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ORIGINAL ARTICLE

Clinical Trials Study

Coexpression of *MYC* and *BCL-2* predicts prognosis in primary gastrointestinal diffuse large B-cell lymphoma

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Abstract

AIM: To investigate whether *MYC* and *BCL-2* coexpression has prognostic significance in primary gastrointestinal diffuse large B-cell lymphoma (PGI-DLBCL) patients, and explore its associations with patients' clinical parameters.

METHODS: Fresh and paraffin-embedded tumor tissue samples from 60 PGI-DLBCL patients who had undergone surgery at the Tianjin Medical University Cancer Institute and Hospital from January 2005 to May 2010 were obtained, and 30 lymphoid tissue samples from reactive lymph nodes of age- and sex-matched patients represented control samples. Staging and diagnostic procedures were conducted according to the Lugano staging system. All patients had been treated with three therapeutic modalities: surgery, chemotherapy, or radiotherapy. Expression of *MYC* and *BCL-2* were detected at both protein and mRNA levels by immunohistochemistry and real-time RT-PCR.

RESULTS: Positive expression levels of MYC and BCL-2 proteins were detected in 35% and 45% of patients, respectively. $MYC^+/BCL\text{-}2^+$ protein was present in 30% of patients. MYC and BCL-2 protein levels were correlated with high *MYC* and *BCL*-2 mRNA expression, respectively (both $P < 0.05$). We found that advanced-stage disease (at Ⅱ E-Ⅳ) was associated with *MYC* and *BCL-2* coexpression levels ($P < 0.05$). In addition, $MYC^+/BCL\text{-}2^+$ patients had more difficulty in achieving complete remission than others ($P < 0.05$). Presence

of MYC protein expression only affected overall survival and progression-free survival (PFS) when BCL-2 protein was coexpressed. The adverse prognostic impact of $MYC^+/BCL\text{-}2^+$ protein on PFS remained significant ($P < 0.05$) even after adjusting for age, Lugano stage, international prognostic index, and BCL-2 protein expression in a multivariable model.

CONCLUSION: $MYC^+/BCL\text{-}2^+$ patients have worse chemotherapy response and poorer prognosis than patients who only express one of the two proteins, suggesting that assessment of MYC and BCL-2 expression by immunohistochemistry has clinical significance in predicting clinical outcomes of PGI-DLBCL patients.

Key words: MYC; BCL-2; Survival; Primary gastrointestinal diffuse large B-cell lymphoma; Prognosis

Core tip: We investigated *MYC* and *BCL-2* coexpression in primary gastrointestinal diffuse large B-cell lymphoma (PGI-DLBCL) and explored its associations with patients' clinical parameters. In contrast to previously published results about *MYC*/*BCL-2* coexpression in DLBCL, this study focused on PGI-DLBCL. Although PGI-DLBCL is rare, we had a large collection of 60 PGI-DLBCL cases to test the protein and mRNA levels of MYC and BCL-2. We found that $MYC^+/BCL\text{-}2^+$ patients have worse chemotherapy response and poorer prognosis than patients who only express one of the two proteins, suggesting that assessment of *MYC* and *BCL-2* expression has clinical significance in predicting clinical outcomes of PGI-DLBCL patients.

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INTRODUCTION

Primary gastrointestinal (PGI) lymphoma is the most common type of extranodal lymphoma, comprising about 30%-40% of all extranodal lymphomas, but only accounts for 1%-8% of all gastrointestinal (GI) malignancies[1]. The most frequent pathological type is mucosa-associated lymphoid tissue lymphoma and diffuse large B-cell lymphoma (DLBCL)[2]. The most common primary site is the stomach (60%-70%), followed by the small bowel (20%-30%), colorectum (5%-10%), and esophagus (< 1%)[2,3]. In China, DLBCL is the most common type of lymphoma as well as a highly heterogeneous disease, comprising approximately 30%-40% of adult non-Hodgkin lymphoma patients. PGI-DLBCL is a relatively rare disease, comprising only 1%-4% of those with gastrointestinal malignancies. Given the location of the GI tract and GI lymphoma association with infections such as *H. pylori* infection, celiac disease, inflammatory bowel disease and autoimmune diseases, we consider PGI-DLBCL as a distinct disease since their evaluation, diagnosis, management and prognosis are different from DLBCL of lymph node origin. Thus, our study here is focused on primary gastrointestinal DLBCL. MYC, an oncogenic transcription factor, has been recognized as one of the most frequently affected genes in human malignancies, with about 70% of all human malignancies showing overexpression of *MYC*[4]. MYC is also a critical player during lymphoma development, and its potential role in oncogenesis of hematopoietic cells was first demonstrated during the mid-1980s. Burkitt lymphoma is the first known example of MYC-induced lymphomagenesis[4]. In the past decade, our understanding of *MYC*-induced lymphomagenesis has been greatly improved.

BCL-2, an anti-apoptotic gene, has been implicated in conferring chemotherapy resistance in non-Hodgkin's lymphoma and has been extensively studied as a prognostic biomarker in DLBCL[5]. It was found to have an adverse effect on the prognosis of a subgroup of patients with germinal center B-cell-like (GCB) DLBCL who had been treated with CHOP-like therapy and rituximab[6].

