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Prognostic and biological significance of survivin expression in patients with diffuse large B-cell lymphoma treated with rituximab-CHOP therapy

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Survivin, a member of the inhibitor of apoptosis protein family, is overexpressed in a variety of human neoplasms. The prognostic significance of survivin expression in diffuse large B-cell lymphoma patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) is unclear. We used standard immunohistochemistry methods to quantify survivin expression in 463 patients with *de novo* diffuse large B-cell lymphoma who received the R-CHOP. Of the 463 patients, 269 (58%) had survivin overexpression with a cutoff of $>25\%$, associated with an International Prognostic Index score of >2 ($P=0.015$), disease in ≥ 2 extranodal sites ($P=0.011$), and a high Ki-67 index ($P<0.0001$). Among patients with activated B cell-like disease, the overall survival rate of survivin-positive patients was significantly lower than that of survivin-negative patients ($P=0.033$); multivariate analysis confirmed that in these patients, survivin overexpression was an independent prognostic factor for survival. Among patients with wild-type p53 overexpression, the overall survival and progression-free survival rates of the survivin-positive group were significantly lower than those of the survivin-negative group ($P=0.035$ and $P=0.04$ respectively). In STAT3-positive patients, survivin overexpression was associated with significantly better survival. Among patients with activated B cell-like disease, survivin-positive compared with survivin-negative groups had significantly different gene expression signatures, including genes involved in mitosis or tumor cell proliferation. Our results indicate that survivin is an

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independent prognostic factor for poor outcome in patients with activated B cell-like disease treated with the R-CHOP regimen, and patients with survivin-positive activated B cell-like diffuse large B-cell lymphoma seem to benefit less from this treatment and may require additional novel agents.
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Diffuse large B-cell lymphoma, the most common B-cell lymphoma, comprises ~31% of all lymphomas in Western countries and 37% of B-cell tumors worldwide.[1–3] In the 1970s, a subset of patients with diffuse large B-cell lymphoma was first cured with anthracycline-based combination chemotherapy regimens.[4] Subsequently, the cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) regimen became the standard treatment for diffuse large B-cell lymphoma patients because of its equivalent response and survival rates and low toxicity.[5] With the development of anti-CD20 monoclonal antibody rituximab, CHOP plus rituximab (R-CHOP) regimen replaced CHOP, based on the improved complete remission rate and event-free survival rate of R-CHOP, currently the standard treatment for diffuse large B-cell lymphoma.[6–9] However, despite many breakthroughs in the treatment of diffuse large B-cell lymphoma, up to one-third of diffuse large B-cell lymphoma patients still suffer from relapse or primary refractory disease.[10]

The variable clinical presentations of diffuse large B-cell lymphoma reflect the disease's molecular heterogeneity. Gene expression profiling studies have identified two molecularly distinct forms of diffuse large B-cell lymphoma: germinal center B cell-like diffuse large B-cell lymphoma and activated B cell-like diffuse large B-cell lymphoma.[11] Patients with germinal center B cell-like diffuse large B-cell lymphoma have significantly better overall survival than those with activated B cell-like diffuse large B-cell lymphoma.[11] A refined gene expression profiling-based classification based on B cell-associated gene signatures from the normal B-cell hierarchy[12] and presence of *MYC* and *BCL2* rearrangements by fluorescent *in situ* hybridization (FISH) are additional promising prognostic factors.[13,14] However, the clinical International Prognostic Index is still the gold standard in clinic for prognostic stratification of diffuse large B-cell lymphoma.[15]

Survivin, which is encoded by *BIRC5* (ref. 16) and a member of the inhibitor of apoptosis protein family, was identified in 1997.[17] Experimental evidence suggests that survivin is involved in multiple signaling pathways in tumors and might globally affect multiple signaling pathways and surpass the heterogeneity or complexity of tumors. Survivin lacks a caspase-associated recruiting domain that is critical for binding and inactivating caspases.[18] However, survivin inhibits caspase 9 by binding to and stabilizing the X-linked inhibitor of apoptosis,[19] and this inhibitory effect is negatively regulated by second mitochondria-derived activator of caspases.[20] Survivin also interacts with hepatitis B X-interacting protein that binds to caspase 9 and inhibits mitochondrial-driven apoptosis.[21]

In addition to its functions in apoptosis, survivin is also involved in cell division. Survivin is a chromosomal passenger protein[22] and interacts with other proteins in the chromosomal passenger complex, such as aurora kinase B, inner centromere protein, and borealin.[23] Signal transducer and activator of transcription 3 (STAT3) and p53 proteins regulate survivin expression.[24,25] Cyclin-dependent kinase1 and heat shock protein 90 participate in posttranslational modifications of survivin by regulating survivin's stability.[26,27]

Survivin is expressed during fetal development but is undetectable in normal terminally differentiated adult tissues. Previous studies have shown that survivin is highly expressed in most human solid cancers, such as carcinomas of lung, colon, rectum, breast, and bladder, and neuroblastoma,[17,28,29] and survivin expression is relevant to the prognosis or therapeutic response of these cancers.[28–32] Survivin is also expressed in hematological malignancies[33,34] and survivin expression has been identified as a prognostic factor in diffuse large B-cell lymphoma patients treated with the CHOP regimen.[35] However, in the R-CHOP era, studies on the prognostic significance of survivin in diffuse large B-cell lymphoma reported in the literature are conflicting.[36,37] In this study, we investigated survivin expression and its prognostic impact in 463 patients with *de novo* diffuse large B-cell lymphoma who were treated with the standard R-CHOP regimen.

Materials and methods

Patient Selection

We reviewed the cases of 463 patients with *de novo* diffuse large B-cell lymphoma who were diagnosed between 2000 and 2010 and treated with R-CHOP regimen. These cases were organized as a part of the International diffuse large B-cell lymphoma rituximab-CHOP Consortium Program study. All cases were diagnosed according to World Health Organization classification criteria and reviewed by a group of hematopathologists. Patients with transformation from low-grade B-cell lymphoma, primary cutaneous diffuse large B-cell lymphoma, primary central nervous system diffuse large B-cell lymphoma, or primary mediastinal large B-cell lymphoma were excluded, as were patients with AIDS/

HIV. Patients were classified as either germinal center B cell-like or activated B cell-like subtypes mainly according to gene expression profiling and/or by the immunohistochemical Visco–Young algorithm.[38] The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of each participating center. The overall collaboration was approved by the Institutional Review Board of the University of Texas MD Anderson Cancer Center in Houston, TX, USA.

Tissue Microarray and Immunohistochemistry Studies

Hematoxylin and eosin-stained slides from each of the 463 diffuse large B-cell lymphoma patients were reviewed. Tumor cell-rich areas were selected for tissue microarray analysis and prepared using a tissue microarrayer (Beecher Instruments). Immunohistochemistry studies using antibodies for survivin (EP2880Y, Epitomics), p53 (DO-7, DAKO), pSTAT3 (EP2147Y, Abcam), Myc (clone Y69, Epitomics), Bcl-2 (Clone-124, DAKO), Ki-67 (MIB-1, DAKO), Blimp-1 (EPR16655, Epitomics), pAKT (726E11, CST), CD10 (56C6, Vantana), Bcl-6 (PG-B6p, DAKO), MUM1 (MUM1P, DAKO), and FOXP1 (EPR4113, Abcam) were performed using a streptavidin-biotin complex technique on 4 μm tissue microarray sections in all cases.[38–44] A list of antibodies and methods used for immunostaining are summarized in Supplementary Table S1. The formalin-fixed, paraffin-embedded tissue slides underwent deparaffinization and heat-induced antigen retrieval techniques. An endogenous biotin-blocking kit (Ventana) was used to decrease background staining. Following antigen retrieval and primary antibody incubation, the reaction was completed in a Ventana ES instrument using a diaminobenzidine immunoperoxidase detection kit (Ventana). The immunohistochemical stains were scored with 5% increments independently by two pathologists (ZL and KHY), and disagreements were resolved by joint review at a multiheaded microscope. A cutoff value for each marker's overexpression was established from an analysis of receiver-operating characteristic (ROC) curves and/or X-Tile analysis as described previously to achieve maximum specificity and sensitivity. When an optimal cutoff could not be determined by ROC curve and X-Tile analyses, a conventional cutoff value for individual markers was decided on the basis of previous reports in the literature or mean values. The optimal cutoff thresholds were determined using X-Tile statistical software (http://www.tissuearray.org/rimmlab), based on the association of the biomarker with the optimal χ^2 value for overall survival. The distribution of specific biomarker IHC data could produce a cut point that corresponds to the highest χ^2 value by X-Tile. By using X-Tile, the optimal threshold could be independently determined for many biomarkers. However, no cutoff value could be obtained for rare biomarker, such as Ki-67 in X-Tile, because none of the cutoff value is associated with overall survival or progression-free survival. Thus, 70% threshold value was used for Ki-67. The cutoff value for survivin overexpression was defined as 25% positive tumor cells. The cutoff values for p53, pSTAT3, Ki-67, and cell-of-origin markers have been described previously: 20% for p53, 50% for pSTAT3, 30% for CD10, Bcl-6, and MUM1, 60% for FOXP1, and 70% for Ki-67 (Supplementary Table S1).[38,39,41]

Gene Expression Profiling

Total RNAs were extracted from formalin-fixed, paraffin-embedded tissue specimens of 425 patients. Gene expression profiling data sets were acquired using Affymetrix GeneChip Human Genome U133 Plus 2.0 as previously described.[38] The CEL files are deposited in the National Center for Biotechnology Information Gene Expression Omnibus repository (GSE#31312).[38] The parameters of percent, scaling factor, and background (average, min, max) were used to assess the quality of the arrays. During the quality control analysis, the samples with present calls $< 20\%$ were filtered from the data set, leaving 384 samples. The CEL data were normalized using robust multiarray analysis and patients were assigned a molecular subtype (germinal center B-cell type, activated B-cell type, or unclassifiable) according to the expression of classifier genes reported previously.[11,45,46] An analysis of differential expression gene expression was performed using *t*-tests between the contrasts of interest. The contrasts were visualized with heatmaps using significant probe sets.

