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ORIGINAL ARTICLE

Loss of *PRDM1*/BLIMP-1 function contributes to poor prognosis of activated B-cell-like diffuse large B-cell lymphoma

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PRDM1/BLIMP-1, a master regulator of plasma-cell differentiation, is frequently inactivated in activated B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL) patients. Little is known about its genetic aberrations and relevant clinical implications. A large series of patients with *de novo* DLBCL was effectively evaluated for *PRDM1*/BLIMP-1 deletion, mutation, and protein expression. BLIMP-1 expression was frequently associated with the ABC phenotype and plasmablastic morphologic subtype of DLBCL, yet 63% of the ABC-DLBCL patients were negative for BLIMP-1 protein expression. In these patients, loss of BLIMP-1 was associated with Myc overexpression and decreased expression of p53 pathway molecules. In addition, homozygous *PRDM1* deletions and *PRDM1* mutations within exons 1 and 2, which encode for domains crucial for transcriptional repression, were found to show a poor prognostic impact in patients with ABC-DLBCL but not in those with germinal center B-cell-like DLBCL (GCB-DLBCL). Gene expression profiling revealed that loss of *PRDM1*/BLIMP-1 expression correlated with a decreased plasma-cell differentiation signature and upregulation of genes involved in B-cell receptor signaling and tumor-cell proliferation. In conclusion, these results provide novel clinical and biological insight into the tumor-suppressive role of *PRDM1*/BLIMP-1 in ABC-DLBCL patients and suggest that loss of *PRDM1*/BLIMP-1 function contributes to the overall poor prognosis of ABC-DLBCL patients.

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoid malignancy, accounting for 30–40% of all non-Hodgkin lymphoma.[1] Anthracycline-based combination chemotherapy, first introduced in the 1970 s, is the backbone of therapy for patients with DLBCL, and currently the standard therapy regimen includes the anti-CD20 antibody rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone.[2–4] Despite encouraging complete remission and overall survival (OS) rates using this regimen, up to one-third of DLBCL patients suffer from relapse or refractory primary disease.[5]

The heterogeneous clinical outcome of DLBCL results in part from variable genetic profiles of this tumor. Gene expression profiling (GEP) has identified two distinct types of DLBCL: germinal center B-cell-like and activated B-cell-like (GCB and ABC). Patients with ABC-DLBCL have markedly poorer survival than do patients with GCB-DLBCL.[6,7] However, the molecular mechanism responsible for this difference is not completely understood.

ABC-DLBCL is characterized by constitutive nuclear factor-κB (NF-κB) activation and genetic alterations that interfere with terminal B-cell differentiation.[8] *PRDM1*, located on chromosome 6q21, encodes for BLIMP-1, a zinc-finger-containing DNA-binding transcriptional repressor. BLIMP-1 expression is required for the development of immunoglobulin-secreting cells and maintenance of long-lived plasma cells.[9] Conditional *PRDM1*-knockout mice do not have production of plasma cells or serum immunoglobulins.[10] *PRDM1* is frequently inactivated in ABC-DLBCL cases as a result of genetic deletions or mutations or transcriptional repression of it.[11,12] Studies have demonstrated that an inactivation mutation of *PRDM1* is recurrent in ~25% of ABC-DLBCL cases.[11–14] Moreover, conditional deletion of *PRDM1* in murine B cells facilitates the development of lymphoproliferative disease resembling human ABC-DLBCL.[8,11]

Limited research has been carried out on the clinical implications of *PRDM1*/BLIMP-1 abnormality in DLBCL patients. In the present study, we investigated *PRDM1* deletion and mutation and

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BLIMP-1 protein expression in a large cohort of *de novo* DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone. Our results demonstrated that disruption of *PRDM1*/BLIMP-1 is associated with poor prognosis for ABC-DLBCL. We further characterized the potential molecular mechanisms underlying the tumor-suppressive function of *PRDM1*/BLIMP-1 using GEP.

MATERIALS AND METHODS

Patients

We studied 520 biopsy specimens obtained from rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone-treated patients with *de novo* DLBCL. The diagnostic criteria for DLBCL, patient-selection process, therapy and treatment responses were described previously.[15] The study was approved by the institutional review boards of the participating institutions.

Immunohistochemistry

Tissue microarrays prepared using formalin-fixed, paraffin-embedded tissue blocks of the diagnostic biopsy specimens obtained from the studied patients were stained with an anti-BLIMP-1 antibody (EPR16655; Epitomics, Burlingame, CA, USA). BLIMP-1 expression levels were determined by estimating the percentage of BLIMP-1$^+$ tumor cells in the tissue sections. Evaluation of other biomarkers was performed using immunohistochemistry with corresponding antibodies. Details of the immunohistochemical procedures and scoring processes were described previously.[15–19]

Fluorescence *in situ* hybridization, and *PRDM1* and *TP53* sequencing

Fluorescence *in situ* hybridization (FISH) probes spanning the *PRDM1* gene and chromosome 6 centromere were generated as described previously.[13] Dual-color FISH was performed using standard procedures. Briefly, 4-μm-thick tissue sections were baked overnight at 56 °C, deparaffinized in CitriSolv, treated with 0.2 N hydrochloric acid for 20 min at room temperature, pretreated with 1 N sodium thiocyanate at 80 °C for 30 min, protease-digested at 37 °C for 50 min, refixed in 10% buffered formalin for 10 min and derehydrated in a 70, 85 and 100% ethanol series before hybridization. The tissue sections were codenatured with the addition of a probe for 8 min at 74 °C and incubated for 24 h at 37 °C in a hybridization oven (ThermoBrite, Buffalo Grove, IL, USA). Posthybridization washes were performed in 0.4x standard saline citrate/0.3% NP-40 for 2 min and 2x standard saline citrate/0.1% NP-40 for 1 min at 72 °C. Slides were then mounted with 4,6-diamidino-2-phenylindole (0.5 g/ml) containing an antifade solution. For one slide containing 25 cases, 200 nuclei of cells were evaluated independently by two observers (AT and VM). The overall concordance of their evaluations was nearly perfect ($\kappa = 0.95$), thus all other slides (200 nuclei per case) were scored by AT only. The ratio of *PRDM1* signals (red) to CEP6 signals (green) was calculated. If this ratio was lower than 0.72, heterozygous *PRDM1* deletion was considered to be present. Ratios lower than 0.38 were considered to be suggestive of homozygous *PRDM1* deletions. These ratios were calculated as ratios below the mean plus three standard deviations of green to red signal ratios in reference cases (tonsils; $n = 6$) and subtraction of tumor-infiltrating T cells, which accounted for ~30% of undeleted alleles. *MYC*, *BCL2* and *BCL6* gene arrangements and copy-number aberrations were detected using FISH, as well.[16,20]

Sequencing of *PRDM1* coding regions was performed by Polymorphic DNA Technologies (Alameda, CA, USA) using Sanger sequencing. Sequencing results were compared with the NCBI NM_001198 reference sequence, followed by exclusion of all the single-nucleotide polymorphisms documented by the NCBI dbSNP database (build 147). The remaining variates detected by sequencing were considered as *PRDM1* somatic mutations. *TP53* was sequenced using a p53 AmpliChip (Roche Molecular Diagnostics, Pleasanton, CA, USA) as described previously.[21]

GEP and GCB/ABC classification

GEP was performed using a GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA, USA) with total RNAs as described previously.[15,21] The CEL files were deposited in the National Center for Biotechnology Information Gene Expression Omnibus repository (GSE no. 31312). GEP classified 407 cases as GCB/ABC subtypes, and the cell of origin of another 111 cases was determined and compared by immunohistochemical algorithms (Hans, Visco-Young and Choi).[15] Cell-of-origin classification based on B-cell-associated gene signatures has been described previously.[22]

MicroRNA profiling

An HTG EdgeSeq Whole Transcriptome Assay (HTG Molecular Diagnostics, Tucson, AZ, USA) coupled with a HiSeq system (Illumina, San Diego, CA, USA) was used for measuring the expression of microRNAs (miRNAs) in formalin-fixed, paraffin-embedded tissues (unpublished data). Selection of regulatory miRNAs for BLIMP-1 expression was based on TargetScan and published data.[23–27]

Statistical analysis

Clinical and molecular features of patient tumors were compared using the Fisher's exact or χ^2 test. OS and progression-free survival (PFS) in the study population were analyzed using the Kaplan–Meier method, and differences between DLBCL subgroups were compared using the log-rank test with the Prism 6 software program (GraphPad Software, San Diego, CA, USA). Multivariate analysis was performed using the Cox proportional hazards regression model with the SPSS software program (version 19.0; IBM Corporation, Armonk, NY, USA). *P*-values of up to 0.05 were considered statistically significant.