MYC translocations, with or without *BCL-2* translocations, have been associated with inferior prognosis in DLBCL. We, in this study, focus on examining MYC and BCL2 protein expression by using recently developed and commercially available monoclonal antibodies. The MYC antibody targets the N-terminus of the MYC protein and has been shown to predict *MYC* rearrangements and has been validated for use in FFPE tissues. We believe that molecular confirmation of the immunohistochemistry for MYC and BCL-2 is more practical, relevant and closely representative. This is based on the concept that, in addition to translocations, *MYC* can also be deregulated by amplifications, mutations, or by microRNA-dependent mechanisms. Although *MYC* translocations can be detected by karyotype and fluorescence *in situ* hybridization (FISH), FISH fails to detect *MYC* deregulation caused by mechanisms other than translocation. This suggests that mechanisms other than gene rearrangements are responsible for elevated protein expression in a considerable proportion of DLBCL cases.

DLBCL patients with both MYC and BCL-2 protein coexpression have shown inferior overall survival (OS) and progression-free survival (PFS)[7]. This concurrent expression of MYC and BCL-2 proteins in patients with PGI-DLBCL has thus far not been clearly understood. In the present study, we used real-time quantitative PCR to measure expression levels of *MYC* and *BCL-2* mRNA and used our results to investigate whether

the coexpression of MYC and BCL-2 proteins has prognostic significance in patients with PGI-DLBCL. Furthermore, we explored associations among these coexpression levels and patients' clinical parameters.

MATERIALS AND METHODS

Patients

We obtained fresh and paraffin-embedded tumor tissue samples from 60 PGI-DLBCL patients who had undergone surgery at the Tianjin Medical University Cancer Institute and Hospital from January 2005 to May 2010. In addition, our study included 30 lymphoid tissue samples from reactive lymph nodes of age- and sex-matched patients, which represented control samples. This study was approved by the hospital-based ethics committee. The diagnoses of all patients were reevaluated by at least two experienced hematopathologists. Patients provided informed consent to allow complete clinical information and follow-up data to be obtained.

Staging and diagnostic procedures

All patients were staged according to the Lugano staging system[8], which is modified from the Ann Arbor criteria for primary gastrointestinal non-Hodgkin's lymphoma. The staging system included the patients' history, physical examination, chest X-ray, gastrointestinal endoscopy, abdominal ultrasound, bone marrow aspiration, biopsy, and computed tomography (CT) scans of the neck, chest, abdomen, and pelvis. Routine laboratory tests included the measurement of hemoglobin and serum lactate dehydrogenase (LDH) levels. Low hemoglobin was defined as < 120 g/L in men and < 110 g/L in women. High LDH was defined as > 245 U/L.

Treatment and response assessment

All patients had been treated with three therapeutic modalities: surgery, chemotherapy, or radiotherapy. All patients had been suspected as having other gastrointestinal malignancies but were diagnosed with PGI-DLBCL by postoperative pathology. None of the patients had preoperative chemotherapy, radiotherapy, or other treatment history, and all patients received at least four cycles of a standard-dose R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone)-like regimen. The status of chemotherapy response was evaluated by Cheson's standard criteria.

RNA and cDNA preparation

The fresh tissue specimens had been immediately frozen in liquid nitrogen until use. Approximately 25 mg of fresh tissue was used for RNA extraction, pulverized under liquid nitrogen with a pestle and mortar. Total RNA was isolated from tissue samples using RNeasy (Qiagen, Valencia, CA) following the manufacturer's instructions (Qiagen), and the concentration was determined by 260 nm/280 nm absorbance using a Nanodrop® ND-1000 spectrophotometer (Thermo Scientific). The reverse transcription reaction for the mRNA of *MYC* and *BCL-2* was done using Takara Kit (TaKaRa Bio Inc.) according to the manufacturer's protocol. cDNA were stored at -20℃ until use (Table 1).

Quantitative real-time PCR

Amplification for the cDNA of *MYC* and *BCL-2* was performed in a total volume of 20 μL containing 10 μL of kit-supplied QuantiTect™ SYBR® Green RT-PCR Master mix (Applied-Biosystems), 0.4 μL of each primer (Table 1), 2 μL of cDNA, and 7.2 μL ddH_2O. The PCR cycling parameters were set as follows: 95 ℃ for 30 s followed by 40 cycles of PCR reaction at 95 ℃ for 5 s and 60 ℃ for 34 s. The reactions were performed in the Bio-Rad CM9600 real-time PCR detection system (Bio-Rad, Hercules, CA). β-Actin was chosen as an internal standard. All PCR reactions were repeated 3 times. The relative amount of the mRNA was calculated by subtracting the average Ct value of the internal standard from the average Ct value of the target mRNA, yielding a ΔCt value. The ΔΔCt value was then calculated by subtracting the average ΔCt value of the controls from the respective ΔCt values of each patient sample. Relative expression levels were expressed as $2^{-\Delta\Delta Ct}$.

Immunohistochemistry

All tissue biopsies were fixed in 10% buffered formalin, embedded in paraffin, and cut into 4-μm sections. After deparaffinization, heat-induced antigen retrieval techniques were used. Endogenous peroxidase activity was then blocked with 0.5% H_2O_2. After being washed in phosphate-buffered saline, the sections were stained for the following antibodies: MYC (ab51154, Abcam), BCL-2 (ZM0010, ZSGB-BIO), CD10 (ZM0283, ZSGB-BIO), BCL-6 (ZM-0011,ZSGB-BIO), and MUM-1 (ZA-0583, ZSGB-BIO), CD5 (ZM0283, ZSGB-BIO), CD20 (ZM0039, ZSGB-BIO). The reaction was carried out at 4 ℃ overnight. After the sections were washed in phosphate-buffered saline again, the secondary antibodies (PV6000, ZSGB-BIO) were dropped. The cell nucleus was restained with hematoxylin after diaminobenzidine showed color, the results were judged as positive if 30% or more were stained.