Statistical Analysis

Different diffuse large B-cell lymphoma subgroups' clinical and laboratory features at the time of presentation were compared using the χ^2 test. The overall survival duration was calculated from the date of diagnosis to the date of last follow-up or death. The progression-free survival duration was calculated from the date of diagnosis to the time of progression or death. Different groups' overall survival and progression-free survival curves were analyzed by using the Kaplan–Meier method that was performed with GraphPad Prism 6 software. Between-group differences in overall survival and progression-free survival were compared using the log-rank (Mantel–Cox) test. Univariate and multivariate analyses for survival were performed using the Cox proportional hazards regression model; IBM SPSS statistics V22 was used to conduct the analyses. Unpaired *t*-tests were used to compare different groups' expressions of various protein and BIRC5 mRNA levels. All differences with *P*-values < 0.05 were considered to be statistically significant.

Results

Patient Characteristics

The main clinical characteristics of the patients in the study group are summarized in Table 1 according to survivin expression. The median age of patients in the study was 64 years (range, 16–92 years); 273 patients (59%) were >60 years old and 268 (58%) were men. A total of 227 patients (49%) were classified as germinal center B cell-like subtype and 233 patients (51%) were classified as activated B cell-like subtype.

Survivin Expression and Clinical Parameters

The immunohistochemical results (Figure 1a–j) and the frequency of survivin expression in the activated B cell-like diffuse large B-cell lymphoma and germinal center B cell-like diffuse large B-cell lymphoma subgroups are shown (Figure 1k–m). The staining pattern of survivin is predominantly nuclear. Equivocal cytoplasmic staining may be present in ~10% cases, but very weak staining precludes meaningful analysis. Survivin overexpression was demonstrated in 194 of 463 (42%) diffuse large B-cell lymphoma patients (Table 1). The *BIRC5* mRNA level of survivin-positive diffuse large B-cell lymphoma group was significantly higher than that of the survivin-negative diffuse large B-cell lymphoma group (Figure 1n), and similar results were found in the activated B cell-like diffuse large B-cell lymphoma and germinal center B cell-like diffuse large B-cell lymphoma subgroups (Figure 1o). The differential *BIRC5* mRNA expression between survivin-positive and -negative diffuse large B-cell lymphoma samples supported the cutoff value of survivin overexpression by immunohistochemistry. In addition, the difference in survivin expression between the germinal center B cell-like diffuse large B-cell lymphoma and activated B-cell-like diffuse large B-cell lymphoma subgroups was not significant ($P=0.19$; Figure 1p). Consistent with this result, the difference in *BIRC5* mRNA level ($P=0.24$) between the germinal center B cell-like diffuse large B-cell lymphoma and activated B cell-like diffuse large B-cell lymphoma subgroups was not significant (Figure 1q).

In diffuse large B-cell lymphoma, univariate analysis demonstrated a significant association between survivin overexpression and ≥2 extranodal disease sites ($P=0.011$), International Prognostic Index score >2 ($P=0.015$), high Ki-67 index ($P<0.0001$), and *TP53* mutation ($P=0.023$; Table 1). In activated B cell-like diffuse large B-cell lymphoma, survivin overexpression was associated with therapy response ($P=0.037$), high Ki-67 index ($P<0.0001$), and p53 overexpression ($P=0.0048$); in germinal center B cell-like diffuse large B-cell lymphoma, survivin overexpression was associated with ≥2 extranodal disease sites ($P=0.0026$), International Prognostic Index score >2 ($P=0.0392$), and high Ki-67 index ($P<0.0001$; Table 1).

Relationship of Survivin Expression and Other Tumor Markers

We compared the expression of a number of tumor markers in the survivin-positive *versus* -negative diffuse large B-cell lymphoma groups. We found significant differences between the survivin-negative and survivin-positive groups in the expression of p53 ($P=0.0024$), Myc ($P<0.0001$), B lymphocyte-induced maturation protein-1 (Blimp-1; $P=0.034$), Ki-67 ($P<0.0001$), phosphorylated protein kinase B (pAKT; $P=0.0038$), and B-cell lymphoma 2 (Bcl-2; $P=0.049$; Figure 2a–f). We found similar results for Myc, Ki-67, and pAKT expression after stratifying patients into activated B cell-like diffuse large B-cell lymphoma and germinal center B cell-like diffuse large B-cell lymphoma subgroups (Figure 2h, j, and k). p53 and Blimp-1 expression was significantly different only between survivin-positive and survivin-negative groups of activated B cell-like diffuse large B-cell lymphoma patients ($P=0.0006$, $P=0.021$; Figure 2g and i). In both activated B cell-like diffuse large B-cell lymphoma and germinal center B cell-like diffuse large B-cell lymphoma patients, the difference in Bcl-2 expression between the survivin-positive and survivin-negative groups was not significant ($P=0.10$, $P=0.32$; Figure 2l).

Prognostic Significance of Survivin Overexpression

The median follow-up time was 44 months. The overall survival and progression-free survival rates of patients with survivin-positive diffuse large B-cell lymphoma did not differ significantly from those of patients with survivin-negative diffuse large B-cell lymphoma ($P=0.08$, $P=0.35$; Figure 3a and b). Among germinal center B cell-like diffuse large B-cell lymphoma patients, there was no significant difference between the survivin-positive and survivin-negative groups ($P=0.69$, $P=0.74$; Figure 3c and d). However, among the activated B cell-like diffuse large B-cell lymphoma patients, the overall survival rate was significantly lower in patients with survivin-positive diffuse large B-cell lymphoma than it was in patients with survivin-negative diffuse large B-cell lymphoma ($P=0.033$; Figure 3e and f).

Furthermore, survivin overexpression affected overall survival in patients with stage III or IV disease ($P=0.03$) but not stage I or II disease (Supplementary Figure S1A–D). Among patients stratified according to other clinical characteristics, including International Prognostic Index score, lactate dehydrogenase level, size of largest tumor, and age, the overall survival and progression-free survival of patients with survivin-positive diffuse large B-cell lymphoma did not differ significantly from those of patients with survivin-negative

Table 1 Characteristics of the 463 patients with diffuse large B cell lymphoma, activated B cell-like diffuse large B-cell lymphoma, and germinal center B cell-like diffuse large B-cell lymphoma according to survivin expression