RESULTS

Patients

The clinical characteristics of the study patients with DLBCL ($n = 520$) are summarized in Table 1. Their median age was 65 years (range, 16–82 years), the median follow-up duration was 46.7 months (range, 20.0–186.7 months) and the male:female ratio was 1.2:1. Two hundred sixty-eight patients (52%) had GCB-DLBCL, and 250 patients (48%) had ABC-DLBCL.

Homozygous *PRDM1* deletion predicts poor prognosis for ABC-DLBCL

We detected homozygous deletion of *PRDM1* in 19 (7%) of 292 patients with available FISH results (Figure 1a and Supplementary Table 1). Patients with homozygous *PRDM1* deletions had lower BLIMP-1 protein expression than did those without these deletions ($P = 0.055$; Figure 1a). Homozygous *PRDM1* deletion was not associated with the ABC phenotype ($P = 0.34$) or any other clinical parameters. OS was significantly shorter in patients with homozygous *PRDM1* deletions than in those with normal or heterozygous deletions of *PRDM1* or monosomy 6 ($P = 0.037$) (Figure 1b). This difference was remarkable in patients with ABC-DLBCL ($P = 0.004$) but not in those with GCB-DLBCL ($P = 0.98$) (Figure 1b). Homozygous *PRDM1* deletion was also associated with significantly poorer PFS in the entire DLBCL cohort ($P = 0.0048$) and in ABC-DLBCL ($P = 0.036$) (Figure 1c). Multivariate analysis adjusting clinical features confirmed homozygous *PRDM1* deletion as an independent prognostic factor for both OS ($P = 0.032$) and PFS ($P = 0.037$). In the ABC-DLBCL patients, homozygous *PRDM1* deletion was an independent prognostic factor for OS ($P = 0.032$) but not for PFS ($P = 0.13$) (Table 2).

PRDM1 mutation predicts poor prognosis for ABC-DLBCL

We studied *PRDM1* mutations in 368 patients with available genomic DNA. Using a quality score cutoff of 16 (97% confidence), we identified that 94 patients (25.5%) had *PRDM1* mutations within the coding DNA sequence region of *PRDM1* (Supplementary Table 2). Of these, seven patients had truncating (nonsense) mutations and two had a frameshift mutation. Seven of these nine patients had ABC-DLBCL, including four patients

Table 1. Clinical features of DLBCL patients with BLIMP-1 expression, *PRDM1* mutation and homozygous deletion

Variables	*BLIMP-1 protein expression*			PRDM1 *mutation*			*Homozygous* PRDM1 *deletion*		
	Positive	*Negative*	P-*value*	*Positive*	*Negative*	P-*value*	*Positive*	*Negative*	P-*value*
No. of patients	132	388		94	274		19	273	
Cell-of-origin									
GCB	39 (30%)	229 (59%)	< **0.0001**	48 (51%)	141 (52%)	0.91	7 (37%)	138 (51%)	0.34
ABC	92 (70%)	158 (41%)		46 (49%)	131 (48%)		12 (63%)	134 (49%)	
Plasmablastic subtype									
No	55 (47%)	259 (94%)	< **0.0001**	25 (80%)	48 (87%)	0.41	9 (100%)	185 (87%)	0.27
Yes	29 (53%)	15 (6%)		6 (20%)	7 (13%)		0	25 (13%)	
Age (years)									
< 60	53 (40%)	166 (43%)	0.61	39 (41%)	117 (43%)	0.90	10 (53%)	114 (42%)	0.47
⩾ 60	79 (60%)	222 (57%)		55 (59%)	157 (57%)		9 (47%)	159 (58%)	
Gender									
Male	81 (61%)	224 (58%)	0.46	51 (54%)	162 (59%)	0.47	11 (58%)	143 (52%)	0.81
Female	51 (39%)	164 (42%)		43 (46%)	112 (41%)		8 (42%)	130 (48%)	
Stage									
I and II	60 (47%)	179 (47%)	0.96	36 (41%)	120 (45%)	0.54	11 (58%)	126 (48%)	0.48
III and IV	67 (53%)	198 (53%)		52 (59%)	147 (55%)		8 (42%)	135 (52%)	
B symptoms									
No	78 (61%)	244 (67%)	0.24	48 (55%)	176 (68%)	**0.039**	15 (79%)	165 (63%)	0.22
Yes	50 (39%)	122 (33%)		39 (45%)	84 (32%)		4 (21%)	98 (37%)	
LDH level									
Normal	49 (41%)	134 (38%)	0.58	30 (34%)	107 (43%)	0.17	8 (44%)	102 (40%)	0.80
Elevated	72 (59%)	222 (62%)		57 (66%)	140 (57%)		10 (56%)	156 (60%)	
No. of extranodal sites									
0–1	93 (74%)	287 (77%)	0.51	65 (72%)	200 (77%)	0.39	17 (94%)	197 (78%)	0.13
⩾ 2	33 (26%)	87 (23%)		25 (28%)	59 (23%)		1 (6%)	57 (22%)	
ECOG performance status									
0–1	99 (84%)	289 (83%)	0.83	61 (72%)	208 (86%)	**0.005**	16 (89%)	204 (83%)	0.75
⩾ 2	19 (16%)	59 (17%)		24 (28%)	34 (14%)		2 (11%)	43 (17%)	
Size of largest tumor (cm)									
< 5	61 (64%)	162 (56%)	0.19	38 (61%)	129 (59%)	0.88	10 (67%)	129 (54%)	0.43
⩾ 5	35 (36%)	128 (44%)		24 (39%)	88 (41%)		5 (33%)	108 (46%)	
IPI score									
0–2	77 (59%)	231 (62%)	0.59	50 (54%)	166 (63%)	0.14	13 (68%)	160 (61%)	0.51
3–5	53 (41%)	142 (38%)		42 (46%)	96 (37%)		6 (32%)	103 (39%)	
Therapy response									
CR	103 (78%)	286 (74%)	0.32	65 (69%)	206 (75%)	0.28	14 (74%)	214 (78%)	0.58
PR	17 (13%)	52 (13%)		18 (19%)	35 (13%)		4 (21%)	29 (11%)	
SD	6 (4.5%)	18 (5%)		4 (4%)	12 (4%)		1 (5%)	11 (4%)	
PD	6 (4.5%)	32 (8%)		7 (7%)	21 (8%)		0	19 (7%)	

Abbreviations: ABC, activated B-cell-like; CR, complete response; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-cell-like; IPI, International Prognostic Index; LDH, lactate dehydrogenase; PD, progressive disease; PR, partial response; SD, stable disease. Bold values are the significant *P* values or those showing trends of significance.

with short OS and PFS (Supplementary Table 3). *PRDM1* mutation was not associated with the ABC phenotype ($P = 0.91$) and did not impact survival in the overall cohort or in GCB-DLBCL patients in particular (Supplementary Figure 1). However, *PRDM1* mutation did correlate with poorer prognosis for ABC-DLBCL (Figure 2a). Moreover, ABC-DLBCL patients harboring *PRDM1* mutations within exon 1 or 2 had markedly shorter survival durations than did patients with mutations in exons 3–7 or wild-type *PRDM1* (Figures 2b and c). In comparison, the prognostic impact of exon 1 and 2 *PRDM1* mutations on GCB-DLBCL was minimal (Supplementary Figure 2). Consistently, we found that patients with exon 1 and 2 *PRDM1* mutations were more likely to have overexpression of both Myc and Bcl-2 than were patients with exon 3–7 mutations in the overall cohort ($P = 0.03$) and those with wild-type *PRDM1* in ABC-DLBCL ($P = 0.05$) but not in those with GCB-DLBCL ($P = 0.44$). Multivariate analysis of clinical parameters, Myc/Bcl-2 expression scores, and *PRDM1* mutations indicated that Myc/Bcl-2 coexpression (hazard ratio: 2.9, $P < 0.001$), but not *PRDM1* mutations (hazard ratio: 1.54, $P = 0.12$), was an independent prognostic factor in ABC-DLBCL.

