

# FOXP1 expression and its clinicopathologic significance in nodal and extranodal diffuse large B-cell lymphoma

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**Abstract** The aims of this study were to investigate FOXP1 expression in nodal and extranodal diffuse large B-cell lymphoma (DLBCL) and its association with the subclassification and other clinicopathologic parameters of DLBCL. Expression of FOXP1, CD10, Bcl6, MUM1, and Bcl2 was detected by immunohistochemistry on tissue microarray sections. The Kaplan–Meier method was used to estimate the overall survival of patients, and the log-rank test was used to compare survival differences between groups with different FOXP1 protein expressions. Expression of FOXP1 was detected in 67.4% (95/141) of

DLBCLs. FOXP1 expression in non-GCB (67/90, 74.4%) was significantly higher than that in GCB (28/51, 54.9%) ( $p < 0.05$ ). FOXP1 expression in MUM1-positive cases (62/81, 76.5%) was significantly higher than that in MUM1-negative cases (33/60, 55%) ( $p < 0.01$ ). FOXP1 expression was positively correlated with Bcl2 ( $p < 0.05$ ) in non-GCB among nodal DLBCL cases. Among the extranodal group, patients with FOXP1 expression had a significantly inferior OS compared to those with negative FOXP1 expression ( $p < 0.05$ ), which was not seen in nodal group. In conclusion, FOXP1 expression might be involved in the tumorigenesis of both nodal and extranodal DLBCL. The most striking finding of this study was that FOXP1 expression had an adverse effect on survival of patients with extranodal DLBCL, which indicated that FOXP1 function might be mediated by different mechanisms in nodal and extranodal DLBCLs. FOXP1 might play a role in the pathogenesis of nodal non-GCB DLBCL through the pathways in which Bcl2 was involved, and it might be a second important biomarker for non-GCB.

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## Introduction

Diffuse large B-cell lymphoma (DLBCL) is one of the most common non-Hodgkin lymphomas (NHLs), accounting for 30–40% of NHL in Western countries and over 40% of NHL in Asia [1]. The overall survival of DLBCL is unfavorable and its prognosis is very poor [1]. Approximately 60% of patients with DLBCL relapse after conventional chemotherapy [1–3]. Given its considerable heterogeneity, DLBCL has been subclassified into three

phenotypes according to gene expression profiling studies: germinal center B-cell-like (GCB), activated B-cell-like (ABC), and the third type. GCB phenotype has the best 5-year survival rate [4–7]. Although DLBCL molecular subclassification by gene profiling suggests its progression and prognosis, it is not practical to conduct gene profiling in routine work. Thus a number of immunostaining panels to classify DLBCL have been proposed, among which the panel of CD10, Bcl6, and MUM1 proposed by Hans et al. is the most popular [8–12]. However, more reliable biomarkers with high repeatability and refined predictive power are urgently needed to diagnose high-risk patients and develop alternative strategies for treatment.

FOXP1 (Forkhead box protein P1), a novel winged helix transcription factor, is a member of the FOXP subfamily, which belongs to the forkhead/winged helix transcription factor family [13, 14]. FOXP1 has been shown to be expressed in normal activated B cells, mantle zone, and some germinal center B cells using genomic-scale expression profiling or immunohistochemistry [15, 16]. Recently, several studies have investigated the expression of FOXP1 in DLBCL. FOXP1 protein was predominantly overexpressed in non-GCB DLBCL compared with GCB DLBCL [11, 17, 18]. A few studies have assessed the correlation between FOXP1 expression and the survival. However, the results are conflicting. Some studies showed a significantly lower survival for the FOXP1-positive group [17, 18], whereas others found that FOXP1 had no effect on clinical outcomes [11]. In addition, some of these previous studies were limited to nodal DLBCL, while others did not differentiate the nodal or extranodal origin clearly.

