese Patients

Of the 124 Chinese DLBCL cases, expression of CD10 was seen in 16 cases (12.9%), BCL6 in 45 (36.3%), MUM1 (cutoff ≥30%) in 80 (64.5%), MUM1 (cutoff ≥80%) in 48 (38.7%), GCET1 in 16 (12.9%), and FOXP1 in 58 (46.8%). Using the algorithm of Hans et al,[5] 27 Chinese cases (22.1%) showed a GCB phenotype, whereas 97 (78.2%) had a non-GCB phenotype. Using the new algorithm developed by Choi et al,[6] 34 cases (27.4%) were considered GCB and 90 (72.6%) were considered the non-GCB subtype. Both algorithms showed that the GCB subtype of DLBCL was significantly less common than the non-GCB subtype in Chinese patients ($P < .0001$) **Table 3**.

Of 114 DLBCL cases from the Western countries, 60 cases (52.6%) were considered GCB and 54 (47.4%) were considered non-GCB using the algorithm of Hans et al.[5] When using the algorithm of Choi et al,[6] 64 cases (56.1%) were considered GCB and 50 (43.9%) were considered non-GCB. Compared with the Western cohort, the frequency of the GCB subtype was much lower in the Chinese cohort (34/124 vs 64/114; $P = .0001$) **Table 4**.

In our analysis, we used the algorithms of Hans et al[5] and Choi et al[6] but achieved similar results. Whereas the algorithm by Hans et al[5] had a misclassification rate of 14% compared with the GEP classification, which is considered the "gold standard," the algorithm by Choi et al[6] had an approximately 7% misclassification rate. We present the results based on the algorithm of Choi et al[6] in the following studies.

BCL2 Expression in DLBCL in Chinese Cases

Expression of BCL2 protein was seen in 68.5% of the Chinese DLBCL cases (85/124) but in only 50.0% of the Western DLBCL cases (57/114; $P = .0054$). Within the GCB subgroup, the percentage of cases with BCL2 overexpression in the Chinese cohort was similar to that in the Western cohort (16/34 [47%] vs 31/64 [48%]). However, the expression of BCL2 protein was more often seen in the non-GCB subgroup in the Chinese cohort compared with the non-GCB subgroup of the Western cohort (69/90 [77%] vs 26/50 [52%]; $P = .005$). In the Western DLBCL cases, the percentage of cases with BCL2 overexpression in the GCB subgroup was similar to that in the non-GCB subgroup ($P = .8503$); however, the expression of BCL2 protein was more frequent in the non-GCB subgroup than the GCB subgroup ($P = .0032$) in the Chinese cohort **Table 5**.

Table 4
Distributions of GCB DLBCL and Non-GCB DLBCL in the Chinese and Western Cohorts*

Subtype/Algorithm	Chinese DLBCL (n = 124)	Western DLBCL (n = 114)†	*P*
GCB			
Hans et al[5]	27 (21.8)	60 (52.6)	.0001
Choi et al[6]	34 (27.4)	64 (56.1)	.0001
Non-GCB			
Hans et al[5]	97 (78.2)	54 (47.4)	.0001
Choi et al[6]	90 (72.6)	50 (43.9)	.0001

DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell subtype.
* Data are given as number (percentage).
† Untreated Western de novo DLBCL cases previously characterized by gene expression profiling using Lymphochip complementary DNA microarray.[18]

Table 3
Subclassification of Chinese Diffuse Large B-Cell Lymphoma Cases*

		Hans et al[5] Algorithm		Choi et al[6] Algorithm	
	All Cases (n = 124 [100.0%])	GCB (n = 27 [21.8%])	Non-GCB (n = 97 [78.2%])	GCB (n = 34 [27.4%])	Non-GCB (n = 90 [72.6%])
CD10+	16 (12.9)	16 (59)	0 (0)	15 (44)	1 (1)
BCL6+	45 (36.3)	19 (70)	26 (27)	24 (71)	21 (23)
MUM1+ (≥30%)	80 (64.5)	9 (33)	71 (73)	—	—
MUM1+ (≥80%)	48 (38.7)	—	—	8 (24)	40 (44)
GCET1+	16 (12.9)	11 (41)	5 (5)	12 (35)	4 (4)
FOXP1+	58 (46.8)	7 (26)	51 (53)	5 (15)	53 (59)

GCB, germinal center B-cell subtype; FOXP1, Forkhead box-P1; GCET1, germinal center B-cell expressed transcript 1; MUM1, multiple myeloma oncogene 1.
* Data are given as number (percentage).