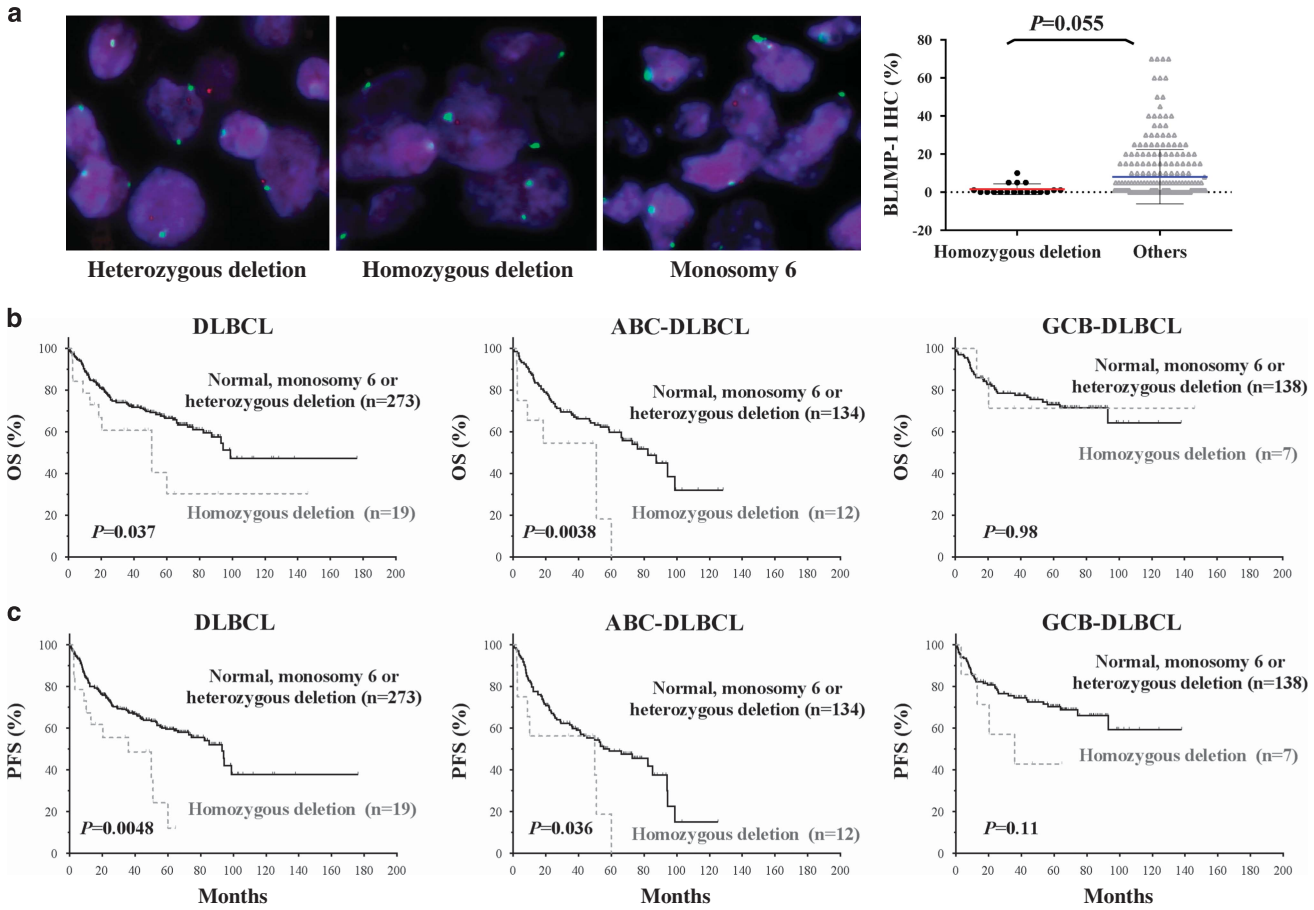

Figure 1. Homozygous *PRDM1* deletion in DLBCL cases. (**a**) Representative examples of FISH results with heterozygous *PRDM1* deletion (of note are a mixture of cells with two green signals, corresponding to centromere 6, but lacking red signals, corresponding to *PRDM1*, and cells with two green signals and one red signal), homozygous *PRDM1* deletion (of note are two green signals but a lack of red signals in the majority of the cells; one cell in the center of the microphotograph has both red and green signals and serves as an internal positive control) and monosomy 6 (all cells have only one green and one red signal). DLBCL patients with homozygous *PRDM1* deletions had lower levels of BLIMP-1 protein expression than did the rest of the studied patients. (**b** and **c**) The impact of homozygous *PRDM1* deletion on OS and PFS in all patients with DLBCL, patients with ABC-DLBCL and patients with GCB-DLBCL. Patients with this deletion had shorter OS and PFS durations than did patients with normal FISH signals, heterozygous *PRDM1* deletions or monosomy 6. This trend was greater in patients with ABC-DLBCL than in GCB-DLBCL.

Table 2. Multivariate analysis for homozygous *PRDM1* deletions in DLBCL and ABC-DLBCL

	DLBCL						*ABC-DLBCL*					
	Overall survival			*Progression-free survival*			*Overall survival*			*Progression-free survival*		
	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*
Female sex	0.60	0.89	0.58–1.37	0.69	0.92	0.62–1.37	0.63	1.15	0.66–1.99	0.71	1.10	0.67–1.82
B symptoms	**0.031**	1.62	1.04–2.50	0.072	1.45	0.97–2.19	0.26	1.37	0.79–2.37	0.21	1.39	0.83–2.32
IPI > 2	< **0.001**	3.02	1.96–4.64	< **0.001**	2.62	1.76–3.89	< **0.001**	2.67	1.57–4.53	**0.001**	2.29	1.40–3.75
Tumor size > 5 cm	0.18	1.33	0.87–2.02	0.56	1.12	0.64–1.66	0.26	1.35	0.80–2.26	0.60	1.14	0.70–1.84
PRDM1 homozygous deletion	**0.032**	2.23	1.07–4.65	**0.037**	2.09	1.05–4.18	**0.032**	2.58	1.08–6.15	**0.13**	1.95	0.83–4.59

Abbreviations: ABC, activated B-cell like; HR, hazard ratio; CI, confidence interval; DLBCL, diffuse large B-cell lymphoma; IPI, International Prognostic Index. Bold values are the significant *P* values or those showing trends of significance.

We also studied the correlations between various types of *PRDM1* mutations and BLIMP-1 protein expression, as well as *PRDM1* deletion. Patients with nonsense mutations or exon 1/2 mutations had lower BLIMP-1 expression than patients without these aberrations, but the *P*-values for the differences were not significant (Supplementary Figure 3). No significant associations were observed between *PRDM1* mutations and *PRDM1* allelic deletions.