In this study, we examined the expression of FOXP1 and other molecular markers (Bcl2, CD10, Bcl6, and MUM1) in a large series of DLBCL (derived from nodal and extranodal cases) by constructing tissue microarray (TMA) and immunohistochemistry analysis. Then we compared the expression of FOXP1 in nodal and extranodal DLBCLs, and analyzed the association of FOXP1 expression with molecular subgroups of DLBCL and overall survival to determine the possible value of FOXP1 in the pathogenesis, subclassification, and prognosis of DLBCL.

## Materials and methods

### Patient population and tissue microarray construction

We retrospectively studied 150 cases with a diagnosis of de novo nodal or extranodal DLBCL, who had been treated with CHOP (cyclophosphamide, epirubicin, vincristine, and prednisone) based regimens at Department of Oncology, Fudan University Shanghai Cancer Center between 1995 and 2004 (Shanghai, China) and whose formalin-fixed,

paraffin-embedded pathologic material was available and sufficient for TMA analysis. In addition, lymph reactive hyperplasia cases ( $n=15$ ) were obtained from the same department. The study was approved by the Institutional Review Board. All the cases were histologically reviewed by two senior pathologists according to the World Health Organization classification of tumors of hematopoietic and lymphoid tissues to confirm the diagnosis. The clinical data were also collected. For the TMA construction, H&E stained sections from each formalin-fixed paraffin-embedded block were firstly observed to define representative tumor cell-rich areas and then two representative 0.6-mm cores were obtained from each case and inserted into a recipient paraffin block in a grid using a tissue arrayer (Beecher Instruments, Sliver Spring, MD, USA). Sections (4- $\mu$ m thick) were then cut from TMA blocks and stained with H&E and immunohistochemistry. The H&E section was used to verify adequate representations of diagnostic biopsies.

### Immunohistochemistry (IHC)

Following deparaffinization, TMA sections were subjected to heat-mediated antigen retrieval in 1 mM EDTA (pH 8.0). IHC was carried out using the Envision system (DAKO, Glostrup, Denmark) followed by incubation with the primary antibodies against CD10 (clone 56C6; DAKO; dilution 1:40), Bcl6 (clone PG-B6P; DAKO; dilution 1:10), MUM1 (clone MUM1p; DAKO; dilution 1:100), Bcl2 (clone 124; DAKO; dilution 1:50), and FOXP1 (clone JC12; AbD seroTec; dilution 1:500) overnight. The stained sections were subsequently counterstained with hematoxylin. Reactive tonsil was used as a positive control for FOXP1. For negative controls, the primary antibodies were omitted. Both positive and negative controls were carried out simultaneously on independent slides. The IHC results were reviewed by two independent certified pathologists.

Nuclear staining of tumor cells for FOXP1 was considered as positive. Scoring of the FOXP1 immunostaining was performed according to the literatures as negative (with occasional cells having weak nuclear FOXP1 expression), moderately positive (with part of the tumor cells featuring nuclear FOXP1 expression with variable intensity), or strongly positive (with nearly all tumor cells showing strong, uniform nuclear FOXP1 expression) [19, 20]. Duplicate cores that did not have identical results for FOXP1 staining were averaged [17].

For Bcl6 and MUM1, cases were considered as positive if 30% or more tumor cells were stained on nuclear. Cases were considered as positive for staining of CD10 and Bcl2 on the membrane and cytoplasm, respectively [11]. DLBCLs were then divided into two subtypes, GCB type and non-GCB type, based on the expression of CD10, Bcl6,

and MUM1 as previously reported [11]. The criteria were as follows. Cases were assigned to the GCB subgroup if CD10 alone was positive or both CD10 and Bcl6 were positive. If both were negative, the case was assigned to non-GCB group. If CD10 was negative and Bcl6 was positive, the expression of MUM1 was used to determine which group the case belonged to. If MUM1 was negative, the case was assigned to GCB group; otherwise, the case was assigned to non-GCB group.

#### Statistical analysis

The Spearman rank test was applied to examine the patients' characteristics and the relationships between variables. Overall survival (OS) was defined as the time from initial diagnosis to the time of death or the last contact. Patients still alive were censored at the last known date of contact. The Kaplan–Meier method was used to estimate the overall survival of patients, and the log-rank test was used to compare survival differences between the groups with different FOXP1 protein expressions. The statistical analysis was performed using the SPSS software package (SPSS version 11.5; SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant if *p* values were less than 0.05.