Characteristics	*DLBCL*			*ABC-DLBCL*			*GCB-DLBCL*		
	Survivin$^+$	*Survivin*$^-$	P-*value*	*Survivin*$^+$	*Survivin*$^-$	P-*value*	*Survivin*$^+$	*Survivin*$^-$	P-*value*
No. of patients	194	269		95	138		98	129	
Age, years									
< 60	78 (40.2%)	112 (41.6%)	0.76	29 (30.5%)	47 (34.1%)	0.67	48 (49.0%)	64 (49.6%)	1.00
≥ 60	116 (59.8%)	157 (58.4%)		66 (69.5%)	91 (65.9%)		50 (51.0%)	65 (50.4%)	
Gender									
F	80 (41.5%)	114 (42.4%)	0.84	40 (42.1%)	56 (40.6%)	0.89	40 (40.8%)	57 (44.2%)	0.68
M	113 (58.5%)	155 (57.6%)		55 (57.9%)	82 (59.4%)		58 (59.2%)	72 (55.8%)	
Stage									
I–II	80 (43.2%)	130 (49.4%)	0.20	33 (35.9%)	57 (42.5%)	0.41	46 (50.0%)	72 (56.7%)	0.34
III–IV	105 (56.8%)	133 (50.6%)		59 (64.1%)	77 (57.5%)		46 (50.0%)	55 (43.3%)	
B-symptoms									
No	115 (63.5%)	164 (64.1%)	0.91	53 (58.2%)	78 (59.1%)	0.78	62 (69.7%)	84 (68.9%)	1.00
Yes	66 (36.5%)	92 (35.9%)		38 (41.8%)	54 (40.9%)		27 (30.3%)	38 (31.1%)	
LDH level									
Normal	66 (38.2%)	98 (39.4%)	0.80	31 (35.2%)	50 (39.4%)	0.57	35 (41.7%)	47 (39.2%)	0.77
Elevated	107 (61.8%)	151 (60.6%)		57 (64.8%)	77 (60.6%)		49 (58.3%)	73 (60.8%)	
Extranodal sites, no.									
0–1	129 (72.1%)	217 (82.2%)	**0.011**	68 (74.7%)	104 (77.0%)	0.64	61 (70.1%)	111 (87.4%)	**0.0026**
≥ 2	50 (27.9%)	47 (17.8%)		23 (25.3%)	31 (23.0%)		26 (29.9%)	16 (12.6%)	
ECOG performance status									
0–1	133 (80.6%)	211 (85.8%)	0.16	69 (80.2%)	106 (82.8%)	0.72	63 (80.8%)	103 (88.8%)	0.15
≥ 2	32 (19.4%)	35 (14.2%)		17 (19.8%)	22 (17.2%)		15 (19.2%)	13 (11.2%)	
Size of largest tumor									
< 5 cm	77 (51.7%)	116 (60.1%)	0.12	41 (52.6%)	58 (58.0%)	0.45	36 (50.7%)	57 (62.0%)	0.16
≥ 5 cm	72 (48.3%)	77 (39.9%)		37 (47.4%)	42 (42.0%)		35 (49.3%)	35 (38.0%)	
IPI score									
0–2	101 (54.9%)	175 (66.3%)	**0.015**	45 (48.9%)	79 (58.5%)	0.18	55 (60.4%)	94 (74.0%)	**0.0392**
3–5	83 (45.1%)	89 (33.7%)		47 (51.1%)	56 (41.5%)		36 (39.6%)	33 (26.0%)	
Therapy response									
CR	142 (73.2%)	213 (79.5%)	0.11	67 (70.5%)	114 (82.6%)	**0.037**	74 (75.5%)	97 (75.2%)	1.00
PR	27 (13.9%)	26 (9.7%)		18 (18.9%)	13 (9.4%)		9 (9.2%)	14 (10.9%)	
SD	9 (4.6%)	12 (4.5%)		5 (5.3%)	3 (2.2%)		4 (4.1%)	9 (7.0%)	
PD	16 (8.2%)	17 (6.3%)		5 (5.3%)	8 (5.8%)		11 (11.2%)	9 (7.0%)	
Ki-67 index									
< 70%	30 (15.5%)	130 (49.1%)	< **0.0001**	15 (15.8%)	59 (43.1%)	< **0.0001**	15 (15.3%)	71 (55.5%)	< **0.0001**
≥ 70%	164 (84.5%)	135 (50.9%)		80 (84.2%)	78 (56.9%)		83 (84.7%)	57 (44.5%)	
Cell-of-origin									
GCB	98 (50.8%)	129 (48.3%)	0.57						
ABC	95 (49.2%)	138 (51.7%)							
TP53 mutations									
No	129 (72.5%)	194 (81.9%)	**0.023**	68 (78.2%)	107 (87.0%)	0.09	61 (67.0%)	85 (75.9%)	0.21
Yes	49 (27.5%)	43 (18.1%)		19 (21.8%)	16 (13.0%)		30 (33.0%)	27 (24.1%)	
p53 overexpression									
< 20%	102 (57.6%)	160 (68.7%)	**0.018**	47 (54.0%)	90 (73.8%)	**0.0048**	55 (60.4%)	70 (63.1%)	0.77
≥ 20%	75 (42.4%)	73 (31.3%)		40 (46.0%)	32 (26.2%)		36 (39.6%)	41 (36.9%)	

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. If comparison of the groups is biostatistically significant, *P*-value is highlighted with bold.

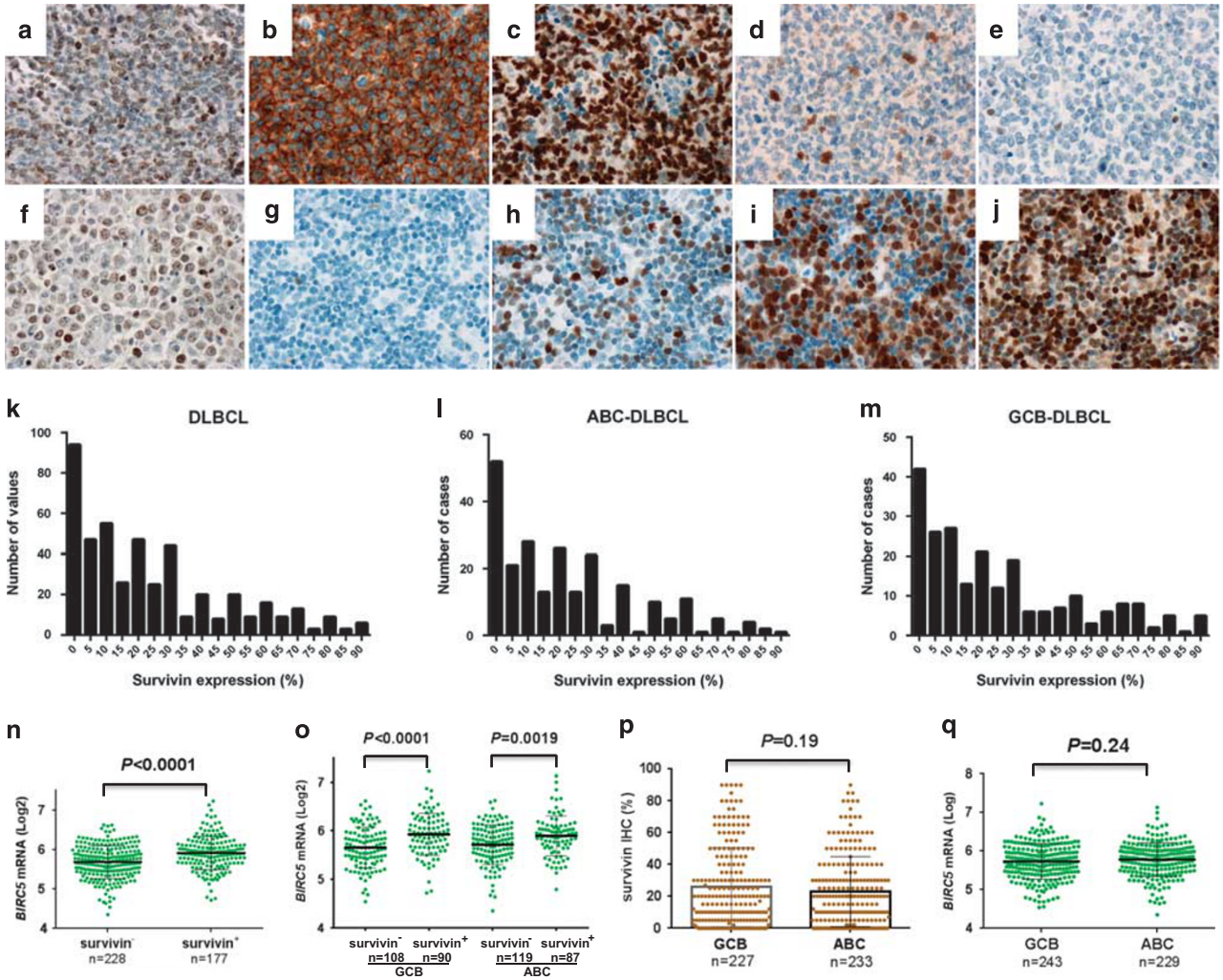

Figure 1 Representative examples of immunohistochemical staining in diffuse large B-cell lymphoma (magnification ×40). (**a–e**) Representative example of germinal center B cell-like diffuse large B-cell lymphoma positive for survivin (**a**), cluster of differentiation 10 (CD10; **b**), and B-cell lymphoma 6 (Bcl-6; **c**), and negative for multiple myeloma 1 (MUM1; **d**) and forkhead box P1 (FOXP1; **e**). (**f–j**) Representative example of active B cell-like diffuse large B-cell lymphoma positive for survivin (**f**), Bcl-6 (**h**), MUM1 (**i**), and FOXP1 (**j**), and negative for CD10 (**g**). Frequency distribution of survivin expression in diffuse large B-cell lymphoma (**k**), activated B cell-like diffuse large B-cell lymphoma (**l**), and germinal center B cell-like diffuse large B-cell lymphoma (**m**). (**n** and **o**) Relationship between *BIRC5* mRNA level and survivin expression in diffuse large B-cell lymphoma (**n**) and in germinal center B cell-like diffuse large B-cell lymphoma or activated B cell-like diffuse large B-cell lymphoma (**o**). (**p** and **q**) Expression of survivin (**p**) and *BIRC5* mRNA (**q**) in germinal center B cell-like diffuse large B-cell lymphoma and activated B cell-like diffuse large B-cell lymphoma.

diffuse large B-cell lymphoma (Supplementary Figure S1E–T).

There was a significant difference in *BIRC5* mRNA level between the wild-type p53 (*WT-p53*) and p53 mutant (*MUT-p53*) subgroup of diffuse large B-cell lymphoma ($P=0.0025$; Figure 4a). Survivin immunoexpression results between the *WT-p53* and *MUT-p53* subgroups of diffuse large B-cell lymphoma were consistent with the *BIRC5* mRNA results ($P=0.041$; Figure 4b). In the *WT-p53* subgroup of diffuse large B-cell lymphoma, survivin overexpression was associated with ≥2 extranodal disease sites ($P=0.0083$), International Prognostic Index score >2 ($P=0.041$), and high Ki-67 index ($P<0.0001$); survivin overexpression was associated with high Ki-67 index ($P<0.0001$) in the *MUT-p53* subgroup of diffuse large B-cell lymphoma (Table 2).