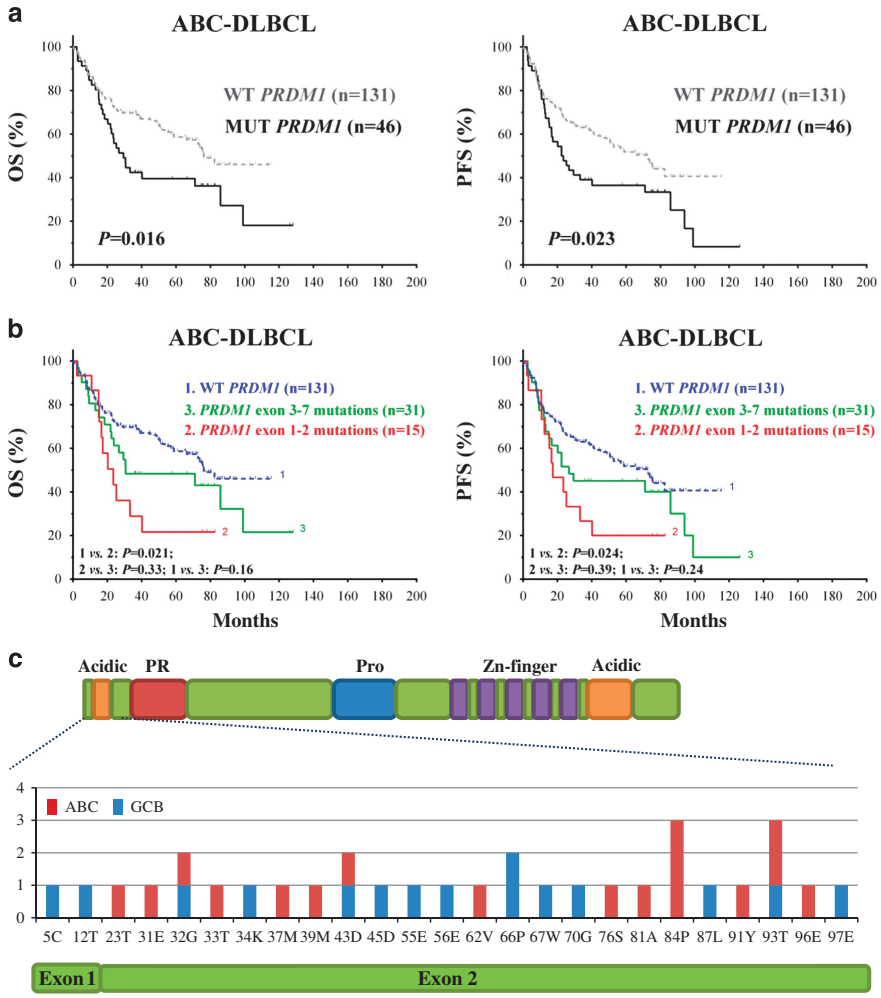

Figure 2. Correlation of *PRDM1* mutations with poor prognosis for DLBCL. (**a**) In ABC-DLBCL, patients with *PRDM1* mutations had shorter OS and PFS durations than did patients with wild-type *PRDM1*. (**b**) *PRDM1* mutations within exons 1 and 2 had even greater poor impact on OS and PFS compared with the overall mutation cohort. (**c**) The detailed distribution of *PRDM1* mutations within exons 1 and 2.

Loss of BLIMP-1 protein expression correlates with Myc overexpression and decreased p53 pathway molecule expression in ABC-DLBCLs

Representative immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections for BLIMP-1 protein expression is shown in Figure 3a. Using a cutoff of 10%, we found that 132 (25%) of the 520 patients were positive for BLIMP-1. BLIMP-1 protein expression was more common in patients with the ABC phenotype (37% *vs* 15% in GCB-DLBCL, $P < 0.0001$) and plasmablast subtype[22] of DLBCL than others ($P < 0.0001$; Table 1). The histogram of BLIMP-1 expression in the study cohort, and distribution of *PRDM1* deletions and mutations and BLIMP-1 protein expression in 180 patients with all data available is shown in Figure 3b. Ninety-six of these patients were in the ABC subgroup, 59 of whom (61%) had BLIMP-1$^-$ DLBCL. Thirty-seven (63%) of the BLIMP-1$^-$ABC-DLBCL cases had no detectable *PRDM1* deletions or mutations. MiRNA profiling identified that miR-30d-3p, miR-30d-5p and miR-30b-5p were significantly upregulated in BLIMP-1$^-$ patients without apparent *PRDM1* genetic aberrations, suggesting that epigenetic regulations may also have a role in loss of *PRDM1*/BLIMP-1 expression.

In the ABC subgroup, BLIMP-1 negativity was associated with reduced protein expression for the NF-κB pathway component p65 ($P = 0.028$), p53 ($P = 0.024$; only in subjects wild type for *P53*), p53 downstream targets MDM2 ($P = 0.002$) and p21 ($P = 0.074$), and phosphorylated AKT ($P = 0.0095$). BLIMP-1 negativity also was more common in patients without expression of CD30 ($P = 0.0082$) (Table 3). Of note, Myc expression was increased in BLIMP-1$^-$ cases, with a trend of significance ($P = 0.085$), and this negative correlation between BLIMP-1 and Myc overexpression in ABC-DLBCL was statistically significant when using a higher cutoff (⩾20%) for BLIMP-1$^+$ ($P = 0.0062$).

In the GCB subgroup, BLIMP-1 negativity was associated with *MYC* translocation ($P = 0.049$), mutated p53 overexpression, and decreased p21 expression ($P = 0.0028$) (Table 3). We found several other alterations associated with BLIMP-1 negativity in the overall DBLCL cohort, including *BCL2* translocation ($P = 0.004$), *TP53* mutation ($P = 0.005$), decreased expression of p63 ($P = 0.023$), nuclear p50 ($P = 0.023$) and IRF4/MUM1 ($P < 0.0001$), increased expression of GCET1 ($P = 0.039$) and CD10 ($P = 0.019$), and a lack of *BCL6* translocation. Bcl-6 expression tended to be upregulated in BLIMP-1$^-$ DLBCLs ($P = 0.092$), especially in patients with mutated *TP53* ($P = 0.075$).

We did not observe correlations between BLIMP-1 expression and survival in the study cohort (Supplementary Figure 4). However, BLIMP-1 negativity had an unfavorable impact on OS and PFS in DLBCL patients with *TP53* mutations, although the impact was not significant (OS, $P = 0.12$; PFS, $P = 0.074$) (Figure 3c).

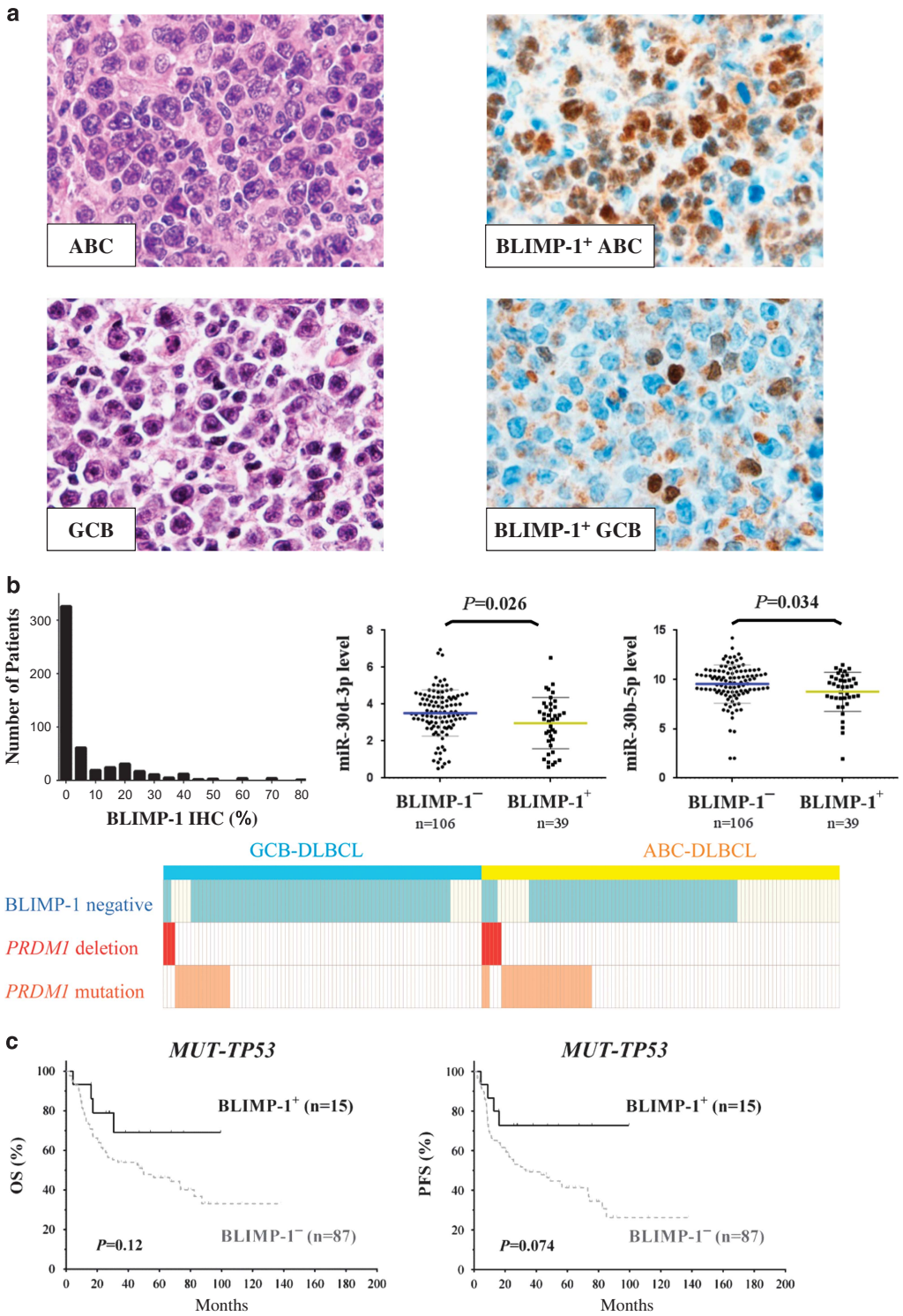

Figure 3. BLIMP-1 protein expression in DLBCL. (**a**) Representative immunohistochemical stains of DLBCL sections for BLIMP-1 protein expression. (**b**) The histograms of BLIMP-1 protein expression in all DLBCL patients, and the distribution of *PRDM1* deletions and mutations and BLIMP-1 protein expression in 180 patients with all these three data available. Expression of miR-30d-3p and miR-30b-5p was elevated in BLIMP-1$^-$ DLBCL patients without *PRDM1* genetic alterations. (**c**) The impact of BLIMP-1 protein expression on OS and PFS in DLBCL patients with mutated *TP*53.