Statistical analysis

SPSS 17.0 was used for statistical analysis. The results of *MYC* and *BCL-2* mRNA expression are presented as mean ± SD. The relative expression levels between patients with PGI-DLBCL and healthy controls were analyzed by the nonparametric Mann-Whitney *U*-test. Fisher's exact test and χ^2 test were used to determine differences in proportions regarding different clinical characteristics of the same groups. Unpaired *t*-test was used to find statistical significances between the PGI-DLBCL group and the normal control group. Univariate

Table 1 Primer sequences

Genes	Forward sequence (5′→3′)	Reverse sequence (5′→3′)
MYC	CCTCCACTCGGAAGGACTATC	TGTTCGCCTCTTGACATTCTC
BCL-2	GTGGATGACTGAGTACCTGAACC	AGACAGCCAGGAGAAATCAAAC
β-actin	CCTGGCACCCAGCACAAT	GGGCCGGACTCGTCATAC

Table 2 Clinical characteristics of primary gastrointestinal diffuse large B-cell lymphoma patients

Clinical characteristics	*n*	DP	Non-DP	χ^2	*P*
Patients	60	18	42		
Age (yr)				2.534	0.111
≤ 60	34	13	21		
> 60	26	5	21		
Gender				0.051	0.821
Male	32	10	22		
Female	28	8	20		
Primary site				0.013	0.908
Stomach	36	11	25		
Intestinal	24	7	17		
Lugano staging system				22.781	< 0.0001
Ⅰ-Ⅱ2	36	2	34		
ⅡE-Ⅳ	24	16	8		
LDH				0.207	0.649
Normal	34	11	23		
Elevated	26	7	19		
B symptoms				0.277	0.599
Positive	7	1	6		
Negative	53	17	36		
IPI				2.286	0.131
0-2	50	13	37		
3-5	10	5	5		
Pathological type				2.188	0.139
Non-GCB	48	17	31		
GCB	12	1	11		
CD10 status				0.013	0.908
Positive	36	117	25		
Negative/NA	24		17		
CD5 status				2.265	0.127
Positive	49	13	36		
Negative/NA	11	5	6		
CD20 status				0.207	0.649
Positive	34	11	23		
Negative/NA	26	7	19		
Anemia				0.003	0.955
Present	33	10	23		
Absent	27	8	19		
Treatment				2.017	0.156
ST + CT	51	13	38		
ST + CT + RT	9	5	4		
Therapeutic evaluation					
CR	48	9	39	11.910	0.001
PR/SD/PD	12	9	3		

DP: MYC/BCL-2 protein double positive; Non-DP: Non-MYC/BCL-2 protein double positive; NA: Not available; ST: Surgery; CT: Chemotherapy; RT: Radiotherapy; CR: Complete remission; PR: Partial remission; SD: Stable disease; PD: Progressive disease.

analysis of survival was done with the Kaplan-Meier method, which was carried out on PFS times and OS times. PFS was measured from the time of diagnosis to the date of treatment failure, clinical relapse, metastasis, disease progression, or death due to any cause. OS was calculated from the date of diagnosis until date of last follow-up or death. The significant factors ($P < 0.05$) of univariate analysis were included into the Cox regression model for multivariate analysis, in order to decide which factor could be the independent prognostic factor for survival. $P < 0.05$ was judged as statistically significant.

RESULTS

Clinical characteristics

Patients had a median age of 57 years (range: 23-79 years), with similar distribution of both sexes (32 males and 28 females; ratio = 1.1). Using the Lugano staging system, 36 patients (60%) presented with stage Ⅰ-Ⅱ2 and 24 patients (40%) presented with stage ⅡE-Ⅳ (advanced stage). Patients were divided into subgroups according to clinical parameters, such as age, primary site, LDH level, Lugano staging system, international prognostic index (IPI) score, anemia, and DLBCL pathological type. Table 2 shows the clinical characteristics of the 60 patients with PGI-DLBCL.

Concurrent expression of MYC and BCL-2 proteins in patients with PGI-DLBCL

In our patient group, 21 (35%) samples were positive for MYC and 27 (45%) samples were positive for BCL-2 (Figure 1). Concurrent expression of MYC and BCL-2 proteins was present in 18 (30%) patients. When we grouped patients according to the clinical characteristics, concurrent expression of MYC and BCL-2 proteins was significantly closely related to Lugano staging system, with a higher proportion of patients with stage ⅡE-Ⅳ showing both MYC and BCL-2 expression ($P < 0.05$; Table 2). No significant differences were observed in other clinical characteristic subgroups. Distribution by gender, age, origin, LDH, B symptoms, IPI, and anemia was similar among the MYC and BCL-2 protein coexpression group, as well as for the other expression groups ($P > 0.05$; Table 2). When we grouped patients into germinal center B-cell-like (GCB) DLBCL and non-GCB DLBCL groups based on the immunophenotypic profile, we found that the proportion of GCB and non-GCB did not differ significantly ($P > 0.05$).