In diffuse large B-cell lymphoma patients with *WT-p53*, the overall survival but not progression-free survival of the survivin-positive group was significantly lower than that of the survivin-negative group ($P=0.048$, $P=0.15$; Figure 4c and Supplementary Figure S2A). In diffuse large B-cell lymphoma patients with *MUT-p53*, the overall survival and progression-free survival of the survivin-positive and survivin-negative groups did not differ significantly ($P=0.98$, $P=0.82$; Figure 4d and Supplementary Figure S2B). Of the 463 patients in the study, 82 (18%) had WT-p53 overexpression. In this patients subset, WT-p53/survivin-positive diffuse large

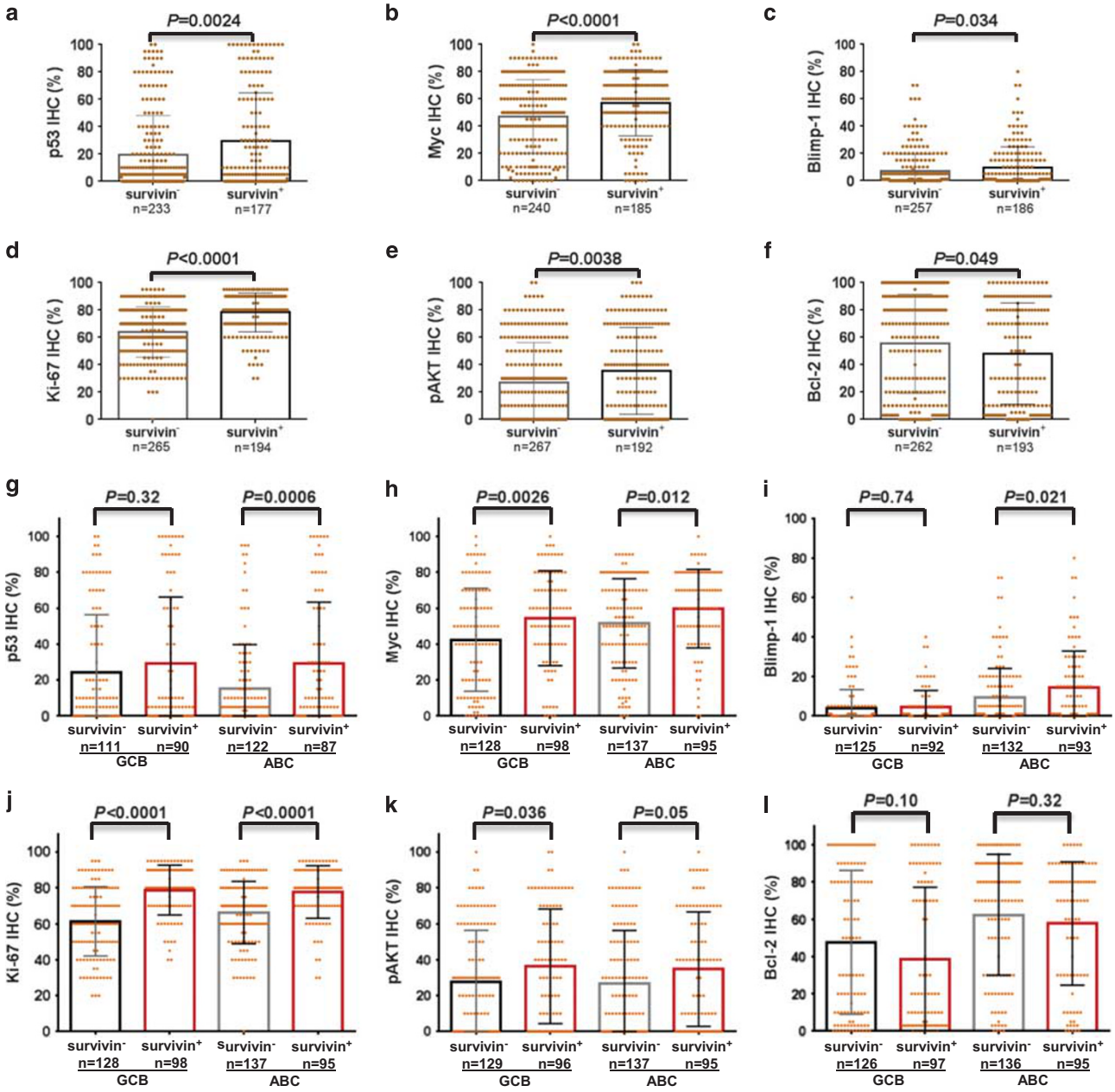

Figure 2 The relationship between survivin expression and the expression of p53 (**a**), Myc (**b**), B lymphocyte-induced maturation protein-1 (Blimp-1; **c**), Ki-67 (**d**), phosphorylated protein kinase B (pAKT; **e**), and B-cell lymphoma 2 (Bcl-2; **f**) in diffuse large B-cell lymphoma. (**g–l**) The relationship between survivin expression and the expression of p53 (**g**), Myc (**h**), Blimp-1 (**i**), Ki-67 (**j**), pAKT (**k**), and Bcl-2 (**l**) in germinal center B cell-like diffuse large B-cell lymphoma and activated B cell-like diffuse large B-cell lymphoma.

B-cell lymphoma was associated with inferior overall survival ($P=0.04$) and progression-free survival ($P=0.035$) compared with WT-p53-positive survivin-negative diffuse large B-cell lymphoma (Figure 4e and Supplementary Figure S2C).

In the STAT3-positive diffuse large B-cell lymphoma subgroup, survivin overexpression was associated with B symptoms ($P=0.036$) and high Ki-67 index ($P=0.03$); in the STAT3-negative subgroup, survivin overexpression was associated with International Prognostic Index score >2 ($P=0.016$) and high Ki-67 index ($P<0.0001$; Table 3). Interestingly, in the 64 patients with STAT3-positive diffuse large B-cell lymphoma (14%), the coexpression of STAT3 and survivin was associated with significantly better overall survival ($P=0.015$) and progression-free survival ($P=0.019$; Figure 5a and b). We found no significant prognostic effect of survivin overexpression in the WT-p53-negative ($P=0.4$ and $P=0.94$ for overall- and progression-free survival respectively; Figure 4f and Supplementary Figure S2D) or STAT3-negative diffuse large B-cell lymphoma subgroups

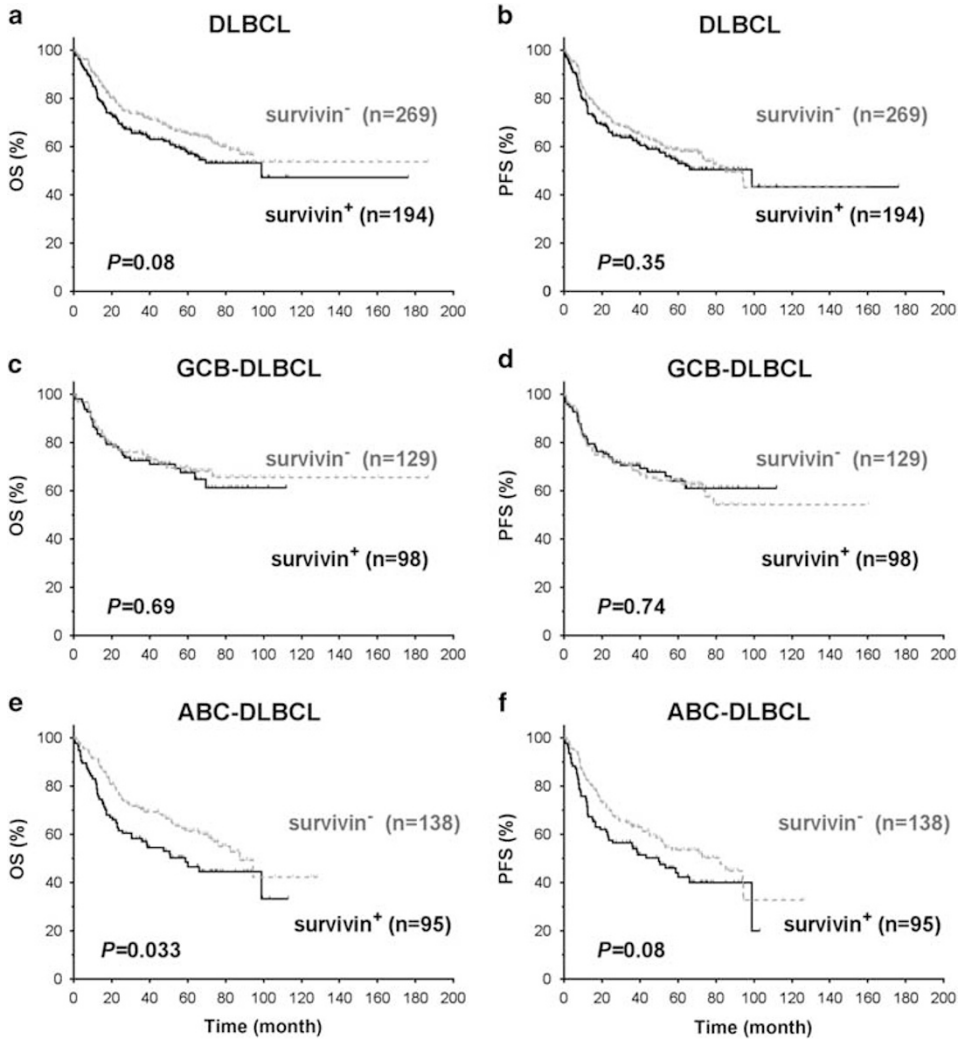

Figure 3 Impact of survivin expression on the overall survival and progression-free survival of patients with diffuse large B-cell lymphoma (**a** and **b**), patients with germinal center B cell-like diffuse large B-cell lymphoma (**c** and **d**), and patients with activated B cell-like diffuse large B-cell lymphoma (**e** and **f**).

(marginal $P = 0.068$ for overall survival and $P = 0.28$ for progression-free survival; Figure 5c and d).