Homozygous *PRDM1* deletion contributes to gene expression signatures of transcriptional activation

We identified 25 differentially expressed genes (DEGs) between homozygous *PRDM1* deletion-positive and -negative DLBCL cases (Figure 4a). Expression of all of the DEGs was upregulated. Subjects with homozygous *PRDM1* deletions had robust transcriptional activity with upregulation of transcription factors *STAT3*, *HLF*, *TCF19*, *TSHZ1* and *HMGA2*. Accordingly, analysis of the ABC

Table 3. BLIMP-1 protein expression and its correlation with pathologic and biological biomarkers in DLBCL

Variables	*Total*	*ABC-DLBCL*			*GCB-DLBCL*			*Overall DLBCL*		
		BLIMP-1$^+$	*BLIMP-1*$^-$	P-*value*	*BLIMP-1*$^+$	*BLIMP-1*$^-$	P-*value*	*BLIMP-1* $^+$	*BLIMP-1*$^-$	P-*value*
Patient no.	520	$n=92$	$n=158$		$n=39$	$n=229$		$n=132$	$n=388$	
ABC subtype	518	—	—	—	—	—	—	70% (92/131)	41% (158/387)	$<$ **0.0001**
Ki-67 $\geqslant$ 70%	516	76% (70/92)	65% (102/158)	**0.066**	56% (22/39)	60% (136/225)	0.72	71% (93/132)	62% (236/384)	**0.092**
BCL2 translocation	417	3.8% (3/80)	4% (5/127)	1.0	22% (8/36)	35% (60/173)	0.17	9% (11/116)	22% (65/301)	**0.0043**
Bcl-2 $\geqslant$ 70%	514	55% (50/91)	59% (93/157)	0.60	28% (11/39)	47% (96/225)	0.11	47% (61/131)	49% (189/383)	0.61
MYC translocation	332	9% (6/70)	7% (7/101)	0.77	3% (1/30)	18% (24/130)	**0.049**	7% (7/100)	13% (31/232)	0.13
Myc $\geqslant$ 70%	513	31% (28/91)	42% (66/158)	**0.085**	18% (7/39)	29% (65/223)	0.15	28% (36/131)	34% (131/382)	0.15
BCL6 translocation	362	44% (30/69)	39% (39/101)	0.53	40% (12/31)	23% (36/159)	0.06	42% (42/101)	29% (75/261)	**0.019**
Bcl-6 $\geqslant$ 50%	510	52% (47/90)	49% (77/157)	0.69	66% (26/39)	76% (171/224)	0.23	57% (73/129)	65% (248/381)	**0.092**
TP53 mutation	459	13% (10/80)	21% (30/142)	0.15	16% (6/38)	30% (59/198)	**0.077**	14% (16/118)	26% (89/341)	**0.005**
p53 $\geqslant$ 20%	450	39% (31/80)	33% (46/139)	0.46	26% (10/38)	38% (75/193)	0.20	35% (41/118)	36% (121/332)	0.82
Wild-type p53 $\geqslant$ 20%	348	36% (25/70)	20% (22/110)	**0.024**	28% (9/32)	26% (35/136)	0.82	33% (34/102)	23% (57/246)	**0.049**
Mutated p53 $\geqslant$ 20%	102	60% (6/10)	83% (24/29)	0.20	17% (1/6)	70% (40/57)	**0.017**	44% (7/16)	74% (64/86)	**0.02**
p63 $\geqslant$ 10%	499	51% (45/89)	41% (63/155)	0.15	53% (20/38)	38% (81/215)	0.10	51% (65/128)	39% (145/371)	**0.023**
MDM2 $>$ 10%	513	55% (50/91)	35% (24/156)	**0.0018**	46% (18/39)	34% (77/226)	0.15	52% (68/131)	34% (131/382)	**0.0004**
p21 $>$ 5%	450	40% (32/80)	28% (39/139)	**0.074**	42% (16/38)	19% (26/193)	**0.0028**	41% (48/118)	23% (75/332)	**0.0003**
IRF4/MUM1 $>$ 30%	515	84% (76/91)	73% (115/158)	**0.054**	35% (14/39)	22% (51/227)	**0.062**	69% (90/130)	43% (166/385)	$<$ **0.0001**
GCET1 $\geqslant$ 50%	511	19% (17/90)	8% (13/155)	**0.025**	38% (15/39)	54% (122/226)	**0.084**	25% (32/129)	35% (133/382)	**0.039**
CD10 $>$ 30%	517	10% (9/92)	7% (11/158)	0.47	64% (25/39)	70% (159/228)	0.57	26% (34/131)	44% (170/386)	**0.0003**
Nuclear p65+	467	66% (55/84)	50% (75/149)	**0.028**	62% (21/34)	61% (121/198)	1.0	65% (77/119)	56% (196/348)	0.13
Nuclear p50 $\geqslant$ 20%	452	46% (36/79)	39% (55/141)	0.39	37% (13/35)	26% (50/196)	0.16	44% (50/115)	31% (105/337)	**0.023**
pAKT $\geqslant$ 30%	493	51% (45/88)	34% (51/151)	**0.0095**	51% (19/37)	37% (79/215)	0.1	51% (64/126)	35% (130/367)	**0.0003**
CD30+	516	23% (21/92)	10% (15/156)	**0.0082**	18% (7/39)	16% (37/227)	0.82	21% (28/132)	14% (52/384)	**0.05**

Abbreviations: ABC, activated B-cell-like; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell-like. Bold values are the significant *P* values or those showing trends of significance.

subgroup also identified increased transcriptional signatures (Table 4). In contrast, we found no DEGs for homozygous *PRDM1* deletion in the GCB subgroup.

BLIMP-1 expression signatures in the overall cohort resemble those in ABC-DLBCL

We compared GEP of BLIMP-1$^-$ and BLIMP-1$^+$ DLBCL cases in the overall DLBCL cohort, in wild-type and mutated *PRDM1* subsets (Figure 4a). DEGs were identified in the overall DLBCL and the subset with wild-type *PRDM1* (147 and 32 DEGs, respectively, with a false discovery rate (FDR) threshold of 0.01), but not in the subset with mutated *PRDM1*, suggesting loss of wild-type BLIMP-1 function (Table 4). Loss of BLIMP-1 expression in DLBCL was associated with downregulation of *PRDM1*, X-box binding protein 1 (*XBP1*), which encodes for a critical regulator of plasma differentiation,[28] and its downstream target genes involved in endoplasmic reticulum, protein synthesis and transportation.[29,30] Moreover, expression of genes related to immunoglobulin production (*ELL2*, *MGC29506/MZB1* and *ARID3A*), cell differentiation (*BATF*, *IRF4*, which transactivates *PRDM1* and represses *BCL6* expression[31]) and B/T cell receptor signaling inhibition (*LAX1*) was also downregulated in BLIMP-1$^-$ DLBCLs. In contrast, expression of *CD22*, *MS4A1*, which encodes for CD20, *BCL11A* (a B-cell proto-oncogene and cofactor with Bcl-6, upregulated during hematopoietic cell differentiation) and *BLK*, which is involved in B-cell receptor (BCR) signaling was markedly upregulated (1.42-, 1.54-, 1.67- and 1.2-fold, respectively).