## Results

#### General information of the DLBCLs

Among the total cases, nine DLBCL cases were failed to be investigated because of tissue's dropping off during experiments. Among the 141 DLBCL cases, 77 were male and 64 were female. Their mean age was 54.3 years, ranging from 9 to 82 years. Seventy-one of the cases occurred within lymph nodes; 70 cases occurred in extranodal sites, including stomach, colon, breast, salivary glands, greater omentum, ovary, spleen, thyroid, testis, and skin. Clinical data were available for 85 cases, among which follow-up data were available for 61 cases, including 26 nodal lymphomas and 35 extranodal lymphomas.

#### FOXP1 expression in nodal and extranodal DLBCL

To assess the expression of FOXP1, IHC was performed using JC12, the specific monoclonal antibody against FOXP1. We assessed and compared the results of the two cores for every case. Our findings revealed that the results of the two cores were consistent with each other in 136 of 141 cases; only five cases showed different outcomes of staining. We combined and averaged the two cores' results as the final results for the FOXP1 immunostaining of these

five cases. FOXP1 was expressed in 67.4% (95 of 141) of total DLBCL patients (Table 1, Fig. 1). The cases with strong expression accounted for 7.1% (10 of 141). Only one of 15 RH cases showed moderate positive expression of FOXP1 and one case was weak positive in variable proportion of B cells (negative according to the criteria above), and the other 13 cases were negative.

The cases with FOXP1 expression (both moderate and strong positive) in nodal DLBCL accounted for 71.8% (51 of 71), among which the percentage of strong positive cases was 12.7%. The cases with expression of FOXP1 in extranodal DLBCL accounted for 62.9% (44 of 70), among which the percentage of strong positive cases was only 1.4%. There was no correlation between FOXP1 and the primary sites when FOXP1 expression level was classified into two groups, positive and negative ( $p=0.169$ ) (Table 1). However, FOXP1 expression was correlated with primary sites when the expression level was classified into three groups, negative, moderate positive, and strong positive ( $p=0.025$ ) (Table 2), especially the strong expression of FOXP1, which was significantly higher in nodal cases than that in extranodal cases ( $p=0.009$ ).

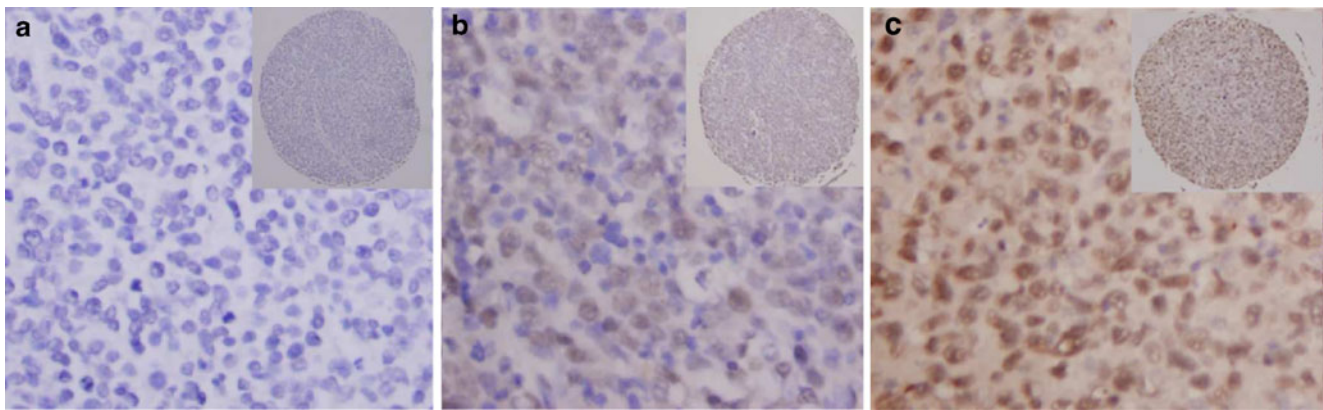
#### DLBCL subclassification and its correlation with FOXP1 expression