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Lower Frequency of t(14;18)(q32;q21) in the GCB Subgroup in Chinese DLBCL Cases

Of the 124 Chinese DLBCL cases, 118 had successful cytogenetic analysis and FISH studies for the t(14;18). Only 4 cases (4/118 [3.4%]) showed the t(14;18) by conventional cytogenetic studies, which was further confirmed by interphase FISH analysis. None of the other cases showed t(14;18) by cytogenetic or interphase FISH analysis. Of the 4 positive cases, 3 were classified as GCB DLBCL and 1 as non-GCB DLBCL. Of the 114 cases in the Western cohort, 25 cases (21.9%) were positive for the t(14;18) detected by interphase FISH, 23 of which were classified as GCB DLBCL and the other 2 as non-GCB DLBCL. The t(14;18) occurred at a much lower frequency in the Chinese DLBCL cohort than in the Western cohort (4/118 in the Chinese vs 25/114 in the Western cohort; $P = .0001$). Within the GCB subtypes, the t(14;18) was also significantly less frequent in the Chinese DLBCL cohort than in the Western cohort (3/34 vs 23/64; $P = .0068$).

Frequent Gains of Chromosome 18/18q and 3/3q in BCL2+ DLBCLs

Of the 118 Chinese cases, chromosomal karyotyping showed that 28 (23.7%) were near-triploid or near-tetraploid with high chromosome numbers, ranging from 58 to 101. The remaining 90 cases (76.3%) were near-diploid. To avoid errors in the scoring of chromosome imbalances secondary to high chromosome numbers, we used 90 cases with near-diploidy for chromosomal imbalance analysis.

The frequencies of chromosomal gains and losses in BCL2+ and BCL2– DLBCL are summarized and illustrated in **Image 2**. In the Chinese DLBCL cases, frequent recurrent genomic imbalances (≥15%) in the BCL2+ group were gains of 1q, 3p, 3q, 3, 7p, 7q, 7, and 18 and losses of 6q, 17p, and Y. Frequent recurrent genomic imbalances (≥15%) in the BCL2– group were gains of 1q and X and losses of 1p, 6q, 15, 18q, and Y. The BCL2+ subgroup had significantly more frequent gain of 3/3q and 18/18q than the BCL2– subgroup ($P < .05$; Fisher exact test). On the other hand, the BCL2– subgroup had a significantly more frequent loss of 1p than the BCL2+ subgroup ($P < .05$; Fisher exact test) (Images 2A and 2B).

We also analyzed genomic imbalances detected by CGH in BCL2+ and BCL2– DLBCL in the Western cohort. The frequent recurrent genomic imbalances (≥15%) in the BCL2+ group were gains of 1q, 2p, and 18 and losses of 6q. Frequent recurrent genomic imbalances (≥15%) in the BCL2– group were gains of 1q and X and losses of 2p and 6q. The BCL2+ subgroup also had significantly more frequent gain of 3/3q and 18q than the BCL2– subgroup in the Western cohort ($P < .05$; Fisher exact test) (Images 2C and 2D). However, the BCL2+ subgroup in the Chinese cohort had significantly more frequent gains of 3/3q and 18/18q than the BCL2+ subgroup in the Western cohort ($P < .05$).

Table 5
BCL2 Expression in DLBCL Subgroups of Chinese and Western Cohorts*

DLBCL Cohort	Entire Cohort	GCB	Non-GCB	*P*
Chinese	85/124 (68.5)	16/34 (47)	69/90 (77)	.0032
Western	57/114 (50.0)	31/64 (48)	26/50 (52)	.8503
P	.0054	.8965	.005	

DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell subtype.
* Data are given as number of BCL2+ cases/total number of cases (percentage).

Of the 90 Chinese DLBCL cases with near-diploidy, 26 (29%) had gains of chromosome 18/18q and 27 (30%) had gains of chromosome 3/3q. A total of 35 cases (39%) showed gains of 18/18q and/or 3/3q, and 18 (51%) of 35 cases had coexistent gains of chromosome 18/18q and 3/3q. Of the 114 Western DLBCL cases, 13 (11.4%) had gains of chromosome 18/18q and 15 (13.2%) had gains of chromosome 3/3q. A total of 19 cases (16.7%) had gains of 18/18q and/or 3/3q. Among these 19 cases, 9 (47%) had coexistent gains of chromosome 18/18q and 3/3q.