Multivariate analysis confirmed the prognostic significance of International Prognostic Index score and *TP53* mutation in diffuse large B-cell lymphoma (Table 4) and showed that survivin overexpression is an independent prognostic factor for the survival of patients with activated B cell-like diffuse large B-cell lymphoma ($P = 0.026$, $P = 0.033$; Table 4).

Differential Gene Expression

To clarify the molecular basis underlying the aggressive clinical course of survivin-positive activated B cell-like diffuse large B-cell lymphoma, we compared the gene expression profiling results of the survivin-positive activated B cell-like diffuse large B-cell lymphoma patients with those of the survivin-negative activated B cell-like diffuse large B-cell lymphoma patients. A total of 86 genes were differentially expressed; 61 genes were upregulated in survivin-positive group and 25 genes were upregulated in the survivin-negative group (FDR = 0.1; Figure 6a and Supplementary Table S2). Of the 61 upregulated genes in the survivin-positive group, 18 (30%) were involved in DNA replication and repair, mitosis, cell cycle regulation, and/or proliferation. In contrast, only one upregulated gene in the survivin-negative group was involved in these processes (Table 5). In addition, among the upregulated genes in the survivin-positive group, nine were involved in metabolism and nine were involved in transcription and translation regulation. Interestingly, *TRIM35*, which may play a role as a tumor suppressor, was upregulated in the survivin-positive activated B cell-like diffuse large B-cell lymphoma group (Table 5).

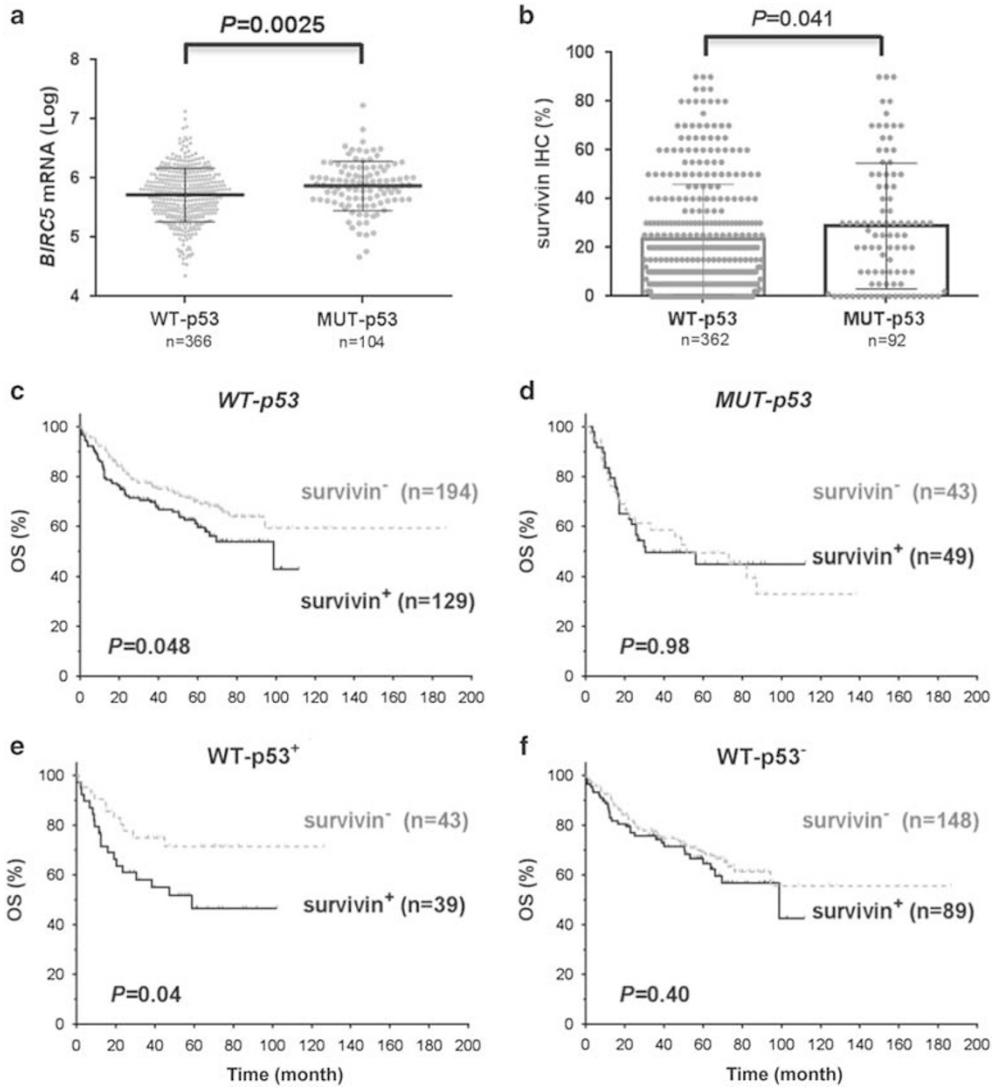

Figure 4 *BIRC5* mRNA and survivin expression in wild-type p53 (WT-p53) and p53 mutant (MUT-p53) groups and impact of survivin expression on overall survival and progression-free survival in diffuse large B-cell lymphoma patients with different p53 expression statuses. The expression of *BIRC5* mRNA and survivin in the WT-p53 subgroup was significantly different from that in the MUT-p53 group (**a** and **b**). In patients with WT-p53, survivin overexpression was associated with worse overall survival (**c** and **d**). Among patients with MUT-p53, differences in overall survival and progression-free survival between the survivin-positive and survivin-negative groups were not significant (**e** and **f**).

We also compared the gene expression profiling results of subgroups of germinal center B cell-like diffuse large B-cell lymphoma patients, in whom survivin was not associated with poor prognosis. Interestingly, we found that the gene expression profiling of the germinal center B cell-like diffuse large B-cell lymphoma group was very similar to that of the activated B cell-like diffuse large B-cell lymphoma group (Figure 6b and Table 5). Between the survivin-positive germinal center B cell-like diffuse large B-cell lymphoma patients and survivin-negative germinal center B cell-like diffuse large B-cell lymphoma patients, 58 genes were differentially expressed; 49 (84%) were upregulated in the survivin-positive group and 9 (16%) were upregulated in the survivin-negative group (Supplementary Table S3). Six genes—*CEP55*, *RAD51*, *TOP2A*, *H2AFX*, *H2AFZ*, and *TAF5*—were upregulated in both the survivin-positive activated B cell-like diffuse large B-cell lymphoma and survivin-positive germinal center B cell-like diffuse large B-cell lymphoma groups (Table 5). As in the survivin-positive activated B cell-like diffuse large B-cell lymphoma group, most of the other genes upregulated in the survivin-positive germinal center B cell-like diffuse large B-cell lymphoma group were involved in mitosis, cell cycle regulation, gene transcription, translation, and/or metabolism

Table 2 Characteristics of diffuse large B-cell lymphoma patients with WT-p53 or MUT-p53

Characteristics	*WT-p53*			*MUT-p53*		
	Survivin$^+$	*Survivin*$^-$	P-*value*	*Survivin*$^+$	*Survivin*$^-$	P-*value*
No. of patients	129	194		49	43	
Age, years						
<60	48 (37.2%)	78 (40.2%)	0.64	21 (42.9%)	16 (37.2%)	0.67
≥ 60	81 (62.8%)	116 (59.8%)		28 (57.1%)	27 (62.8%)	
Gender						
F	52 (40.3%)	82 (42.3%)	0.73	21 (42.9%)	17 (39.5%)	0.83
M	77 (59.7%)	112 (57.7%)		28 (57.1%)	26 (60.5%)	
Stage						
I–II	54 (44.6%)	90 (47.9%)	0.64	19 (38.8%)	23 (53.5%)	0.21
III–IV	67 (55.4%)	98 (52.1%)		30 (61.2%)	20 (46.5%)	
B-symptoms						
No	75 (62.5%)	125 (67.6%)	0.39	33 (71.7%)	24 (60.0%)	0.26
Yes	45 (37.5%)	60 (32.4%)		13 (28.3%)	16 (40.0%)	
LDH level						
Normal	46 (40.0%)	74 (42.3%)	0.72	18 (40.9%)	14 (33.3%)	0.51
Elevated	69 (60.0%)	101 (57.7%)		26 (59.1%)	28 (66.7%)	
Extranodal sites, no.						
0–1	84 (71.8%)	160 (84.7%)	**0.0083**	35 (74.5%)	36 (83.7%)	0.31
≥ 2	33 (28.2%)	29 (15.3%)		12 (25.5%)	7 (16.3%)	
ECOG performance status						
0–1	88 (80.7%)	153 (87.4%)	0.13	38 (84.4%)	36 (92.3%)	0.33
≥ 2	21 (19.3%)	22 (12.6%)		7 (15.6%)	3 (7.7%)	
Size of largest tumor						
<5 cm	57 (55.9%)	89 (62.7%)	0.29	15 (38.5%)	21 (58.3%)	0.11
≥ 5 cm	45 (44.1%)	53 (37.3%)		24 (61.5%)	15 (41.7%)	
IPI score						
0–2	68 (55.7%)	128 (67.7%)	**0.041**	24 (51.1%)	28 (65.1%)	
3–5	54 (44.3%)	61 (32.3%)		23 (48.9%)	15 (34.9%)	
Therapy response						
CR	102 (79.1%)	163 (84.0%)	0.30	27 (55.1%)	27 (62.8%)	0.53
PR	15 (11.6%)	15 (7.7%)		10 (20.4%)	9 (20.9%)	
SD	5 (3.9%)	4 (2.1%)		4 (8.2%)	3 (7.0%)	
PD	7 (5.4%)	12 (6.2%)		8 (16.3%)	4 (9.3%)	
Ki-67 index						
$<70\%$	26 (20.2%)	95 (49.7%)	$<$ **0.0001**	2 (4.1%)	17 (40.5%)	$<$ **0.0001**
$\geq 70\%$	103 (79.8%)	96 (50.3%)		47 (95.9%)	25 (59.5%)	
Cell-of-origin						
GCB	61 (47.3%)	85 (44.3%)	0.65	30 (61.2%)	27 (62.8%)	1.00
ABC	68 (52.7%)	107 (55.7%)		19 (38.8%)	16 (37.2%)	
p53 overexpression						
$<20\%$	89 (69.5%)	148 (77.5%)	0.12	13 (26.5%)	12 (28.6%)	1.00
$\geq 20\%$	39 (30.5%)	43 (22.5%)		36 (73.5%)	30 (71.4%)	

Abbreviations: CR, complete response; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase; MUT-p53, p53 mutant; PD, progressive disease; PR, partial response; SD, stable disease; WT-p53, wild type p53. If comparison of the groups is biostatistically significant, *P*-value is highlighted with bold.