DEGs were also identified in the ABC and GCB subgroups (38 DEGs in ABC with an FDR threshold of 0.05 (Table 4); 22 DEGs in GCB with an FDR threshold of 0.15; Supplementary Table 4). When analyzed in the wild-type and mutated *PRDM1* subsets separately, DEGs were only identified in ABC-DLBCL (but not GCB-DLBCL) with wild-type *PRDM1* (30 DEGs with a FDR threshold of 0.30; Figure 4a and Table 4). The spectrum of DEGs in the ABC subgroup was similar to that in the overall cohort, including downregulation of *PRDM1*, *XBP1*, *GHITM*, *DUSP4*, *TMEM59*, *APOBEC3B*, *FKBP11*, *DNAJC3*, *DNAJB9*, *MANF*, *SEC24A*, *VDR*, *TXNDC5*, *ITGAL* and *C12orf55*, as well as upregulation of *BCL11A*, *CD22* and *CLLU1* in BLIMP-1$^-$ ABC-DLBCL cases. *IGJ* was downregulated in BLIMP-1$^-$ ABC-DLBCL, whereas *CD37* antigen was upregulated. In comparison, the DEGs identified in the overall GCB-DLBCL (regardless of *PRDM1* mutation status) also included *PRDM1*, *BCL11A* and *DUSP4*, but were more likely to be involved in immune responses (*CTLA4*, *GBP5*, *C1orf38*, *HLA-F* and *SERPINB1*) and metabolism (*ACSL1*, *LPCAT2*, *GTPBP8*, *CTSA* and *METRNL*) (Supplementary Figure 5a).

Considering the potential prognostic impact of BLIMP-1 expression in DLBCL patients with mutated *TP53*, we additionally compared the BLIMP-1 expression signatures in patients with mutated *TP53* and in those wild type for *TP53*. Forty-three and 33 DEGs were identified, respectively, in these two subsets with an FDR threshold of 0.20 (Supplementary Figures 5b–c and Supplementary Table 4). Comparing these two BLIMP-1 GEP signatures, we found that *PRDM1*, *SSR1* and *HSP90B1* were common signatures. Unique BLIMP-1 expression signatures in cases wild type for *TP53* included *MS4A1/CD20*, *LAX1*, *IGJ*, *PRF1*, *DNAJB9*, *MOV10*, *LANCL2*, *CD2BP2* and *IRF1*. In the *TP53*-mutated subgroup, *STAT3* which is a BCL6 target gene was downregulated in BLIMP-1$^-$ cases.[32] Other unique signatures in DLBCL with mutated *TP53* included *CFLAR*, *RBPJ*, *CMAH*, *DNAJC13*, *DLEU2*, *VAMP5*, and many others.

PRDM1 mutations within exon 1/2, but not mutations in exons 3–7, showed gene expression signatures

We further compared GEP between mutated and wild-type *PRDM1* DLBCLs regardless of BLIMP-1 expression. In the overall DLBCL and GCB subset, 103 and 193 DEGs were identified with FDR thresholds of 0.10 and 0.05, respectively. No DEGs were identified in the ABC subset, however, probably because of heterogeneity. Similarly, GEP comparisons between cases with mutated *PRDM1* exon 1/2 and those with wild-type *PRDM1* only identified DEGs in

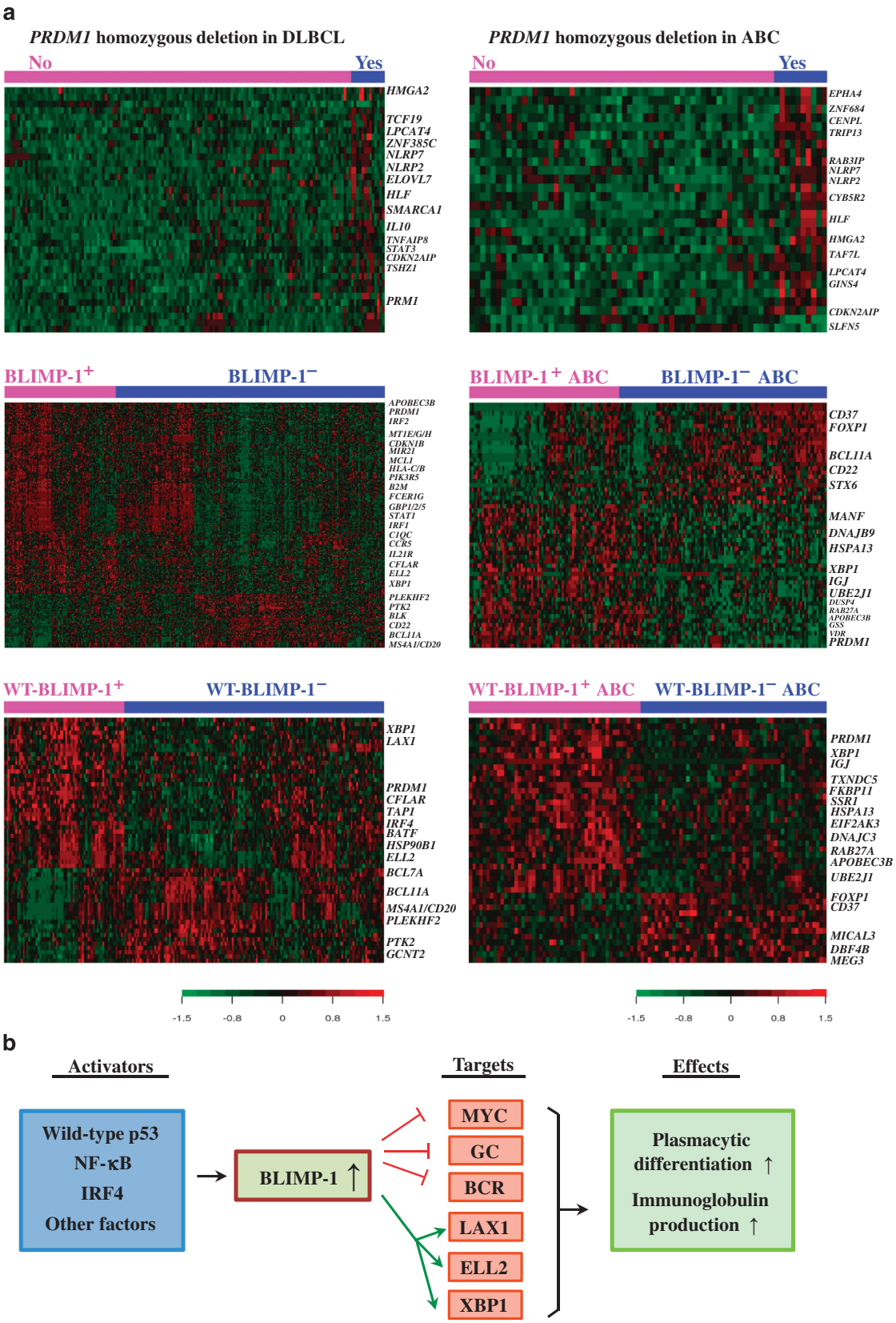

Figure 4. Gene expression profiles for the overall and ABC-DLBCL patients and the BLIMP-1 network in DLBCL. (**a**) Differential expression of genes between patients with and without homozygous *PRDM1* deletions, and between patients with and without BLIMP-1 protein expression. Differential expression of genes were only found between wild-type BLIMP-1$^+$ and BLIMP-1$^-$ DLBCLs but not between mutated BLIMP-1$^+$ and BLIMP-1$^-$ DLBCLs. (**b**) A brief network of BLIMP-1's functions and regulations summarizing our results. BLIMP-1 can be activated by p53, IRF4 and NF-κB signaling. BLIMP-1 represses the transcription of *MYC*, B-cell antigen/surface receptors and germinal center programs, whereas activates *LAX1*, which inhibits BCR signaling; therefore, leading to attenuated BCR signaling and decreased tumor cell proliferation. BLIMP-1 also transactivates *XBP1* and *ELL2*, which results in activation of plasmacytic differentiation and immunoglobulin production.