Gains of Chromosome 18/18q and 3/3q Highly Associated With BCL2 Expression in Chinese DLBCL Cases

Among the Chinese DLBCL cases, all 4 cases with t(14;18) and all 26 cases with gain of 18/18q were positive for BCL2 overexpression by immunostains. Among 10 cases with gain of 3/3q, all cases except 1 were positive for BCL2 protein expression. In the Western cohort, BCL2 was positive in 22 (88%) of 25 cases with the t(14;18), 4 (100%) of 4 cases with gain of only 18/18q, 2 (33%) of 6 cases with gain of only 3/3q, and 8 (100%) of 8 cases with coexistent gains of 18/18q and 3/3q. The t(14;18) and gain of 18/18q were highly associated with BCL2 expression in the Chinese and Western cohorts. It is interesting that gain of 3/3q showed high association with BCL2 expression in the Chinese DLBCL cases.

BCL2 Expression Is Associated With Gains of Chromosome 18/18q and 3/3q in the GCB and Non-GCB Subgroups in Chinese DLBCL Cases

Because gains of chromosome 18/18q and 3/3q were both highly associated with BCL2 expression and the 2 alterations frequently coexisted, we combined them to study their relationship with BCL2 expression in the Chinese and Western cohorts. Within the Chinese cohort, 34 (54%) of 63 BCL2+ cases had gains of 18/18q and/or 3/3q, whereas in the Western cohort, only 15 (26%) of 57 BCL2+ cases had gains of 18/18q and/or 3/3q ($P = .0038$) **Table 6**.

Within the non-GCB DLBCL cases, 29 (57%) of 51 BCL2+ cases in the Chinese cohort and 13 (50%) of 26 BCL2+ cases in the Western cohort had gains of 3/3q and/

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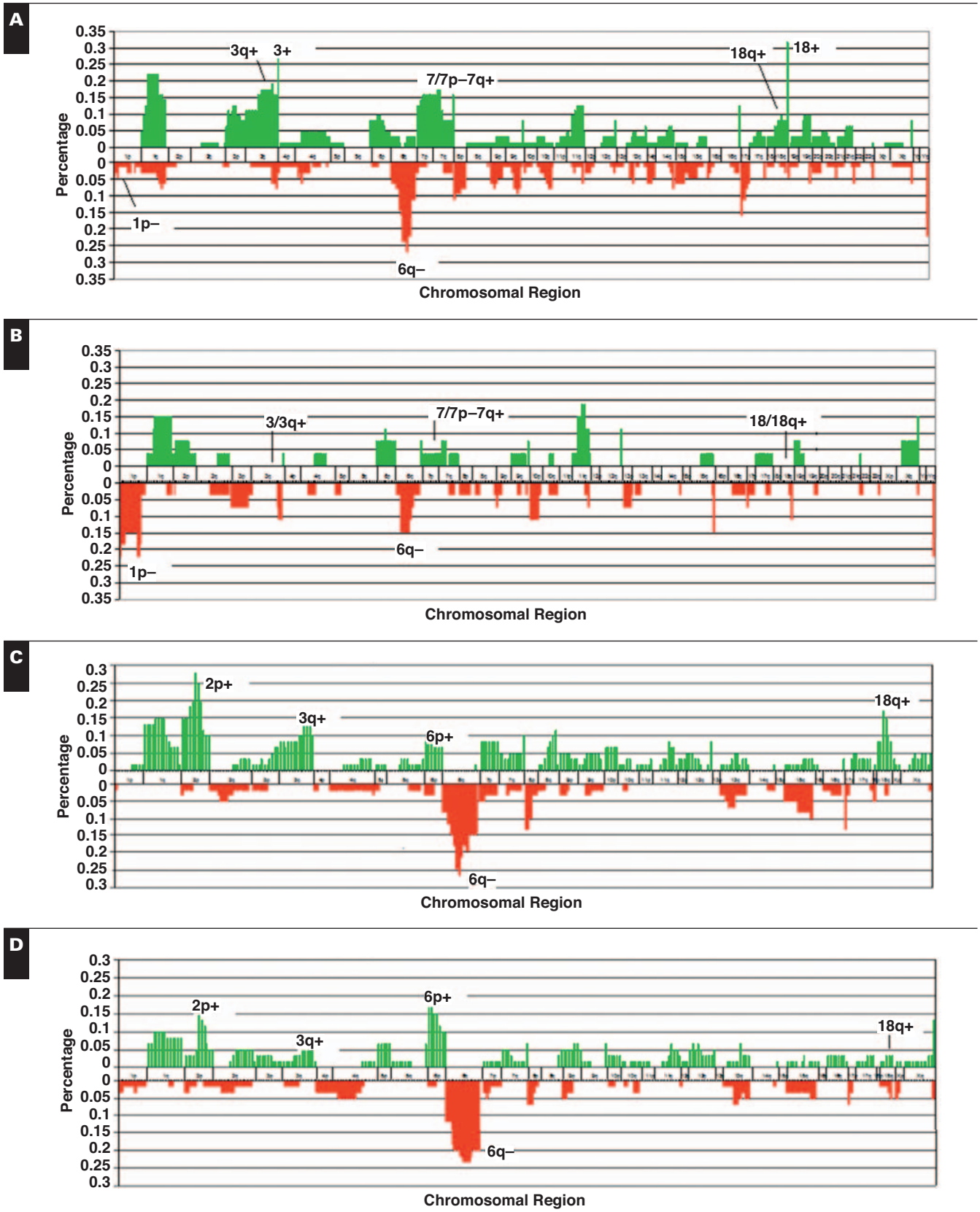