(Table 5). Comparing the survivin positivity gene expression profiling signatures of the germinal center B cell-like and activated B cell-like diffuse large B-cell lymphoma subgroups, the most distinctive feature was a group of eight genes, involved in growth factor, receptor, and signal transduction, upregulated in survivin-negative activated B cell-like diffuse large B-cell lymphoma.

Table 3 Characteristics of diffuse large B-cell lymphoma patients in the STAT3$^+$ or STAT3$^-$ subgroup

Characteristics	*STAT3*$^+$			*STAT3*$^-$		
	Survivin$^+$	*Survivin*$^-$	P-*value*	*Survivin*$^+$	*Survivin*$^-$	P-*value*
No. of patients	30	34		133	196	
Age, years						
<60	10 (33.3%)	14 (41.2%)	0.61	54 (40.6%)	83 (42.3%)	0.82
≥ 60	20 (66.7%)	20 (58.8%)		79 (59.4%)	113 (57.7%)	
Gender						
F	11 (36.7%)	11 (32.4%)	0.79	55 (41.4%)	87 (44.4%)	0.65
M	19 (63.3%)	23 (67.6%)		78 (58.6%)	109 (55.6%)	
Stage						
I–II	8 (27.6%)	14 (42.4%)	0.29	57 (45.2%)	95 (49.7%)	0.49
III–IV	21 (72.4%)	19 (57.6%)		69 (54.8%)	96 (50.3%)	
B-symptoms						
No	21 (75.0%)	15 (45.5%)	**0.036**	77 (61.6%)	122 (65.9%)	0.47
Yes	7 (25.0%)	18 (54.5%)		48 (38.4%)	63 (34.1%)	
LDH level						
Normal	12 (41.4%)	10 (33.3%)	0.60	43 (37.7%)	67 (36.8%)	0.90
Elevated	17 (58.6%)	20 (66.7%)		71 (62.3%)	115 (63.2%)	
Extranodal sites, no.						
0–1	16 (55.2%)	26 (76.5%)	0.11	93 (76.9%)	161 (84.3%)	0.10
≥ 2	13 (44.8%)	8 (23.5%)		28 (23.1%)	30 (15.7%)	
ECOG performance status						
0–1	26 (89.7%)	23 (76.7%)	0.30	85 (79.4%)	155 (87.1%)	0.10
≥ 2	3 (10.3%)	7 (23.3%)		22 (20.6%)	23 (12.9%)	
Size of largest tumor						
<5 cm	14 (56.0%)	13 (56.5%)	1.00	50 (49.0%)	90 (60.4%)	0.09
≥ 5 cm	11 (44.0%)	10 (43.5%)		52 (51.0%)	59 (39.6%)	
IPI score						
0–2	14 (48.3%)	15 (45.5%)	1.00	69 (55.6%)	133 (69.3%)	**0.016**
3–5	15 (51.7%)	18 (54.5%)		55 (44.4%)	59 (30.7%)	
Therapy response						
CR	25 (83.3%)	25 (73.5%)	0.38	99 (74.4%)	156 (79.6%)	0.28
PR	3 (10.0%)	3 (8.8%)		18 (13.5%)	20 (10.2%)	
SD	0 (0.0%)	2 (5.9%)		4 (3.0%)	8 (4.1%)	
PD	2 (6.7%)	4 (11.8%)		12 (9.0%)	12 (6.1%)	
Ki-67 index						
$<70\%$	5 (16.7%)	15 (44.1%)	**0.03**	21 (15.8%)	96 (49.2%)	$<$ **0.0001**
$\geq 70\%$	25 (83.3%)	19 (55.9%)		112 (84.2%)	99 (50.8%)	
Cell-of-origin						
GCB	11 (36.7%)	10 (29.4%)	0.60	70 (52.6%)	103 (52.6%)	1.00
ABC	19 (63.3%)	24 (70.6%)		63 (47.4%)	93 (47.4%)	
TP53 mutations						
No	22 (81.5%)	26 (83.9%)	1.00	85 (70.2%)	143 (80.3%)	0.05
Yes	5 (18.5%)	5 (16.1%)		36 (29.8%)	35 (19.7%)	
p53 overexpression						
$<20\%$	13 (48.1%)	20 (64.5%)	0.29	70 (58.3%)	123 (69.5%)	0.06
$\geq 20\%$	14 (51.9%)	11 (35.5%)		50 (41.7%)	54 (30.5%)	

Abbreviations: CR, complete response; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase; PD, progressive disease; PR, partial response; SD, stable disease. If comparison of the groups is biostatistically significant, *P*-value is highlighted with bold.

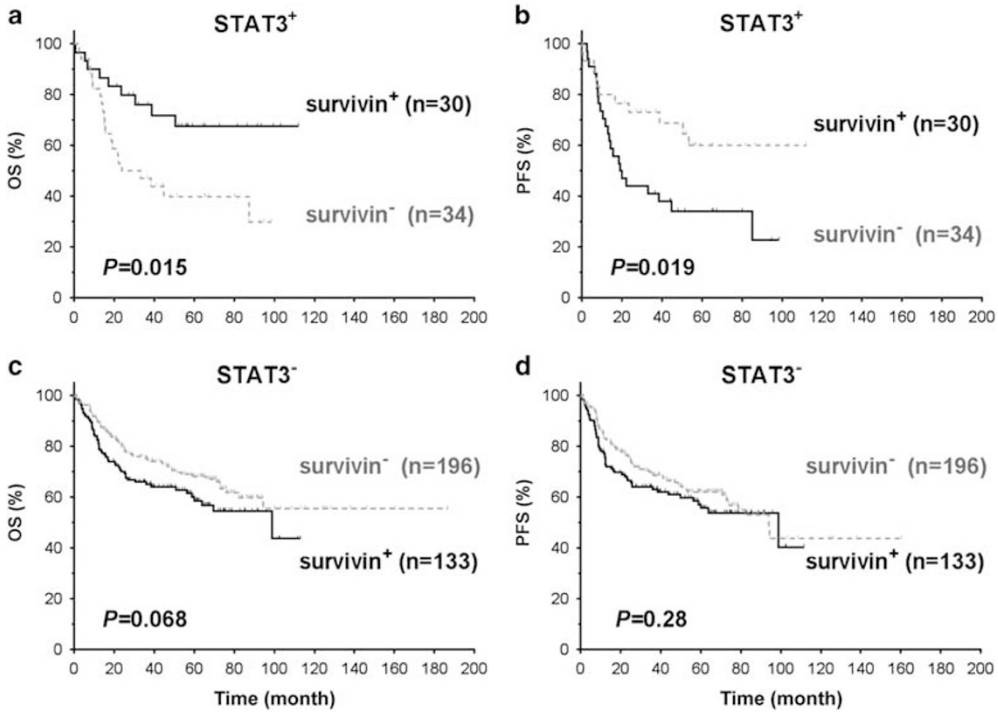

Figure 5 Impact of survivin expression on overall survival and progression-free survival in diffuse large B-cell lymphoma patients with or without signal transducer and activator of transcription 3 (STAT3) overexpression. In patients with STAT3 overexpression, survivin expression was associated with better overall survival and progression-free survival (**a** and **b**). Among patients without STAT3 expression, overall survival and progression-free survival did not differ significantly between the survivin-positive and survivin-negative groups (**c** and **d**).