Table 4. Differentially expressed genes between homozygous *PRDM1* deletion-positive and -negative DLBCL and between BLIMP-1 protein-negative and -positive DLBCL

	Homozygous PRDM1 deletion-positive vs -negative DLBCL		*Overall BLIMP-1 protein-negative vs -positive DLBCL*		*Wild-type BLIMP-1 protein-negative vs -positive DLBCL*	
	Overall DLBCL	*ABC-DLBCL*	*Overall DLBCL*	*ABC-DLBCL*	*Overall DLBCL*	*ABC-DLBCL*
Function category	FDR < 0.1	FDR < 0.3	FDR < 0.01	FDR < 0.05	FDR < 0.01	FDR < 0.3
Cytokine, immune/inflammation related						
Up	*NLRP7, IL10*	*NLRP7*	*CNR2*	—	—	—
Down	—	—	*GZMB, SLAMF7, PRF1, IL2RB, GBP1, GZMA, APOBEC3B, GBP5, GBP2, FAM46C, STAT1, FCER1G, C1QC, IRF1, SRGN, C1orf38, RAB27A, IDO1, CST7, CCR5, IL21R, HLA-C, HLA-B, IRF2, NLRC5, B2M, ZBP1, MGC29506, ZC3HAV1, TAP1*	*IGJ, APOBEC3B, RAB27A*	*PRF1, FAM46C, LANCL2, TAP1*	*IGJ, APOBEC3B, RAB27A*
Differentiation						
Up	*DPPA4*	—	*BCL11A*	*BCL11A, FOXP1*	*BCL11A*	*FOXP1*
Down	—	*SLFN5*	*BATF*	—	*BATF*	—
B/T antigen/surface receptor and regulation						
Up	—	—	*MS4A1, CD22, GCNT2, BLK*	*CD22, CD37*	*MS4A1, GCNT2*	*CD37*
Down	—	—	*TARP /// TRGC2*	—	*LAX1*	—
Transcription factor, RNA processing, protein synthesis, folding, transportation and endoplasmic reticulum stress						
Up	*STAT3, HLF, TCF19, TSHZ1, HMGA2*	*HLF, ZNF684, TAF7L, HMGA2*	*ELL3 /// SERINC4, PLEKHF2, TEAD2, DNAJC10*	—	*ELL3 /// SERINC4, DNAJC10, PLEKHF2, SLC6A16*	*GOLGA8B*
Down	—	—	*PRDM1, ELL2, XBP1, HSP90B1, FKBP11, CRTC3, WARS, RUNX3, SUB1, XRN1, SSR4, DNAJC3, CTSB, VAMP5, NEAT1, SAR1B, DNAJB9, LAP3, ARID3A, LITAF, SEC11C, SSR1, SSR3, ARL4C, RALB, RNF149, MANF, HSPA13, C15orf24, SEC24A, RNF213, EDEM2, UFM1, LRPAP1, SELK*	*PRDM1, UBE2J1, FKBP11, ZBTB38, XBP1, HERPUD1, DNAJC3, SEC24A, HSPA13, ERLEC1, MANF, EIF2AK3, DNAJB9*	*PRDM1, ELL2, IRF4, FKBP11, HSP90B1, XBP1, SSR4, DNAJB9, TRAM2, MANF, DNAJC3*	*PRDM1, FKBP11, UBE2J1, UBE2J1, XBP1, MANF, ERLEC1, HSPA13, SSR1, HSPA5, DNAJB9, EIF2AK3, CD2BP2*
Cell cycle, DNA replication, chromatin structure, DNA damage response, proliferation and signaling						
Up	*NLRP2, CDKN2AIP, EPHA4, SMARCA1, C10orf2, PRM1*	*NLRP2, TRIP13, CDKN2AIP, CENPL, EPHA4, RAB3IP, GINS4*	*CLLU1, SLC6A16*	*CLLU1, GPR39, STX6*	*BCL7A*	*DBF4B*
Down	—	—	*DUSP4, MIR21, VDR, CDKN1B, SERPINA1, SPRY4, PIM2, GIMAP6, TMEM184B, PIK3R5, GRIN3A, CALM1*	*DUSP4, VDR, TRIB1*	—	*VDR*
Cell death and tumor suppression						
Up	*TNFAIP8*	—	*PAWR*	—	—	*MEG3*
Down	—	—	*TXNDC5, CFLAR, TMEM49, MLKL, MCL1, GADD45A, GHITM, ARMCX3*	*TXNDC5, GHITM*	*MLKL, TXNDC5, CFLAR*	*TXNDC5, TMEM59*
Metabolism, autophagy and oxidative stress						
Up	*CYB5R2, SLC2A13, ELOVL7, MOCOS, LPCAT4*	*CYB5R2, LPCAT4, SLC2A13, TGDS*	*RIMKLB, GYLTL1B, CCBL1, CALCA*	*ATP8B1, SLC39A9*	—	*ACSM1, SLC39A9*
Down	—	—	*CHST2, WIPI1, TMEM59, SOD2, TXN, GLUL, ACP2, P4HB, SLC39A8, GLA, SAT1, GALM, GSS, APOL6, GLRX, ACSL1*	*GSS, TMEM59*	—	—

Table 4. (Continued)

	Homozygous PRDM1 deletion-positive vs -negative DLBCL		*Overall BLIMP-1 protein-negative vs -positive DLBCL*		*Wild-type BLIMP-1 protein-negative vs -positive DLBCL*	
	Overall DLBCL	*ABC-DLBCL*	*Overall DLBCL*	*ABC-DLBCL*	*Overall DLBCL*	*ABC-DLBCL*
Adhesion, motility, cytoskeleton and extracellular matrix						
Up	*NRCAM, C14orf45*	—	*PCDHGA4, KANK1, DAAM1, PTK2, MICAL3*	—	*PTK2*	*MICAL3*
Down	—	—	*CADM1, MT2A, MT1H, MT1X, MT1G, MT1E, MT1E/H/M, IQGAP2, ITGAL, TMSB10, FNDC3A, C12orf55*	*ITGAL, C12orf55*	—	—
Unknown						
Up	*ZNF385C, PSORS1C2, LOC4orf45, FAM108C1, hCG_17324*	*ZNF385C, BTF3L4, MGC87042, FAM108C1, LOC401312*	*SYPL1, C8orf37, LOC151162 /// MGAT5*	*FAM129C, SNORA74A, TRIM34 /// TRIM6-TRIM34*	*SYPL1*	—
Down	—	—	*LOC541471 /// NCRNA00152, KIAA1618, NKG7, LOC283922, LOC100289053, KLHDC7B*	—	*C12orf55, C20orf196*	*LOC100129637, FAM122C, C16orf70*

Abbreviations: ABC, activated B-cell-like; DLBCL, diffuse large B-cell lymphoma; FDR, false discovery rate; GCB, germinal center B-cell-like; XBP1, X-box binding protein 1.

the overall DLBCL (Supplementary Figure 5d and Supplementary Table 5) and GCB (156 DEGs with an FDR threshold of 0.01) but not ABC subset. Interestingly, tumor suppressors *PTEN* and *IKZF1* (a transcriptional regulator of lymphocyte differentiation) were significantly downregulated in cases with mutated *PRDM1* exon 1/2. In contrast, no significant genes were found differentially expressed between cases with mutated *PRDM1* exons 3–7 and those with wild-type *PRDM1*.

DISCUSSION

In this study, we analyzed the clinical and experimental data on 520 *de novo* DLBCL cases to determine the tumor-suppressive function of *PRDM1*/BLIMP-1. Previous studies demonstrated that loss of *PRDM1*/BLIMP1 function is critical for the pathogenesis of ABC-DLBCL.[8,11] Herein, we provide evidence that loss of *PRDM1*/BLIMP1 function is a factor for poor prognosis in ABC-DLBCL. We found homozygous *PRDM1* deletions and *PRDM1* mutations within *PRDM1* exons 1 and 2 were poor prognostic factors in patients with ABC-DLBCL. Loss of BLIMP-1 protein expression was common in ABC-DLBCL and associated with a decreased plasma-cell differentiation signature and upregulation of B-cell antigens.

In univariate and multivariate analyses, we found that the prognosis for DLBCL with homozygous *PRDM1* deletion was worse than that for DLBCL without this deletion. However, this homozygous deletion only had prognostic significance in the ABC subgroup, as its impact on prognosis for GCB-DLBCLs was minimal. Although the number of patients harboring homozygous *PRDM1* deletions was small, our GEP still supported reduced transcriptional repression in patients with these deletions as suggested by upregulation of transcription factors, including *ZNF385C*, *HLF* and *HMGA2*. Notably, we only observed these gene expression signatures in the ABC group.