Treatment outcomes

All patients had been treated with surgery and chemotherapy with or without radiotherapy. After the initial chemotherapy, 48 (80%) patients achieved complete remission (CR), with 12 (20%) patients not achieving CR or partial remission (PR), stable disease (SD), and

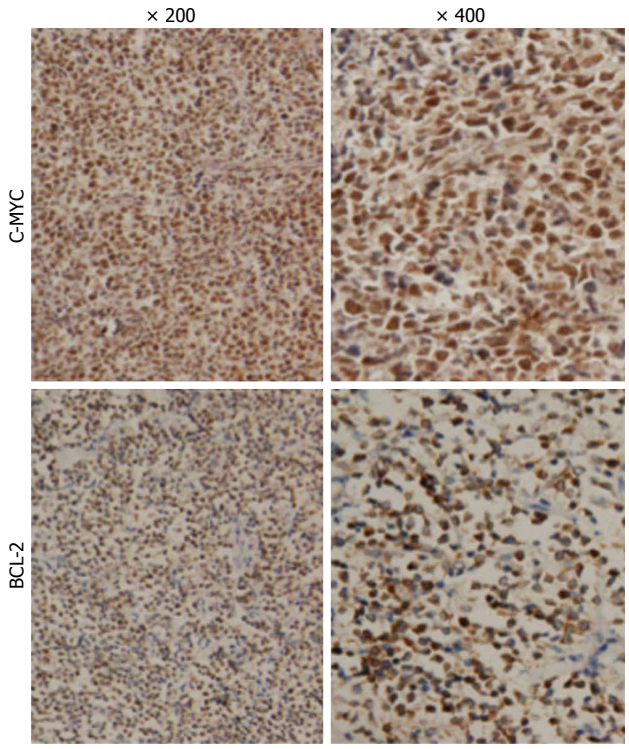

Figure 1 MYC and BCL-2 expression in primary gastrointestinal diffuse large B-cell lymphoma by immunohistochemistry (magnification × 200 and × 400, respectively).

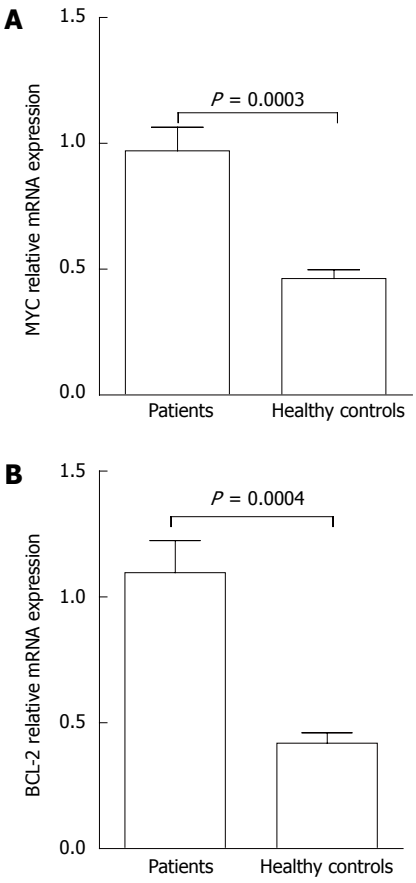

Figure 2 mRNA expression of MYC and BCL-2 in primary gastrointestinal diffuse large B-cell lymphoma patients.

progressive disease (PD). The distribution of patients treated with or without radiotherapy was similar in the MYC and BCL-2 protein coexpression group and in all other groups ($P > 0.05$, Table 2). In addition, we found that *MYC* and *BCL-2* coexpression was associated with inferior chemotherapy response in patients with PGI-DLBCL. Patients who had coexpression of *MYC* and *BCL-2* were less likely to achieve CR than other patient groups, with the difference being statistically significant ($P < 0.05$; Table 2).

mRNA levels of MYC and BCL-2 in patients with PGI-DLBCL

The results of real-time PCR in patient groups showed that the expression level of *MYC* mRNA was 0.97 ± 0.72 and *BCL-2* mRNA was 1.10 ± 0.98, with both significantly higher than those shown in normal controls ($P < 0.05$) (Figure 2). We also observed a significant correlation between BCL-2 mRNA and BCL-2 protein expression in PGI-DLBCL as a group (Spearman correlation of 0.64, $P < 0.05$; Table 3). *MYC* protein was also correlated with presence of high MYC mRNA (Spearman correlation of 0.43, $P < 0.05$; Table 4).

Prognostic analysis

The median follow-up time was 48 mo (range: 6-109 mo). The median PFS was 44.5 mo (95%CI: 63.4-86.8, range: 1-109 mo), and the median OS was 49 mo (95%CI: 83.4-101.2, range: 6-109 mo). The 5-year PFS and OS estimates for all patients were 65% and 82%, respectively (Figure 3). In univariate analysis, advanced-stage disease (ⅡE-Ⅳ), IPI (3-5), BCL-2 protein expression, and coexpression of MYC and BCL-2 proteins were all associated with inferior OS, whereas age (> 60 years), advanced-stage disease (ⅡE-Ⅳ), IPI (3-5), BCL-2 protein expression, and coexpression of MYC and BCL-2 proteins adversely affected PFS. However, radiotherapy could improve PFS significantly (Figure 4, Table 5). Gender, primary site, LDH, B symptoms, pathological type, anemia, and MYC protein expression did not significantly affect prognosis ($P > 0.05$; Table 5). Presence of MYC protein expression only affected OS and PFS when BCL-2 protein was coexpressed; the negative prognostic impact of MYC and BCL-2 was amplified when both variables were present (Figure 5). Prognosis for coexpression of MYC and BCL-2 proteins was worse than for the $MYC^+/BCL\text{-}2^-$ group and $MYC^-/BCL\text{-}2^-$ group. In addition, in the Cox multivariant model, the adverse prognostic impact of concurrent expression of MYC/BCL-2 protein and IPI on PFS existed even after adjusting for age, Lugano stage, IPI, and BCL-2 protein expression, whereas IPI was the only impact factor for OS (Table 6).

