CD21S antigen expression in tumour cells of diffuse large B-cell lymphomas is an independent prognostic factor indicating better overall survival

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Summary

To evaluate the clinical significance of CD21S expression of diffuse large B-cell lymphoma (DLBCL) tumour cells, we compared their clinical features, immunophenotype, response to therapy and outcome in relation to CD21S expression. Between 1987 and 1999, frozen sections from 240 DLBCL cases were examined for CD21S expression by immunohistochemical methods. CD21S expression was detected on the tumour cells of 87 (36%) cases. The median age of the $CD21S^+$ DLBCL cases was 65 years (range: 17–84 years), the male–female ratio was 42:45, and they showed the following clinical features: Eastern Cooperative Oncology Group score >1 in 14%, lactate dehydrogenase greater than normal levels in 38%, extranodal sites >1 in 14%, stages III/IV disease at diagnosis in 29%, B symptoms in 17%, and a high/high–intermediate International Prognostic Index (IPI) in 23%. They also showed a better overall survival ($P = 0{\cdot}00001$, log-rank test) and a better complete remission rate ($P = 0{\cdot}00004$, chi-square test) than $CD21S^-$ DLBCL. Moreover, $CD21S^+$ DLBCL showed a better survival than $CD21S^-$ DLBCL for both low/low–intermediate and high/high–intermediate risk categories of IPI ($P = 0{\cdot}045$ and $P = 0{\cdot}0016$ respectively). Multivariate analysis identified CD21S expression as an independent factor for survival when compared with the five IPI factors. These findings indicate that CD21S expression of DLBCL tumour cells is a useful prognostic factor for survival.

Keywords: CD21, non-Hodgkin's lymphoma, B cells, immunophenotype, prognostic factors.

The CD21/complement receptor type 2 (CR2) is a 145-kDa membrane protein encoded by a gene located at 1q32, and is the receptor for complement activation fragments of C3, specifically iC3b, C3dg and C3d (Iida *et al*, 1983; Weis *et al*, 1984; Cooper *et al*, 1988; Tedder *et al*, 1994). CD21 forms a multimolecular signal transduction complex with CD19, CD81 and CD225 (Leu13) on the membrane of B cells (Matsumoto *et al*, 1991; Bradbury *et al*, 1992). The CD19–CD21 complex plays an important role in the activation and growth regulation of B cells (Tedder *et al*, 1994; Fearon & Carroll, 2000), while CD21 serves as the receptor for the Epstein–Barr virus on B cells (Fingeroth *et al*, 1984) and as a receptor for CD23, a low-affinity receptor for immunoglobulin E (IgE) (Aubry *et al*, 1992).

Human CD21 is expressed on B cells, follicular dendritic cells (FDCs), early thymocytes, a subset of mature T cells, and epithelial cells (Cooper *et al*, 1988; Fearon & Carroll, 2000). FDCs selectively express CD21L, while B cells selectively express a short CD21 lacking exon 10a (CD21S) (Liu *et al*, 1997, Table I). CD21 appears later than CD19 and CD20 in B-cell ontogeny (Tedder *et al*, 1984), is lost from B cells during the early stages of activation, and is absent from activated B cells that still express CD19 and CD20 (Timens *et al*, 1989a; Fearon & Carroll, 2000). In lymphoid tissue, CD21 expression is absent or weak on germinal centre B cells, moderate on mantle zone B cells, and strong on marginal zone B cells (Timens *et al*, 1989a,b). CD21 is therefore regarded as a marginal zone B-cell antigen.

Table I. CD21 isoforms.

	CD21 isoforms	
	CD21S	CD21L
Structure	CD21 lacking exon 10a	CD21 containing exon 10a
Specificity	B cells	FDCs
mAb	Anti-CR_2	Anti-CR_2, R4/23, 7D6, DRC-1, KiM4

mAb, monoclonal antibody; FDCs, follicular dendritic cells.