Table 4 Multivariate survival analysis (International Prognostic Index, gender, tumor size, B-symptoms, TP53 mutation status, p53 overexpression, surviving overexpression) in diffuse large B-cell lymphoma patients or in subgroup of activated B cell-like diffuse large B-cell lymphoma patients

Variables	*Overall survival*			*Progression-free survival*		
	HR	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*
Overall DLBCL						
IPI >2	3.04	2.1–4.39	**0.0001**	2.56	1.81–3.62	**<0.0001**
TP53 mutations	1.76	1.18–2.61	**0.006**	1.67	1.13–2.45	**0.009**
p53 overexpression	1.22	0.81–1.86	0.57	1.12	0.75–1.66	0.58
Survivin overexpression	1.32	0.91–1.32	0.15	1.18	1.83–1.69	0.36
Gender	0.98	0.67–1.43	0.92	1.0	0.70–1.43	0.99
Tumor size >5 cm	1.36	0.80–1.86	0.10	1.81	0.73–1.78	0.17
B-symptoms	1.44	0.97–2.13	0.07	1.40	0.96–2.02	0.08
ABC-DLBCL						
IPI >2	2.79	1.71–4.57	**<0.0001**	2.21	1.39–3.51	**0.0001**
TP53 mutations	2.11	1.10–4.06	**0.025**	1.86	1.05–3.32	**0.035**
p53 overexpression	1.17	0.69–1.97	0.57	1.10	0.67–1.81	0.71
Survivin overexpression	1.71	1.07–2.75	**0.026**	1.63	1.04–2.55	**0.033**
Gender	0.85	0.52–1.40	0.53	0.94	0.59–1.50	0.80
Tumor size >5 cm	1.27	0.80–2.03	0.31	1.14	0.73–1.78	0.56
B-symptoms	1.60	0.97–2.64	0.07	1.62	1.00–2.61	**0.05**

Abbreviations: CI, confidence interval; HR, hazard ratio; IPI, International Prognostic Index. If comparison of the groups is biostatistically significant, *P*-value is highlighted with bold.

Discussion

Earlier studies have shown that survivin expression is significantly associated with poor overall survival in diffuse large B-cell lymphoma patients treated with the CHOP chemotherapy regimen.[35,47,48] However, the significant improvement in diffuse large B-cell lymphoma patients' survival following

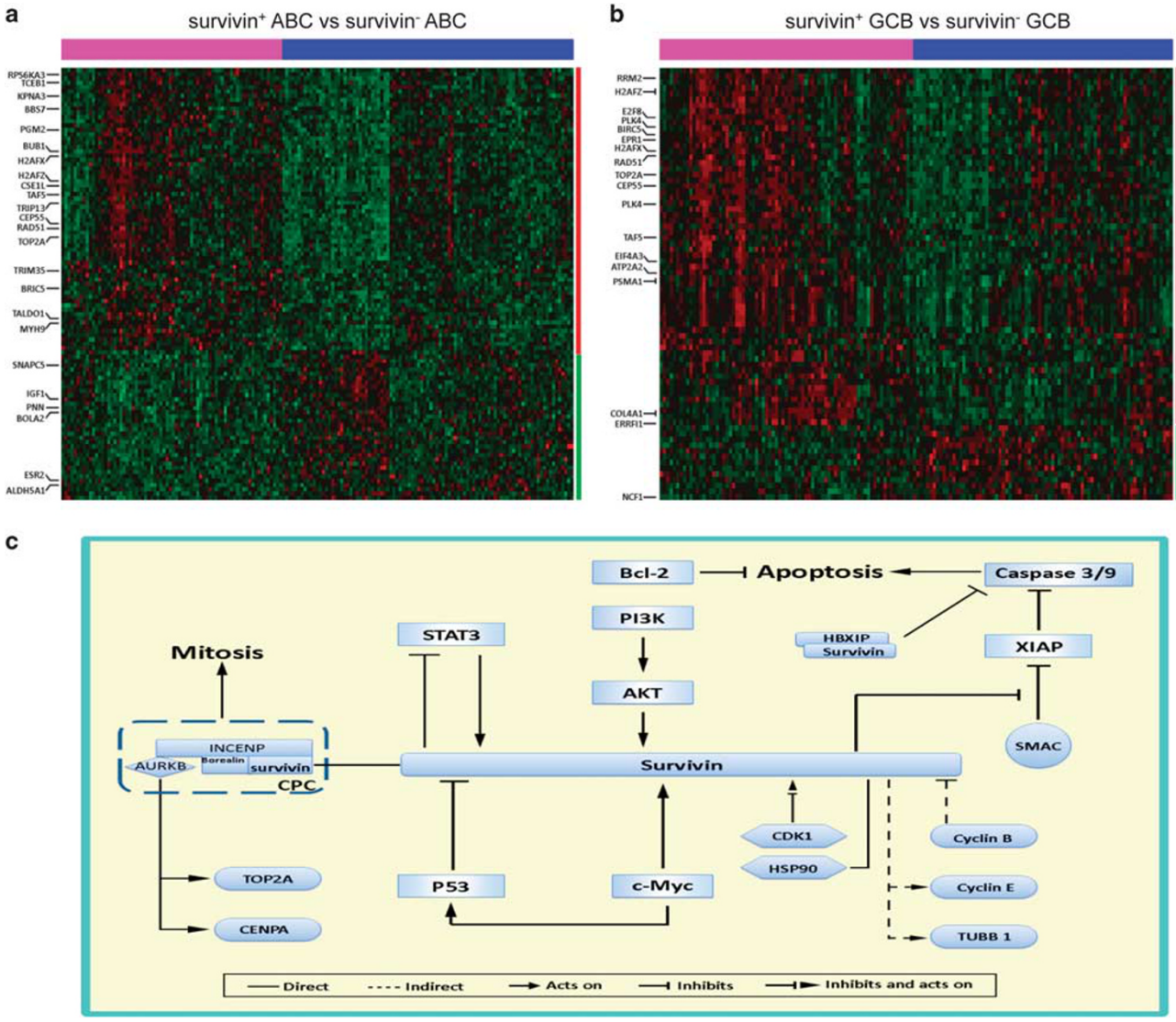

Figure 6 Gene expression profiles of diffuse large B-cell lymphoma subgroups and the network of survivin's functions. Gene expression profiles of survivin-positive and survivin-negative activated B cell-like diffuse large B-cell lymphoma (**a**). A total of 86 genes corresponding to 101 probe sets were differentially expressed between these groups (FDR = 0.1). Gene expression profiles of survivin-positive and survivin-negative germinal center B cell-like diffuse large B-cell lymphoma is shown in (**b**). A total of 58 genes corresponding to 75 probe sets were differentially expressed between these groups (FDR = 0.1). A brief network of survivin's functions generated using published data. AKT, protein kinase B; AURKB, aurora kinase B; Bcl-2, B-cell lymphoma 2; CDK1, cyclin-dependent kinase 1; CENPA, centromere protein A; CPC, chromosomal passenger complex; HBXIP, hepatitis B virus X-interacting protein; HSP90, heat shock protein 90; INCENP, inner centromere protein; PI3K, phosphoinositide 3-kinase; SMAC, small mitochondria-derived activator of caspase; STAT3, signal transducer and activator of transcription 3; TOP2A, topoisomerase IIα; TUBB1, tubulin β1 chain; XIAP, X-linked inhibitor of apoptosis protein (**c**).

the addition of rituximab to CHOP warranted a re-evaluation of such prognostic factors.[49,51,52] The results of studies investigating these factors have been conflicting. Mitrovic *et al*[37] reported that survivin had no prognostic significance in 57 diffuse large B-cell lymphoma patients treated with the R-CHOP regimen. In contrast, Markovic *et al*[36] demonstrated that survivin was an unfavorable factor for therapy response and associated with shorter survival time in 56 diffuse large B-cell lymphoma patients treated with R-CHOP. Given such uncertainty, we conducted the present international multicenter study in a large cohort of diffuse large B-cell lymphoma patients to clarify the prognostic significance of survivin in diffuse large B-cell lymphoma patients who have received R-CHOP. In this study, we found no significant difference in survivin expression between germinal center B cell-like diffuse large B-cell lymphoma and activated B cell-like diffuse large B-cell lymphoma patients, as Markovic *et al*[36] demonstrated, but we did find that survivin expression was associated with higher

Table 5 Differentially expressed genes in activated B cell-like diffuse large B-cell lymphoma group and germinal center B cell-like diffuse large B-cell lymphoma group

Function	*Survivin$^+$ vs survivin$^-$ activated B cell-like diffuse large B-cell lymphoma*		*Survivin$^+$ vs survivin$^-$ germinal center B cell-like diffuse large B-cell lymphoma*	
	Upregulated	*Downregulated*	*Upregulated*	*Downregulated*
DNA replication and repair, mitosis, cell cycle, and proliferation	*CEP55, CENPN, TOP2A, CCNA2, CCNB2, FAM54A, BUB1, CASC5, CSE1L, ECT2, HAUS2, NUSAP1, SKA2, SMC2, TTK, PSMC3IP, RAD51, DCTPP1*	*BOLA2*	*CEP55, RAD51, TOP2A, CENPE, CCNE2, FAM32A, CDC20, CDC6, FEN1, NDC80, PLK4, SPC24, UBE2C, MCM4, KIFC1, MKI67, NCAPG, TYMS, MND1*	
Gene expression, transcription, and translation regulation	*H2AFZ, H2AFX, TAF5, TCEB1, SYNCRIP, CSTF1, NUP160, HIC2, MRPL30*	*SNAPC5, C17orf42, HABP4*	*H2AFZ, H2AFX, TAF5, E2F8, EIF4A3, SMARCA4, SNRPG, TCF4, EXOSC3, SAP30, KEAP1, SNAI2*	*ZNF789, ZC3H6, ZMAT1*
Metabolism	*ATP11C, RPS6KA3, BCAT1, PPP1CB, PGM2, WDR67, FABP5, NSDHL, TALDO1*	*ALDH5A1*	*ESCO2, RRM2, ATP2A2, NDUFAB1, PGAP1*	*NCF1*
Actin, cytoskeleton, adhesion, extracellular matrix, migration	*TMOD3, TUBB, WDR1, MYH9, ELMO2, SNTB2*	*PNN, WASH1*	*TUBA1C, TUBA1B, LAMC1, COL4A1*	
Cytokine, growth factors, receptors, signal transduction	*PAK1IP1, TRIP13, PANK3, CNIH4, AZI2, KPNA3*	*SLC1A4, TSPAN16, KLK2, IGF1, EPHA8, DCAF8, ESR2, IRS2*	*ANXA2, EPR1, SLC20A1, ERRFI1*	
Protein trafficking and degradation	*BBS7*		*PSMA1, TMED9*	
Tumor suppressor, apoptosis	*BIRC5, TRIM35*		*BIRC5*	
Others	*LTV1, DEPDC1B, HBS1L; MAK16, FLII, SMCR8, HN1L, CXorf56, THAP6*	*AQP2, FLJ42875, TECTA, SLAMF9, CRLS1, GPATCH2, CD207, IGHA1; LOC340107, LOC100131088*	*C6orf125, CCDC90A*	*C1orf200, PMS2L2, PMS2L1, FLJ21369, TTC21A*

International Prognostic Index score, higher number of extranodal disease sites, and higher Ki-67 index. Survivin expression predicted shorter overall survival only in the activated B cell-like diffuse large B-cell lymphoma patients and was an independent prognostic factor for activated B cell-like diffuse large B-cell lymphoma.