DISCUSSION

PGI-DLBCL is an aggressive lymphoma that may arise *de novo* or transform from other lymphoma, mostly

Table 3 Associations between *BCL-2* mRNA and BCL-2 protein expression

BCL-2	*BCL-2* mRNA		*r*	*P*
	High	Low		
Positive	18	9	0.640	< 0.0001
Negative	2	31		

Table 4 Associations between *MYC* mRNA and MYC protein expression

MYC	*MYC* mRNA		*r*	*P*
	High	Low		
Positive	17	4	0.430	0.0001
Negative	14	25		

transforming from MALT lymphoma. It most commonly occurs in men with a median age of 50-60 years old[9]. The International Prognostic Index (IPI), which incorporates 5 clinical parameters (age 60 years or older, disease stage Ⅲ/Ⅳ, high LDH level, 2 or more extranodal sites of disease, and an Eastern Cooperative Oncology Group performance status of 2 or more), is recognized as the gold standard for predicting prognosis in patients with DLBCL. However, the IPI evaluation system only integrates some clinical characteristics, and the molecular biology of cancer is not included. Research on gene expression profiling has shown the presence of DLBCL subtypes associated with different cells of origin and clinical outcomes[10,11]. This implies that potential prognostic biomarkers and different pathogenetic pathways may exist among the subtypes.

The proto-oncogene *MYC* is a member of common abnormalities in human malignancies and is a critical player in the development of lymphomas[12-15]. *BCL-2* is an important anti-apoptotic gene that inhibits apoptosis by encoding a mitochondrial protein. In addition, *in vitro* studies suggest that *BCL-2* has a role in drug resistance[16,17]. *MYC* aberrations in DLBCL are usually concurrent with other genetic lesions, such as rearrangements of *BCL-2* and/or *BCL-6*. Patients with these tumors, named as double-hit or triple-hit lymphomas, have an extremely poor prognosis, generally with survival of only 6 mo[18-22]. Moreover, many recent studies have suggested that the co-expression of MYC and BCL-2 proteins contributes to the inferior survival of DLBCL patients[7,23,24]. DLBCL patients with concurrent expression of MYC and BCL-2 proteins have been reported to have a poor prognosis, no matter whether the *MYC* or *BCL-2* gene is rearranged or not[7,25,26].

Johnson *et al*[7] reported a study using c-Myc antibody in combination with an antibody for BCL-2 in a training cohort of 167 and a validation set of another 140 patients with DLBCL. They found *MYC* translocations, high *MYC* mRNA, and MYC protein expression in 11%, 11%, and 33% of the samples,

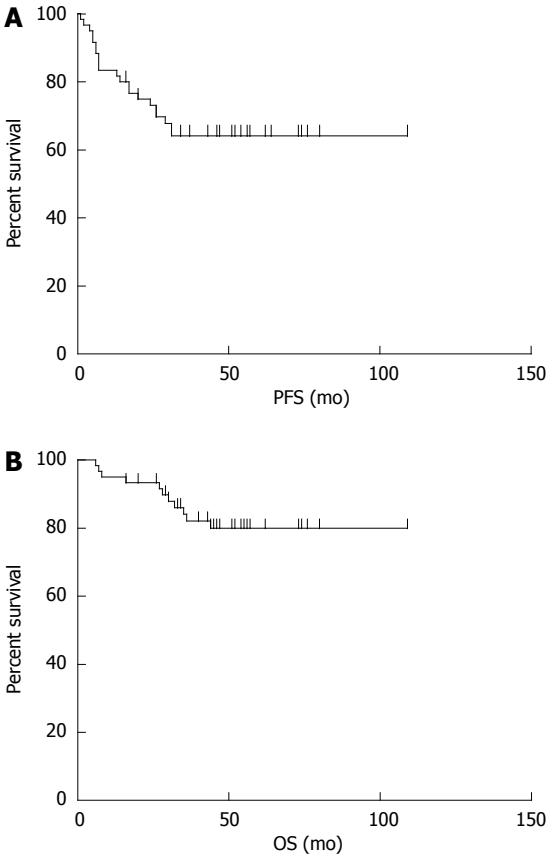

Figure 3 Survival curves for the entire population of patients with primary gastrointestinal diffuse large B-cell lymphoma. A: Progression-free survival (PFS); B: Overall survival (OS).