In order to analyze the subgroups of DLBCL, the expression of CD10, Bcl6, and MUM1 were investigated by IHC and their expression was observed in 19.1% (27 of 141), 51.1% (72 of 141), and 57.4% (81 of 141) DLBCL cases, respectively. Then the cases were divided into two

**Table 1** FOXP1 expression in DLBCL and its correlation with original sites, subgroups, MUM1, and Bcl2 expression when FOXP1 was classified into two groups

Groups	<i>n</i> (141)	FOXP1 expression	
		+ (%)	<i>p</i> value
Total number	141	95 (67.4)	
Sites			0.169
Nodal DLBCL	71	51 (71.8)	
Extranodal DLBCL	70	44 (62.9)	
Subgroups			0.017
GCB	51	28 (54.9)	
Non-GCB	90	67 (74.4)	
MUM1			0.007
Positive	81	62 (76.5)	
Negative	60	33 (55.0)	
Bel2			0.173
Positive	73	53 (72.6)	
Negative	68	42 (61.8)	

+ positive (including both moderate and strong positive) expression of FOXP1



**Fig. 1** FOXP1 immunostaining in DLBCL showed three groups of nuclear expression level of lymphoma cells. **a** Negative. No lymphoma cells or only occasional cells have weak expression of

FOXP1. **b** Moderate positive. Moderate widespread or strong focal staining was seen in lymphoma cells. **c** Strong positive. Almost all the tumor cells show strong, uniform expression of FOXP1

subgroups, GCB and non-GCB, according to the expression of CD10, Bcl6, and MUM1. Of the 141 cases, 36.2% (51 of 141) were assigned to GCB and 63.8% (90 of 141) were non-GCB. The expression of another important molecule Bcl2 was also detected by IHC. Bcl2 was strongly expressed in the cellular membrane and cytoplasm in 51.8% (73 of 141) DLBCL cases. The percentage of FOXP1-positive cases was 54.9% (28/51) in GCB subtype, which was significantly lower than that in the non-GCB subtype (67/90, 74.4%) ( $p=0.017$ ) (Table 1). There was also a significant association between FOXP1 expression and the subgroup when FOXP1 was divided into negative, moderate positive, and strong positive ( $p=0.025$ ) (Table 2).

#### Correlation of FOXP1 expression with MUM1 and Bcl2

Among the MUM1-positive cases, 76.5% (62 of 81) cases expressed FOXP1, which was significantly higher than that

among the MUM1-negative cases (55%, 33 of 60) ( $p=0.007$ ) (Table 1), and this correlation was even stronger when FOXP1 was classified into three groups, negative, moderate positive, and strong positive ( $p=0.002$ ) (Table 2). When FOXP1 was classified into negative and positive groups, there was no significant correlation between FOXP1 and Bcl2 expression ( $p=0.173$ ) (Table 1); whereas the expression of Bcl2 was significantly increased with FOXP1 expression increased from negative to strong positive ( $p=0.036$ ) (Tables 2 and 3). The relationships between FOXP1 and Bcl2 among nodal and extranodal DLBCL were further observed, respectively (Table 3). Among the nodal DLBCL cases, FOXP1 expression was correlated with Bcl2 ( $p=0.016$ ). Among the non-GCB cases in the nodal group, FOXP1 expression was positively correlated with Bcl2 ( $p=0.011$ ), while this was not the case among the GCB cases ( $p=0.640$ ) (Table 3). Among the extranodal DLBCL cases, there was no correlation between

**Table 2** Correlation between FOXP1 expression and original sites, subgroups, MUM1, and Bcl2 expression when FOXP1 was classified into three groups