Similarly, *PRDM1* mutations only influenced survival of DLBCL in the ABC subgroup. Our data further support this by demonstrating that *PRDM1* mutations within exons 1 and 2 still significantly affect survival of ABC- but not GCB-DLBCLs. Therefore, we suggest that intact BLIMP-1 function is critical for the repression of ABC-DLBCLs. Exons 1 and 2 encode for the first 97 amino acids of BLIMP-1, including the N-terminal acidic domain. An intact N-terminal acidic domain is critical for the normal transcriptional repression function of BLIMP-1.[33] Researchers showed that BLIMP-1 lacking this domain do not repress the *MYC* promoter.[34] Interestingly, all but two of the *PRDM1* mutations identified in our cohort were missense mutations. Mandelbaum *et al.*[11] proved that a subset of missense mutations of *PRDM1* can directly impair BLIMP-1's protein stability as well as its transcriptional repression function, including 2 missense mutations at P48 (corresponding to 84P in this article due to different transcripts), the most frequently mutated site within exons 1 and 2 in our ABC-DLBCLs.[11] Although the decreased BLIMP-1 protein expression in patients with *PRDM1* mutations within exons 1 and 2 was not significant, we found that mutations in exons 1 and 2 were closely correlated with Myc and Bcl-2 co-expression, a previously identified marker for poor DLBCL prognosis that was overrepresented in the ABC subgroup.[18] GEP analysis results reinforced the notion that BLIMP-1 mutations especially those in exons 1-2 had lost functions of wild-type BLIMP-1.

At the protein level, although BLIMP-1 expression was frequently associated with the ABC phenotype of DLBCL, 63% of the ABC-DLBCL patients lacked BLIMP-1 protein expression. Over half of these BLIMP-1$^-$ ABC-DLBCL patients had no apparent genetic changes in *PRDM1*. *In vitro* studies have demonstrated that BLIMP-1 can be inactivated by constitutively active Bcl-6.[9,11] We found a negative correlation between Bcl-6 and BLIMP-1 expression with borderline significance. Also, expression of *BCL11A*, a cofactor with Bcl-6, was upregulated in our GEP analysis of both ABC and GCB subtypes of BLIMP-1$^-$ DLBCLs.[35]

Additionally, *PRDM1* can be epigenetically regulated by diverse miRNAs including miR-23, miR-9 and let-7a. [23–27] Using miRNA profiling, we found upregulation of miR-30d and miR-30b-5p in BLIMP-1$^-$ cases without genetic aberrations compared with BLIMP1$^+$ cases. Further functional evidence are needed to elucidate the regulation between these miRNAs and BLIMP-1 expression.

ABC-DLBCL patients without expression of BLIMP-1 had decreased expression of the tumor suppressor protein p53 and its downstream targets MDM2 and p21, as well as increased expression of Myc, which promotes cell proliferation.[36] Therefore, in BLIMP-1$^-$ DLBCL cases, decreased p53 signaling and Myc overexpression may synergistically promote tumor progression. In addition, loss of BLIMP-1 expression was associated with dysregulation of NF-κB pathway molecules. Induction of *PRDM1* mRNA expression in B-cell lymphoma cell lines can be blocked by NF-κB inhibitors.[37] Recently, Heise *et al.*[38] proved that NF-κB subunit p65 (RELA) is required for the induction of BLIMP-1 expression, which is consistent with the positive association of p65 expression and BLIMP-1$^+$ in our ABC DLBCL cohort.

Surprisingly, BLIMP-1 protein expression was not a survival predictor in the present study. Gyory *et al.*[33] found that the alternative splicing protein BLIMP-1β, which lacks the first three exons in the normal BLIMP-1 protein, does not have transcriptional repression activity. Consistently, detection of BLIMP-1β mRNA expression in tumor cells has been associated with poor prognosis for ABC-DLBCL.[39] Using our immunohistochemical assay, we could not discriminate between the normal BLIMP-1 protein and BLIMP-1β. The presence of BLIMP-1β in our cohort may reduce the prognostic impact of normal BLIMP-1 by diminishing its ability to repress the activity of downstream targets such as Myc and Bcl-6.

However, lack of BLIMP-1 expression seemed to be an unfavorable prognostic factor for DLBCL in patients with aberrant p53 statuses. Kusam *et al.*[40] reported that Bcl-6 can immortalize primary B cells and greatly increase B-cell function only in the absence of normal p53 function. BLIMP-1 is a known transcriptional repressor of *BCL6*.[41] We found that BLIMP-1 positivity was closely associated with the absence of Bcl-6 protein expression in our group of DLBCL patients with mutated *TP53*. Therefore, BLIMP-1 may inhibit the proliferation of tumor cells harboring mutated *TP53* by repressing Bcl-6 activity. Consistently, GEP analysis in DLBCL patients with mutated *TP53* found that expression of *IRF4* (an activator of BLIMP-1 and inhibitor of *BCL6*) and *STAT3* (a BCL6 target gene for repression),[31,32] was downregulated in the BLIMP-1$^-$ subgroup.

The BLIMP-1 negativity signature in the overall cohort of DLBCL patients was similar to that in the ABC subgroup: both were characterized by downregulation of plasmacytic differentiation and upregulation of B-cell antigens. BLIMP-1's role in promoting plasmacytic differentiation[27] of DLBCL was indicated by downregulation of *XBP1* along with several of its downstream targets involved in protein synthesis in endoplasmic reticulum and transportation in BLIMP-1$^-$ cases.[42] XBP1 is a transcriptional activator that acts downstream of BLIMP-1. BLIMP-1 and XBP1 are jointly required for the establishment of terminally differentiated plasma cells.[28] Moreover, we found that expression of genes involved in immunoglobulin production (*ELL2*, *MZB1* and *ARID3A*) and cell differentiation (*BATF*) was downregulated in the BLIMP-1$^-$ group. On the other hand, loss of BLIMP-1 expression in DLBCL correlated with upregulation of *CD22* and *BLK* and downregulation of *LAX1* that have important roles in regulating BCR signaling. Importantly, expression of *MS4A1* (encoding CD20) was also upregulated in BLIMP-1$^-$ cases, possibly indicating an increased therapeutic effect of rituximab in patients with BLIMP-1$^-$ DLBCL as suggested previously.[43,44] In line with the role of BLIMP-1 in plasmacytic differentiation suggested by GEP analysis, the cell-of-origin analysis based on B-cell-associated gene signatures[22] also showed positive correlation between BLIMP-1 expression and the plasmablastic subtype of DLBCL (Table 1).

The antiproliferative effect of BLIMP-1 on DLBCL was also suggested by the GEP results as cell-cycle suppressors (e.g., *CDKN1B*, *GADD45A*, *GHITM*, *DUSP4*) were upregulated in BLIMP-1$^+$ DLBCLs. Also, *in vitro* experiments have demonstrated that B-cell proliferation in DLBCL cell lines was promoted upon acute ablation of BLIMP-1 and that BLIMP-1 inactivation led to B-cell lymphomagenesis and shortening of life spans in mouse models of DLBCL.[8,11] Reintroduction of BLIMP-1 into a DLBCL cell line likely represses the cells' proliferation by turning off *MYC* and other genes involved in cell-cycle progression and DNA repair.[45] A brief network of BLIMP-1's functions and regulation generated from our results is presented in Figure 4b.

Collectively, the results of the present study demonstrated that loss of *PRDM1*/BLIMP-1 function contributed to the overall poor prognosis for ABC-DLBCL in three ways. First, genetic *PRDM1* aberrations (including homozygous deletions and mutations) affected the prognosis for ABC- but not GCB-DLBCLs. Second, a lack of BLIMP-1 expression correlated with an impaired p53 signaling pathway and Myc overexpression in ABC-DLBCLs. Third, the GEP signatures of BLIMP-1 expression for the overall DLBCL patient cohort resembled those for the ABC subgroup, both indicating downregulation of plasmacytic differentiation of DLBCLs and upregulation of B antigens and BCR signaling in BLIMP-1$^-$ tumors. These data may suggest that inactivated BLIMP-1 facilitates DLBCL progression through Myc and BCR signaling, which are essential for survival of ABC-DLBCL.[18,46] Therapeutic approaches that restore the normal function of BLIMP-1 may help drive terminal differentiation of tumor cells and overcome the chemoresistance of ABC-DLBCL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)