respectively. MYC protein expression was associated with an inferior progression-free and overall survival only when BCL-2 protein was coexpressed. MYC/BCL-2 protein coexpression was observed in 21% of the DLBCL cases, and the negative impact on prognosis remained significant after adjusting for the presence of high-risk features in a multivariable model that included elevated IPI score. Here, we observed the rate of concurrent expression ($MYC^+/BCL\text{-}2^+$) is higher in PGI-DLBCL than in all of DLBCL (30% *vs* 21%), suggesting that concurrent expression of *MYC* and *BCL-2* is more frequently in PGI-DLBCL. Moreover, our study showed that $MYC^+/BCL\text{-}2^+$ patients had more difficulty in achieving complete remission than others ($P < 0.05$). Presence of MYC protein expression only affected OS and PFS when BCL-2 protein was coexpressed. The adverse prognostic impact of $MYC^+/BCL\text{-}2^+$ protein on PFS remained significant ($P < 0.05$) even after adjusting for age, Lugano stage, IPI, and BCL-2 protein expression in a multivariable model. $MYC^+/BCL\text{-}2^+$ patients have worse chemotherapy response and poorer prognosis than patients who only express one of the two proteins, suggesting that assessment of *MYC* and *BCL-2* expression by immunohistochemistry has clinical significance in predicting prognosis clinical outcomes of PGI-DLBCL patients. Therefore, we speculated that *MYC* and

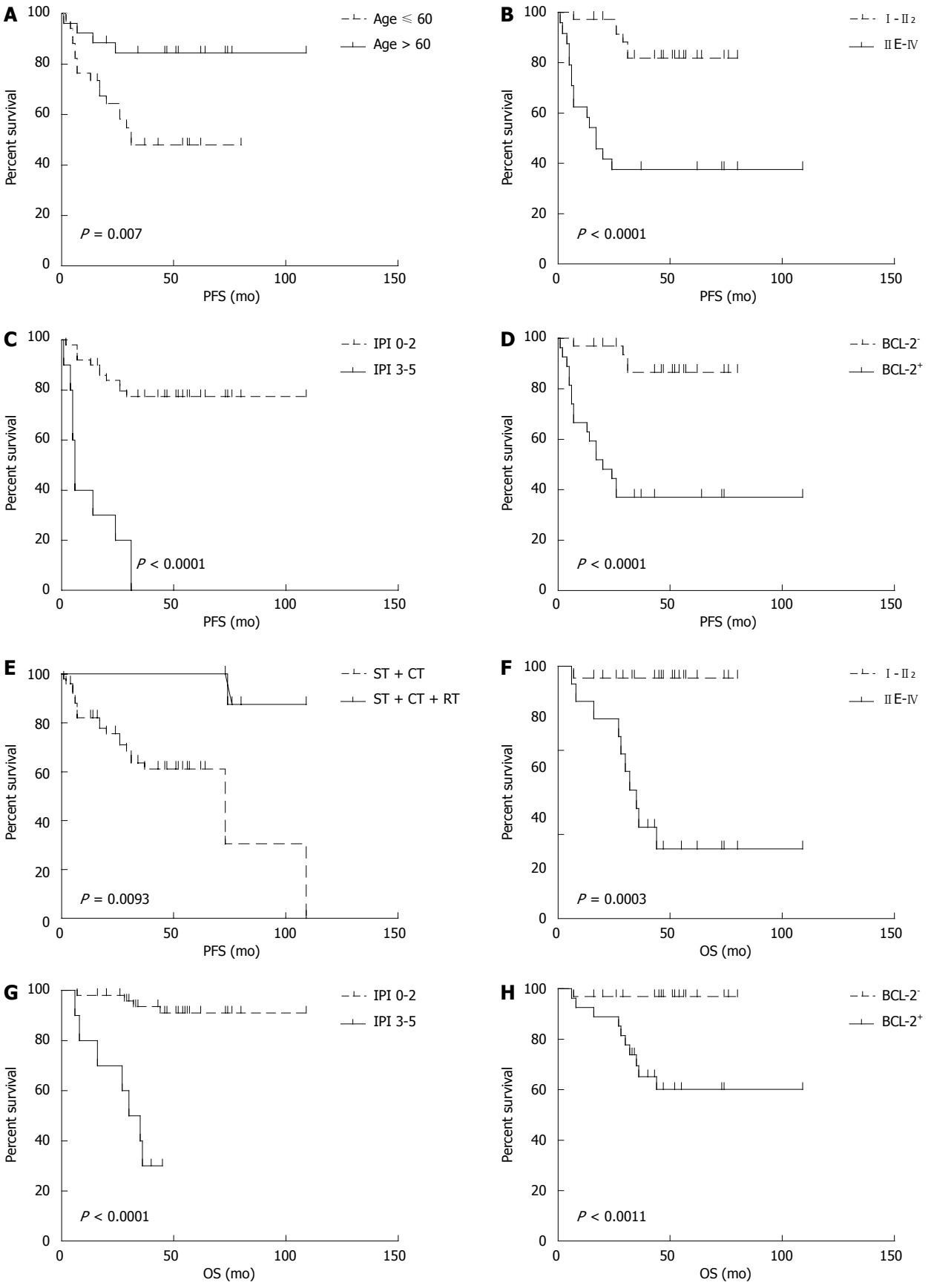

Figure 4 Survival analysis of primary gastrointestinal diffuse large B-cell lymphoma. The effects of age (A), Lugano stage (B), IPI score (C), the expression of BCL-2 protein (D), and treatment (E) on progression-free survival (PFS); And the effects of Lugano stage (F), IPI score (G) and BCL-2 protein expression (H) on overall survival (OS). ST: Surgery; CT: Chemotherapy; RT: Radiotherapy; IPI: International prognostic index.