Image 2 **A** and **B**, Chromosomal imbalances detected by conventional cytogenetics in BCL2+ (n = 63) (**A**) and BCL2– (n = 27) (**B**) subgroups of diffuse large B-cell lymphoma (DLBCL) in a Chinese cohort. **C** and **D**, Chromosomal imbalances detected by comparative genomic hybridization in BCL2+ (n = 61) (**C**) and BCL2– (n = 60) (**D**) subgroups of DLBCL in a Western cohort. Chromosomal gain was shown in green and chromosomal loss in red.

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or 18/18q. Within the GCB DLBCL cases, 5 (42%) of 12 Chinese BCL2+ cases but only 2 (6%) of 31 Western BCL2+ cases had gains of 18/18q and/or 3/3q (P = .0123; Table 6).

Table 6
BCL2 Expression in Relation to the t(14;18) and Gains of 18/18q and 3/3q in Chinese and Western Cohorts

	BCL2 Protein Expression		
Group/Cytogenetic Alterations	**Chinese**	**Western**	***P***
Entire cohort			
No. of cases	63	57	
t(14;18)+	4 (6)	22 (39)	.0001
t(14;18)–; gain of 18/18q and/or 3/3q	34 (54)	15 (26)	.0038
GCB DLBCL			
No. of cases	12	31	
t(14;18)+	3[†] (25)	20[‡] (65)	.0387
t(14;18)–; gain of 18/18q and/or 3/3q	5 (42)	2 (6)	.0123
Non-GCB DLBCL			
No. of cases	51	26	
t(14;18)+	1[§] (2)	2 (8)	.2621
t(14;18)–; gain of 18/18q and/or 3/3q	29 (57)	13 (50)	.7414

DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell subtype.
[*] Data are given as number (percentage) unless otherwise indicated.
[†] One case had t(14;18) and gain of chromosome 3q.
[‡] Two cases had t(14;18) and a gain of chromosome 18q, 2 cases had t(14;18) and a gain of 3q, and 1 case had t(14;18) and gains of 18q and 3q.
[§] This case had t(14;18) and a gain of chromosome 18.

Discussion

In this study, we showed that the GCB subtype of DLBCL was significantly less common than the non-GCB subtype in Chinese patients. Compared with Western DLBCL cases, which were previously characterized by GEP[18] and immunophenotypic[6] studies, the GCB subtype of DLBCL was much less frequent in Chinese patients. However, some limitations may exist in our analysis owing to different methods and different observer biases in interpreting immunohistochemical stains between the present and previous studies.[6]

Several studies have shown that the majority of extranodal DLBCLs have a non-GCB phenotype,[20-23] suggesting that the proportions of GCB and non-GCB subtypes in DLBCL may be affected by the difference in proportions of nodal and extranodal cases. However, the majority of cases in our study presented initially with nodal disease, yet a higher percentage of cases had the non-GCB phenotype. Therefore, the difference in proportions of GCB and non-GCB subtypes of DLBCL in this study is unrelated to nodal vs extranodal disease.