Groups	n (141)	FOXP1 expression			p value
		-	+	++	
<b>Sites</b>					
Nodal DLBCL	71	19 (26.8)	43 (60.6)	9 (12.7)	0.025
Extranodal DLBCL	70	26 (37.1)	43 (61.4)	1 (1.4)	
<b>Subgroups</b>					
GCB	51	23 (45.1)	25 (49.0)	3 (5.9)	0.025
Non-GCB	90	22 (24.4)	61 (67.8)	7 (7.8)	
<b>MUM1 expression</b>					
Positive	81	18 (22.2)	55 (67.9)	8 (9.9)	0.002
Negative	60	27 (45.0)	31 (51.7)	2 (3.3)	
<b>Bcl2 expression</b>					
Positive	73	19 (26.0)	46 (63.0)	8 (11.0)	0.036
Negative	68	26 (38.2)	40 (58.8)	2 (2.9)	

+ moderate positive expression of FOXP1, ++ strong positive expression of FOXP1

**Table 3** Correlation of FOXP1 with Bcl2 expression in total 141 DLBCLs, 71 nodal DLBCLs, and 70 extranodal DLBCLs

Groups	n (141)	FOXP1 expression			p value
		–	+	++	
DLBCL as single entity, n=141					
In total					0.036
Bcl2+	73	19 (26.0)	46 (63.0)	8 (11.0)	
Bcl2–	68	26 (38.2)	40 (58.8)	2 (2.9)	
In non-GCB					0.162
Bcl2+	57	12 (21.2)	39 (68.4)	6 (10.5)	
Bcl2–	33	10 (30.3)	22 (66.7)	1 (3.0)	
In GCB					0.529
Bcl2+	16	7 (43.8)	7 (43.8)	2 (12.5)	
Bcl2–	35	16 (45.7)	18 (51.4)	1 (2.9)	
Nodal DLBCL, n=71					
In total					0.016
Bcl2+	37	7 (18.9)	22 (59.5)	8 (21.6)	
Bcl2–	34	12 (35.3)	21 (61.8)	1 (2.9)	
In non-GCB					0.011
Bcl2+	27	3 (11.1)	18 (66.7)	6 (22.2)	
Bcl2–	19	6 (31.6)	13 (68.4)	0 (0)	
In GCB					0.640
Bcl2+	10	4 (40.0)	4 (40.0)	2 (20.0)	
Bcl2–	15	6 (40.0)	8 (53.3)	1 (6.7)	
Extranodal DLBCL, n=70					
In total					0.692
Bcl2+	36	12 (33.3)	24 (66.6)	0 (0)	
Bcl2–	34	14 (41.2)	19 (55.9)	1 (2.9)	
In non-GCB					0.602
Bcl2+	30	9 (30.0)	21 (70.0)	0 (0)	
Bcl2–	14	6 (31.6)	13 (68.4)	1 (7.1)	
In GCB					1.000
Bcl2+	6	3 (50.0)	3 (50.0)	0 (0)	
Bcl2–	20	10 (50.0)	10 (50.0)	0 (0)	

+ moderate positive expression of FOXP1, ++ strong positive expression of FOXP1

FOXP1 and Bcl2 expression as well as in the GCB and non-GCB subgroups respectively ( $p>0.05$ ).

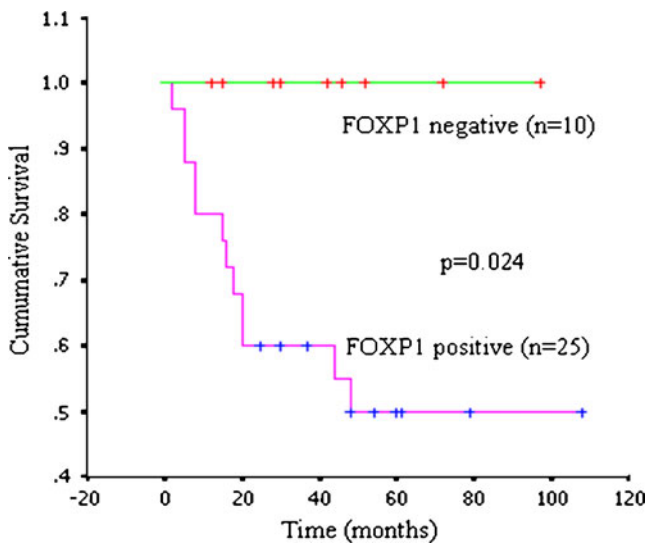
#### Correlation of FOXP1 expression with overall survival