The subcellular localization of survivin helps determine its function; cytoplasmic survivin is involved in apoptosis, whereas nuclear survivin is involved in cell division.[53] However, the findings of studies that have investigated the prognostic significance of survivin in these subcellular pools are inconsistent.[54] Some investigations have shown that nuclear survivin was associated with unfavorable prognosis, whereas other research has shown that cytoplasmic survivin was associated with unfavorable prognosis. In diffuse large B-cell lymphoma, Mitrovic *et al*[37] and Markovic *et al*[36] separately detected the expression of survivin in different subcellular pools. Although both groups found three staining patterns in diffuse large B-cell lymphoma, including cytoplasmic, nuclear, and mixed staining, neither group found a significant difference between any type of survivin expression and patient survival.[36,37] Variations reported from different studies may have resulted from low sensitive immunohistochemical method, low concentration of survivin in the cytoplasm, and different surviving antibodies used in the study. In this study, we only observed predominant nuclear staining pattern of survivin that correlated with poorer survival in activated B cell-like diffuse large B-cell lymphoma and in patients with wild-type p53 overexpression. However, ~10% patients may have very weak cytoplasmic staining but it is technically challenging to be certain and biological analysis based on equivocal cytoplasmic staining is less reliable.

To identify the mechanisms that may play a role in determining the difference in survival between the survivin-positive and survivin-negative activated B cell-like diffuse large B-cell lymphoma groups, we analyzed these two groups by gene expression profiling analysis. Among activated B cell-like diffuse large B-cell lymphoma patients, the gene expression profiling results of the survivin-positive group were significantly different from those of the survivin-negative group. Remarkably, the majority of differentially expressed genes in the survivin-positive group were related to mitosis, cell cycle, and/or metabolism. This gene expression signature may help explain why survivin-positive activated B cell-like diffuse large B-cell lymphoma patients have worse outcomes than survivin-negative activated B cell-like diffuse large B-cell lymphoma patients. Interestingly, we found that the survivin gene expression signature in germinal center B cell-like diffuse large B-cell lymphoma was similar to that in activated B cell-like diffuse large B-cell lymphoma. Although survivin has been identified as an apoptosis inhibitor, it also plays a role in cell division.[55–57]

One study demonstrated that the deletion of the survivin gene resulted in the absence of mitotic spindle, suggesting that survivin regulates chromosome segregation and cytokinesis.[58] In another study, an anti-survivin antibody application caused the premature separation of sister chromatids and the dysregulation of spindle-checkpoint activation.[57] Considering the function of survivin in mitosis, we presumed that the upregulation of genes involved in the cell cycle and in mitosis in survivin-positive activated B cell-like diffuse large B-cell lymphoma is due in part to survivin overexpression but not the cause of survivin overexpression, and underlied the worse survival of survivin-positive activated B cell-like diffuse large B-cell lymphoma patients.

Genes involved in metabolism were also identified in survivin-positive activated B cell-like diffuse large B-cell lymphoma. The transaldolase 1 gene *TALDO1* is a key enzyme of the pentose phosphate pathway, providing ribose-5-phosphate for nucleic acid synthesis and nicotinamide adenine dinucleotide phosphate for lipid biosynthesis.[59] The phosphoglucomutase gene *PGM2* catalyzes the conversion of the nucleoside breakdown products ribose-1-phosphate and deoxyribose-1-phosphate to the corresponding 5-phosphopentoses and may catalyze the interconversion of glucose-1-phosphate and glucose-6-phosphate.[60] The pentose phosphate pathway and glucose-6-phosphate both contribute to the Warburg effect in cancer cell metabolism.[61] However, whether the Warburg effect plays an etiological role in cancer remains unclear.[61,62] Therefore, we cannot conclude whether the genes involved in metabolism that were upregulated in survivin-positive activated B cell-like diffuse large B-cell lymphoma patients should be considered to be a result or the cause of survivin overexpression. Additional studies are needed to clarify the mechanisms underlying the difference in survival between survivin-positive and survivin-negative activated B cell-like diffuse large B-cell lymphoma patients.

We also found that survivin overexpression was associated with inferior overall survival and progression-free survival in patients with *WT-p53* overexpression. The relationship between survivin and *WT-p53* may account for this association. Human p53, which is encoded by the *TP53* gene, is a tumor suppressor that can block cell progression by regulating the cell cycle and/or inducing apoptosis. *WT-p53*, but not *MUT-p53*, represses survivin expression at both the mRNA and protein levels.[24] When a survivin expression plasmid is transfected into cells, the resulting survivin overexpression rescues the cells from p53-induced apoptosis, thereby enabling their proliferation and promoting tumor growth.[24]

The relationship between survivin and STAT3 is somewhat more complicated than that between survivin and p53. STAT3 directly binds to and regulates the survivin promoter,[63] and STAT3 activation induces survivin expression and rescues cells from apoptosis.[25,63] However, survivin can also bind to STAT3 dimers and repress STAT3 transactivation of target gene promoters.[64] Thus, clarifying the mechanism underlying the association of STAT3–survivin coexpression with better overall survival and progression-free survival is difficult.

Our study suggests that activated B cell-like diffuse large B-cell lymphoma patients derive less benefit from R-CHOP than germinal center B cell-like diffuse large B-cell lymphoma patients and therefore require additional effective treatments.[65,66] Agents targeting survivin may provide encouragement for such treatments. The small-molecule YM155, which has shown promising growth inhibitory effects and potent antitumor activities in cell lines and xenograft models,[67,68] is the most well-studied survivin inhibitor. Although the drug's safety and tolerability have been confirmed, single-agent YM155, as monotherapy regimen, demonstrated only limited activity in patients with refractory diffuse large B-cell lymphoma in a phase II clinical study.[69] In contrast, preclinical studies have demonstrated that YM155 plus rituximab, bendamustine, or a STAT3 inhibitor has obvious inhibitory effects on diffuse large B-cell lymphoma.[70,71] Taking into account the negative prognostic impact of survivin expression in activated B cell-like diffuse large B-cell lymphoma patients, assessment of clinical benefits should include stratification into different subgroups when evaluating survivin suppressants, especially when it combines with rituximab.[72,73]

Besides the agents that target survivin, survivin-derived peptide cancer vaccines are another promising choice. Survivin-derived peptide vaccines can elicit immune response through increasing survivin-specific $CD8^+$ cytotoxic T lymphocytes. Survivin-2B80-88 (refs. 74–76) and Survivin-2B[77] have shown the safety and therapeutic potential in cancers, such as breast cancer, colorectal cancer, and gastric cancer. SVN53-67/M57 (SurVaxM) has demonstrated its clinical potential in the treatment of gliomas and, more importantly, this vaccine contains multiple HLA epitopes that may be applicable to a large patient population.[78–80] DPX-Survivac vaccine, which has been proved to be well tolerated and shows the clinical benefits in ovarian cancer in phase I clinical trial,[81] is currently being examined in diffuse large B-cell lymphoma patients with survivin expression in a phase II clinical trial.[82] Furthermore, incomplete Freund's adjuvant and type-I interferon (IFNα) are two adjuvants that can enhance survivin-specific antitumor immunity.[77,83,84]

In summary, we identified survivin as an independent prognostic factor for poor outcome in activated B cell-like diffuse large B-cell lymphoma patients treated with R-CHOP. Our findings suggest that survivin affects the survival of activated B cell-like diffuse large B-cell lymphoma patients by influencing the mitosis and/or proliferation of tumor cells and is a promising therapeutic target in diffuse large B-cell lymphoma and its subgroup patients.

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Author contributions

Conception and design: ZL, ZYX-M, and KHY; research performance: ZL, ZYX-M, and KHY; provision of study materials, key reagents, and technology: ZL, ZYX-M, XC, GCM, XW, AT, YX, XL, CV, LZ, SM-M, KD, AC, AO, YZ, GB, KLR, EDH, WWLC, JH, MP, AJMF, BEP, MBM, MAP, JNW, LJM, and KHY; collection and assembly of data under approved IRB and MTA: ZYXM, AT, CV, SM-M, KD, AC, AO, YZ, GB, KLR, EDH, WWLC, JH, MP, AJMF, BMP, MBM, MAP, JNW, and KHY; data analysis and interpretation: ZL, ZYX-M, LJM, and KHY; manuscript writing: ZL, ZYX-M, LJM, and KHY; final approval of manuscript: all authors.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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