We observed a significantly lower frequency of t(14;18) in Chinese DLBCL cases in the present study. We used the Vysis LSI *IgH*/*BCL2* probe set for the t(14;18), which consists of a 1.5-Mb locus-specific *IgH* probe spanning the entire *IgH* gene and a 750-kb *BCL2* probe spanning the entire *BCL2* gene. This probe covers most if not all common breakpoints. Furthermore, translocations are defined by probe splitting and colocalization, which minimizes the risk of false-positives. FISH results were also compared with conventional G-banding cytogenetic results in all Chinese DLBCL cases with 100% concordance. The probe set used in the present study does not detect a translocation between immunoglobulin light chain genes and the *BCL2* locus, which can be seen in rare DLBCL cases; however, t(2;18)(p11;q21) and t(18;22)(q21;q11) are usually detected with routine G-banding. We did not observe any immunoglobulin light chain/*BCL2* translocations in the present study.

The significantly fewer t(14;18)+ cases seen in Chinese patients would be consistent with the lower frequency of GCB DLBCL in the Chinese cohort because the t(14;18) was found to occur almost exclusively in the GCB subtype.[4,24,25] Compared with the Western cohort, the incidence of the t(14;18) was lower than expected, even in the GCB subgroup of Chinese patients. It is interesting that the incidence of follicular lymphoma (FL) is also reported to be significantly lower in Chinese patients (7%) than in Caucasian patients (22%-30%).[17] The t(14;18)(q32;q21), the characteristic cytogenetics alteration of FL, which has been identified in up to 85% of FL cases in Western populations, occurs in only 37% of FL cases in China.[17] Therefore, the low frequency of FL and GCB DLBCL in Chinese patients may be directly related to the low frequency of t(14;18) translocation.

We observed a higher frequency of BCL2 overexpression in Chinese DLBCL cases in the present study. In the non-GCB subgroup, BCL2 overexpression was highly associated with gains of 18/18q in the Chinese and Western cohorts; a significant association between BCL2 overexpression and 3/3q gain was observed only in the Chinese cohort. Different from Western GCB DLBCL in which BCL2 overexpression is highly associated with the t(14;18) translocation, BCL2 overexpression was also associated with gains of 18/18q and 3/3q in Chinese GCB DLBCL cases.

One of the major mechanisms contributing to BCL2 protein expression is the t(14;18)(q32;q21), which juxtaposes the immunoglobulin enhancers at 14q32 with the *BCL2* locus at 18q21, leading to the overproduction of BCL2 protein. In Western cases, the t(14;18) occurs mainly in GCB DLBCL.[4,24,25] Alternative mechanisms of BCL2 up-regulation, including gain/amplification of 18q21, more frequently seen in ABC DLBCL,[11-13,18] may contribute to BCL2 protein overexpression.[11-13,18,26,27] It may result in BCL2 protein overexpression by directly increasing the copy number of the *BCL2* locus located on 18q21 or by activating nuclear factor-κB (NF-κB), for which *BCL2* is a target. The *MALT1* gene, which lies close to *BCL2* on 18q21, has an important impact

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on NF-κB activation.[28] It is interesting that the NF-κB pathway is constitutively activated in ABC DLBCL and may have a critical role in its pathogenesis.[29] We did not evaluate the activity of the NF-κB pathway in the present study.

In Chinese DLBCL, gain/amplification of 18q21 may thus have an important role in pathogenesis. Moreover, we found that gain of 3/3q was also highly associated with BCL2 overexpression in Chinese DLBCL cases, suggesting that additional unknown factors may also have important roles in the development and progression of DLBCL in Chinese patients. Additional studies using array-based CGH may help narrow down and identify these factors and related molecular mechanisms. Nevertheless, our study included many BCL2+ DLBCL cases lacking the t(14;18) and gain of 18/18q and/or 3/3q. Further studies to elucidate the mechanisms of lymphomagenesis in these cases are warranted.

We observed a significantly lower incidence of the GCB subtype and a lower frequency of the t(14;18) in Chinese DLBCL compared with Western DLBCL cases. The t(14;18) was less frequent in the GCB subgroup of Chinese cases compared with Western cases. There were more cases expressing BCL2 protein in Chinese DLBCL than in the Western cohort. BCL2 expression was associated with gain of chromosome 18/18q in some cases. More interesting is that we found that BCL2 expression was also associated with a gain of 3/3q in Chinese DLBCL cases, whereas this association was less significant in Western cases.

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