To investigate the relationship between FOXP1 and OS, Kaplan–Meier analysis was used. When all the cases were considered as a single entity, no prognostic impact of FOXP1 expression on OS was observed. However, prognostic impact of FOXP1 was observed when the cases were divided into two groups according to the primary site. Among the extranodal group, the median follow-up was 42 months, ranging from 2 months to 108 months. The data showed that 12 out of 25 FOXP1-positive and 0 out of 10 FOXP1-negative patients were dead at the end of the follow-up. Among the 12 dead cases, three occurred in stomach, one in ovary, four in testis, one in thyroid, one in skin, one in greater omentum, and one in spleen. The

median OS of FOXP1-positive patients was 48 months, whereas the median OS of FOXP1-negative patients was not available because all the cases were censored. The 2-year OS rate of FOXP1-negative patients was 100.0%, which was higher than that of FOXP1-positive patients (60.0%). The OS curves showed a dramatic impact of FOXP1 expression status on outcome ( $p=0.024$ ) (Fig. 2). Among the nodal group, the median follow-up was 36 months, ranging from 6 months to 90 months. However, the predictive value was not seen in this group ( $p>0.05$ ).

#### Discussion

Recently the Foxp subfamily has become a research focus and the functional importance of this subfamily is underscored by their spontaneous mutation in mouse and human diseases [21]. Among this subfamily, misregulation of



**Fig. 2** Correlation of FOXP1 protein expression with overall survival in extranodal DLBCL patients. Kaplan–Meier analysis of OS was performed for the 35 patients with extranodal DLBCL stratified according to FOXP1 expression. Patients with positive FOXP1 expression had significantly inferior median OS compared with those with negative expression ( $p=0.024$ )

FOXP1 expression is seen in a variety of tumors, including renal cell carcinoma, breast cancer, follicular lymphoma, and DLBCL [15, 19, 22].

Previously it has been shown that FOXP1 was predominantly expressed in non-GCB subtype of DLBCL [11, 18]. However, the percentage of its expression was reported diversely, from 13% to 61% [11, 17, 18, 20]. The discrepancy between these studies might be due to different methods of tissue preparation, or heterogeneous samples of diverse ethnic or clinical characteristics. Moreover, the criteria for defining FOXP1 positivity might partially account for this discrepancy [11, 17, 18, 20]. In our study, both intensity and extent of positive tumor cells were considered. The proportion of positive (including moderate and strong) cases was 64.7%, which was close to the results of Hans et al. and higher than those of the other previous studies.

Most of the previous studies on FOXP1 expression were confined to nodal DLBCL. However, little was known about the FOXP1 expression in extranodal DLBCL. In the present study, we investigated the expression of FOXP1 in DLBCL occurred both in lymph nodes and in extranodal sites. The results showed that FOXP1 was expressed both in nodal and extranodal DLBCLs. The expression of FOXP1 in nodal DLBCL (71.8%) was slightly higher than that in extranodal DLBCL (62.9%), but there was no statistical significance ( $p>0.05$ ). However, the percentage of strong FOXP1 expression was significantly higher in nodal DLBCL (12.7%) than that in extranodal DLBCL (1.4%) ( $p<0.05$ ). It can be concluded that FOXP1 might be

involved in the development of both nodal and extranodal DLBCL, but the mechanisms and the importance of its function might be different.