Table 5 Univariate analysis of prognostic factors for progression-free survival and overall survival in patients with primary gastrointestinal diffuse large B-cell lymphoma

Prognostic factors	*n*	OS		PFS	
		χ^2	*P*	χ^2	*P*
Age (yr)		0.376	0.540	7.377	0.007
≤ 60	34				
> 60	26				
Gender		0.059	0.808	0.189	0.664
Male	32				
Female	28				
Primary site		0.132	0.717	1.211	0.271
Stomach	36				
Intestinal	24				
Lugano staging system		13.355	< 0.0001	17.581	< 0.0001
Ⅰ-Ⅱ$_2$	36				
ⅡE-Ⅳ	24				
LDH		0.029	0.864	0.118	0.731
Normal	34				
Elevated	26				
B symptoms		1.667	0.197	0.787	0.375
Positive	7				
Negative	53				
IPI		27.098	< 0.0001	39.737	< 0.0001
0-2	50				
3-5	10				
Pathological type		0.033	0.856	0.020	0.888
Non-GCB	48				
GCB	12				
Anemia		0.526	0.468	0.101	0.751
Present	33				
Absent	27				
Treatment		0.249	0.618	6.758	0.0093
ST + CT	51				
ST + CT + RT	9				
MYC protein expression		0.347	0.556	0.078	0.780
MYC$^+$	21				
MYC$^-$	39				
BCL-2 protein expression		10.701	0.001	19.463	< 0.0001
BCL-2$^+$	27				
BCL-2$^-$	33				
MYC/BCL-2 coexpression		10.956	0.001	15.198	< 0.0001
MYC$^+$/BCL-2$^+$	18				
All others	42				

OS: Overall survival; PFS: Progression-free survival; ST: Surgery; CT: Chemotherapy; RT: Radiotherapy; CR: Complete remission; IPI: International prognostic index; PR: Partial remission; SD: Stable disease; PD: Progressive disease.

BCL-2 expression, particularly MYC$^-$/BCL-2 protein coexpression, may play a role in the aggressive pathogenesis of PGI-DLBCL.

Hu *et al*[24] reported that MYC/BCL-2 protein coexpression in DLBCL associated with poor prognosis occurred more frequently in the ABC subgroup. They further confirmed that, after excluding patients with *MYC/BCL-2* coexpression, the clinical outcome of patients with ABC-DLBCL was similar to that of patients with GCB subgroup. Their results suggested *MYC/BCL-2* coexpression appears to account for the inferior prognosis of patients with ABC-DLBCL, and *MYC/BCL-2* coexpression may be a better predictor of prognosis than the cell-of-origin classification.

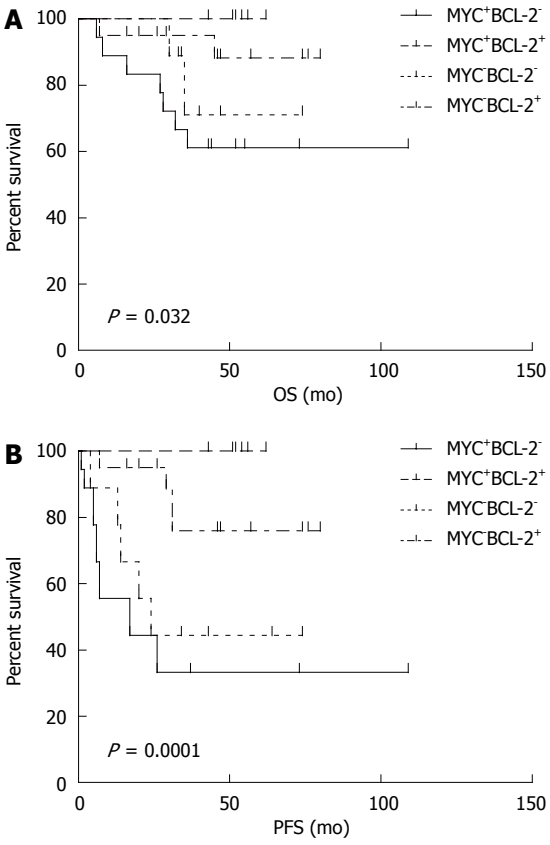

Figure 5 Kaplan-Meier curves represent overall survival (OS) (A) and progression-free survival (B) according to presence of MYC and BCL-2 protein expression. OS: Overall survival; PFS: Progression-free survival.

Table 6 Factors retaining prognostic significance for progression-free survival and overall survival with multivariate and Cox proportional hazards analysis

Prognostic factors	OS			PFS		
	HR	95%CI	*P*	HR	95%CI	*P*
Age	1.570	0.384-6.430	0.530	0.403	0.132-1.224	0.109
Lugano staging system	0.253	0.009-6.990	0.417	1.091	0.243-4.893	0.910
IPI	13.246	2.929-59.903	0.001	15.302	4.119-56.845	< 0.0001
Treatment	1.374	0.247-7.625	0.717	1.542	0.522-4.555	0.434
BCL-2 protein expression	1.056	0.029-37.972	0.976	1.773	0.154-20.434	0.646
MYC/BCL-2 coexpression	4.435	0.728-26.994	0.106	11.371	1.264-102.295	0.030

OS: Overall survival; PFS: Progression-free survival; IPI: International prognostic index.