It is well known that different gene panels are involved in GCB DLBCL and non-GCB DLBCL through different pathways [5, 23]. In the current study, we showed that FOXP1 expression in non-GCB DLBCL (74.4%) was significantly higher than that in GCB DLBCL (54.9%), which was in consistent with the previous studies [11, 18, 20]. These observations suggested that FOXP1 might play a more important role in the development of non-GCB DLBCL, although the underlying mechanism was largely unknown. In addition, the non-GCB subtype accounted for a higher percentage than GCB subtype in Asia, and the expression of FOXP1 was dramatically higher in non-GCB subtype than that in GCB subtype as discussed above, which might partially result in the higher rate of FOXP1 expression in our series of samples.

MUM1 is a lymphoid-specific member of the interferon regulatory factor family of transcriptional regulator, and is thought to be a post-GC marker [24, 25]. The relationship between MUM1 and FOXP1 protein was seldom examined. In the present study, statistical analysis showed that FOXP1 protein expression was 77.8% in MUM1-positive cases and 55% in MUM1-negative cases, and the two biomarkers were positively correlated with each other. Given the biologic function of MUM1 in B-cell differentiation and the close correlation between FOXP1 and MUM1, it appeared that FOXP1 had potential to be a marker of the non-GCB phenotype. These findings were supported by the previous gene expression profiling studies [5, 16], which implied that FOXP1 was the second best predictor gene (behind MUM1) defining the ABC (activated B-cell)-type of DLBCL.

Bcl2 is an antiapoptotic factor which is important in normal B-cell development and differentiation [23]. Bcl-2 protein overexpression has been reported to occur in 24% to 66% of DLBCLs [23, 26, 27]. In the current study, we investigated the association of FOXP1 and Bcl2 in both nodal and extranodal DLBCLs. When all the DLBCLs were considered together, Bcl2 expression was increased with FOXP1 expression increasing from negative to moderate positive to strong positive, indicating that FOXP1 was positively correlated with the expression of Bcl2. However, when the cases were divided into nodal and extranodal groups, this correlation was only observed in nodal DLBCL, but not in extranodal DLBCL. Thus, one can speculate that FOXP1 might function through alternative pathways in nodal and extranodal DLBCLs. We further assessed this association in non-GCB and GCB subgroups within nodal DLBCLs, and the results showed that FOXP1 was closely related to Bcl2 within non-GCB group, while there was no relationship between these two markers within

GCB group. In conclusion, FOXP1 was associated with the expression of Bcl2 mainly in the non-GCB subgroup of nodal DLBCL. This result was in agreement with the previous study in nodal DLBCL, which demonstrated that FOXP1 was predominantly expressed in a subset of DLBCL with positive Bcl2 and negative t(14;18) [18]. As a target gene of NF- $\kappa$ B [28], in many ABC DLBCL patients, Bcl2 upregulation may be mediated through NF- $\kappa$ B pathway [23], which is constitutively expressed in ABC DLBCL and has a critical role in its pathogenesis [29]. Recently, Wlodarska et al. raised a notion that FOXP1 is also involved in the NF- $\kappa$ B pathway, like MALT1 and Bcl10 genes, both of which are known to play crucial roles in activation of NF- $\kappa$ B and thus contribute to the pathogenesis of MALT lymphoma [20]. If FOXP1 is involved in NF- $\kappa$ B pathway, we could hypothesize that FOXP1 might exert the function of Bcl2 regulation through NF- $\kappa$ B pathway in nodal DLBCL. However, further studies are needed to test this hypothesis.

The prognostic significance of FOXP1 expression in DLBCL was controversial. Some studies reported that FOXP1 expression did not predict OS and event-free survival (EFS) in either GCB or non-GCB groups [11]. Meanwhile, some group demonstrated that the median OS was significantly inferior in patients with strong FOXP1 expression compared to those with negative and variable expression, and they elucidated that this apparent discrepancy from previously published data might be attributed to different criteria in defining antigen expression and the poor prognostic effect was confined to patients with uniform high expression which was lost when a 30% cutoff was used [18]. However, another study in which a 30% cutoff was used showed that FOXP1 expression had prognostic significance in patients with de novo DLBCL [17]. Since the same cutoff of 30% nuclear positivity was employed in the two studies undertaken by Hans et al. and Banham et al. [11, 17], the discrepancy in the predictive value of FOXP1 expression might not be caused by the different cutoff. Moreover, the studies discussed above were either confined to nodal DLBCL, or did not identify nodal or extranodal origin clearly. On this basis, we further investigated the potential value of FOXP1 for prediction of DLBCL, including both nodal and extranodal cases. In this study, we divided our cases into FOXP1 negative and positive groups when the Kaplan–Meier analysis was conducted, which was similar to the criteria of 30% cutoff. The most striking observation of this study was that FOXP1-positive patients had a significantly inferior overall survival compared with FOXP1-negative patients in the series of extranodal DLBCL. However, we did not find any correlation between FOXP1 expression and OS in nodal DLBCL patients, which was similar to the results obtained by Hans et al. When all of the cases were considered

together, the predictive value of FOXP1 was also not found. Sagaert et al. found that not only is FOXP1 a significant predictor of unfavorable clinical outcome in MALT lymphoma but also that FOXP1-positive MALT lymphomas, marked by a polymorphic histology and by trisomy 3 and 18, are at risk of transforming into aggressive DLBCLs. In addition, all the five cases with MALT lymphoma with evolution to a DLBCL in their study arose at extranodal sites, but they did not demonstrate directly that strong FOXP1 expression might predict for worse clinical outcome in extranodal DLBCL. Moreover, their number of series was too small (five cases) and all the cases were confined to those transformed by MALT lymphoma instead of de novo DLBCL [19]. Therefore, to the best of our knowledge, our findings were the first report to identify the prognostic significance of FOXP1 in patients with de novo DLBCL. If these findings can be confirmed by further studies in larger series of cases with longer follow-up, it might help explain why some prior studies found a survival difference but others did not, and we might propose that nodal and extranodal cases represent pathophysiologically distinct processes.

Moreover, it is unknown whether the predictive value of FOXP1 in extranodal DLBCLs occurred in different anatomic sites might be distinguished. Recently there are two reports on the prognostic value of FOXP1 expression in cutaneous large B-cell lymphoma. Hoefnagel et al. suggested that the expression of FOXP1 had no prognostic significance in primary cutaneous large B-cell lymphomas, leg type (LBCLLT) [30]. However, Kodama et al. demonstrated that FOXP1 expression was clearly linked to a worse prognosis when all primary cutaneous LBCLs, including LBCLLT, FCLDT (follicle center lymphoma, diffuse type), and LBCLO (LBCL, others), were analyzed together, although they also showed FOXP1 expression failed to reach a statistically significant prognostic value in LBCLLT [31]. In the present study, the predictive value of FOXP1 was more significant ( $p=0.013$ ) when the cases originated from stomach were excluded, probably because DLBCL occurred in stomach had better clinical behavior. As a result, although the dramatic impact of FOXP1 expression on the outcome of extranodal cases in our study was conspicuous and reached statistical significance, the lymphomas arose at different anatomic sites, which do not all share the same clinical behavior. Therefore, our data may require further confirmation by evaluating the FOXP1 expression in larger series of DLBCL based on single extranodal sites.

In summary, frequent expression of FOXP1 in both nodal and extranodal DLBCLs in this study implied that FOXP1 might play a crucial role in the development of DLBCL. The most striking finding of this study was that FOXP1 expression has an adverse effect on survival of the patients with extranodal DLBCL but not on those with nodal DLBCL, which indicated that FOXP1 function might

be mediated by different mechanisms in nodal and extranodal DLBCLs. However, the robustness of this finding needs to be confirmed by further studies with larger series. In addition, we confirmed the gene expression profiling results that FOXP1 might play a role in the pathogenesis of nodal non-GCB DLBCL through pathways in which Bcl2 was involved, and it might be a second important biomarker to identify non-GCB after MUM1. Additional work is required to define the accurate mechanisms underlying the high expression and the function of FOXP1 in DLBCL, especially in extranodal cases, and to identify whether FOXP1 expression may represent a potential new therapeutic target for a certain group of DLBCL.

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