In summary, our results suggested that PGI-DLBCL with concurrent expression of MYC and BCL-2 proteins characterizes a subset of PGI-DLBCL patients with poor prognosis. Previous reports have suggested that chemotherapy regimens that include BCL-2-targeted drugs, such as BH3 mimetics, have shown efficacy in murine *MYC*$^+$/*BCL-2*$^+$ lymphomas[27-29]. BCL-2-targeted therapy may represent a promising new apoptosis-

modulating strategy for $MYC^+/BCL\text{-}2^+$ patients with PGI-DLBCL.

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COMMENTS

Background

Primary gastrointestinal (PGI) lymphoma is the most common type of extranodal lymphoma, comprising about 30%-40% of all extranodal lymphomas. The most frequent pathological type is mucosa-associated lymphoid tissue lymphoma and diffuse large B-cell lymphoma (DLBCL). In China, DLBCL is the most common type of lymphoma as well as a highly heterogeneous disease, comprising approximately 30%-40% of adult non-Hodgkin lymphoma patients. PGI-DLBCL is a relatively rare disease, comprising only 1%-4% of those with gastrointestinal (GI) malignancies. We consider PGI-DLBCL as a distinct disease, since their evaluation, diagnosis, management and prognosis are different from DLBCL of lymph node origin. Thus, this study here is focused on PGI-DLBCL. *MYC*, an oncogenic transcription factor, has been recognized as one of the most frequently affected genes in human malignancies, with about 70% of all human malignancies showing overexpression of *MYC*. *BCL-2*, an anti-apoptotic gene, has been implicated in conferring chemotherapy resistance in non-Hodgkin's lymphoma and has been extensively studied as a prognostic biomarker in DLBCL. DLBCL patients with both MYC and BCL-2 protein coexpression have shown inferior overall survival (OS) and progression-free survival (PFS). This concurrent expression of MYC and BCL-2 proteins in patients with PGI-DLBCL has thus far not been clearly understood.

Research frontiers

Lymphomas with recurrent chromosomal breakpoints activating multiple oncogenes, including *MYC* and *BCL-2* are often referred to as "Dual Hit" or "Double Hit" lymphomas (DHL). DHL make up an important part of this novel WHO category and represent heterogeneous cases of aggressive B-cell lymphoma. PGI-DLBCL is a relatively rare disease. Herein, the authors focus on PGI-DLBCL, and observed that the rate of concurrent expression ($MYC^+/BCL\text{-}2^+$) is higher in PGI-DLBCL than all of DLBCL (30% *vs* 21%), suggesting that concurrent expression of *MYC* and *BCL-2* is more frequently in PGI-DLBCL.

Innovations and breakthroughs

Johnson and colleagues reported a study that used c-Myc antibody in combination with an antibody for BCL2 in a training cohort of 167 and a validation set of another 140 patients with DLBCL. They found *MYC* translocations, high *MYC* mRNA, and MYC protein expression in 11%, 11%, and 33% of the samples, respectively. MYC protein expression was associated with an inferior progression-free and overall survival only when BCL-2 protein was coexpressed. However, most of the DLBCL cases in this study are nodal DLBCL. PGI-DLBCL in this series are relatively small and the incidence and significance of *MYC* or/*BCL-2* expression was not mentioned in this study and has not been reported in the literature. Given the specialized location of the GI tract and GI lymphoma associations with infections such as *H. pylori* infection, celiac disease, inflammatory bowel disease and autoimmune diseases, authors have considered PGI-DLBCL as a distinct entity, since their evaluation, diagnosis, management and prognosis are different from DLBCL of lymph node origin (nodal). Thus, this study is performed to focus on PGI-DLBCL, and it was observed that the rate of concurrent expression ($MYC^+/BCL\text{-}2^+$) was higher in PGI-DLBCL than in all of DLBCL (30% *vs* 21%).

Applications

MYC/*BCL-2* double-hit lymphoma is a highly aggressive lymphoma with generally poor response to first line and salvage treatment, with a median OS of 0.2-1.5 years. These results suggest that PGI-DLBCL with concurrent expression of MYC and BCL-2 proteins characterizes a subset of PGI-DLBCL patients with poor prognosis. Previous reports have suggested that chemotherapy regimens that include BCL-2-targeted drugs, such as BH3 mimetics, have shown efficacy in murine $MYC^+/BCL\text{-}2^+$ lymphomas. *BCL-2*-targeted therapy may represent a promising new apoptosis-modulating strategy for $MYC^+/BCL\text{-}2^+$ patients with PGI-DLBCL.

Terminology

MYC/*BCL-2* double-hit lymphoma: Lymphomas with recurrent chromosomal breakpoints activating multiple oncogenes, including *MYC* and *BCL-2* are often referred to as "Dual Hit" or "Double Hit" lymphomas (DHL). PGI-DLBCL: Primary gastrointestinal lymphoma is the most common type of extranodal lymphoma, comprising about 30%-40% of all extranodal lymphomas, but only accounts for 1%-8% of all gastrointestinal malignancies. The most frequent pathological type is mucosa-associated lymphoid tissue lymphoma and diffuse large B-cell lymphoma.

Peer-review

The paper demonstrated the significance of *MYC* and *BCL-2* co-expression on the prognosis of PGI-DLBCL. Multivariate analysis revealed that *MYC* and *BCL-2* double positive phenotype was the independent factor for poorer response to chemotherapy and poorer prognosis. The presentation of the data was clear and experiments were performed carefully. The precise identification of *MYC*/*BCL-2* expression in PGI-DLBCL is quite important for clinical settings.

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