As for mature B-cell neoplasms, it has been reported that CD21 is expressed by more than half of all B-cell chronic lymphocytic leukaemias (Cossman & Jaffe, 1981; Nadler *et al*, 1981; Tedder *et al*, 1984), follicular lymphomas (FLs) (Schuurman *et al*, 1987; Scoazec *et al*, 1989), and marginal zone B-cell lymphomas (MZBCLs) (Isaacson *et al*, 2001), but by only up to half of diffuse large B-cell lymphomas (DLBCLs; Nadler *et al*, 1981; Tedder *et al*, 1984; Freedman *et al*, 1985; Schuurman *et al*, 1987, 1988; Scoazec *et al*, 1989; Yamaguchi *et al*, 2002). DLBCLs represent the largest category of aggressive lymphomas, and are thought to constitute a clinicopathologically heterogeneous group of lymphomas (Harris *et al*, 1994). While primary mediastinal large B-cell lymphoma, a clinical subtype of DLBCL, is known to be $CD21^-$ (van Besien *et al*, 2001), the overall clinical significance of CD21 expression, especially CD21S expression of the tumour cells in DLBCL, remains to be examined.

In order to determine the clinical significance of CD21S expression of DLBCL tumour cells, we examined CD21S expression by means of immunohistochemistry in frozen sections from 240 cases of DLBCL. We also compared the clinical features and immunophenotype of DLBCL according to CD21S expression.

Materials and methods

Patients

The study comprised 240 patients, all of whom were diagnosed between January 1987 and October 1999 as having DLBCL according to the Revised European-American lymphoma (REAL) classification (Harris *et al*, 1994), and were consecutively examined for CD21 expression by means of immunohistochemistry at the Mie University School of Medicine. They had no past history of any other lymphoproliferative disorders. We took special care to eliminate cases of grade 3 follicular lymphoma from the present study. All specimens for histological and immunophenotypic studies were obtained at the initial presentation of the patients. All patients received medical treatment with similar procedures at Mie University Hospital or nearby affiliated hospitals.

Table II. Anti-CD21 antibodies used in this study.

	Anti-CD21 antibody	
	Anti-CR_2	R4/23
B cells	+	–
FDCs	+	+

FDCs, follicular dendritic cells.

Histopathology and immunophenotypic study

Tissue was fixed in 10% formalin and embedded in paraffin, and 5 μm thick sections were stained with haematoxylin, eosin and Giemsa.

The immunophenotypic study of the tumour cells used a labelled avidin-biotin method for frozen sections as described previously (Oka *et al*, 1994). The monoclonal antibodies used were Leu4 (CD3), Leu1 (CD5), Leu12 (CD19) and anti-CR_2 (CD21) (Becton Dickinson, San Jose, CA, USA); CALLA (CD10), L26 (CD20), MHM6 (CD23), anti-IgD and R4/23 (CD21L) (DAKO Cytomation, Glostrup, Denmark); and anti-IgG, anti-IgA, anti-IgM, anti-kappa and anti-lambda (Biosource International, Camarillo, CA, USA). Anti-CR_2 reacts to both CD21S and CD21L, while R4/23 specifically recognizes CD21L (Table I), so that, DLBCL cells that express CD21S react with anti-CR_2 and not with R4/23 (Table II). In all cases, the tumour cells expressed CD19 and/or CD20, but not CD3. For the purposes of this study, tumour cells that were more than 20% positive for a given antibody were assumed to be positive for the corresponding protein.

CD21S antigen expression in DLBCL tumour cells

The CD21 antigen is expressed on both B cells and FDCs. First, we examined CD21 expression by means of immunohistochemistry using two kinds of anti-CD21 monoclonal antibodies, anti-CR_2 and R4/23, in 30 consecutively diagnosed cases of DLBCL (data not shown). Since various amounts of $R4/23^+$ FDCs were found interspersed among the tumour cells, we decided to immunostain with R4/23 when most cells in the specimen reacted to anti-CR_2 (Fig 1). No cases of grade 3 follicular lymphoma were included in our study. The incidence of $CD21S^+$ DLBCL was 36% (87 of 240) in our series of 240 DLBCL cases, which also included one case each of intravascular large B-cell lymphoma and primary mediastinal large B-cell lymphoma. In both these cases the tumour cells were negative for CD21S.

Statistical analysis

Correlations between two groups were examined with the chi-square test, the Fisher exact test, the Student's *t*-test, and the Mann–Whitney *U*-test. Patient survival data were analysed with the Kaplan–Meier method and compared by means of the log-rank test. Univariate and multivariate analyses were performed with the Cox proportional hazard regression model,

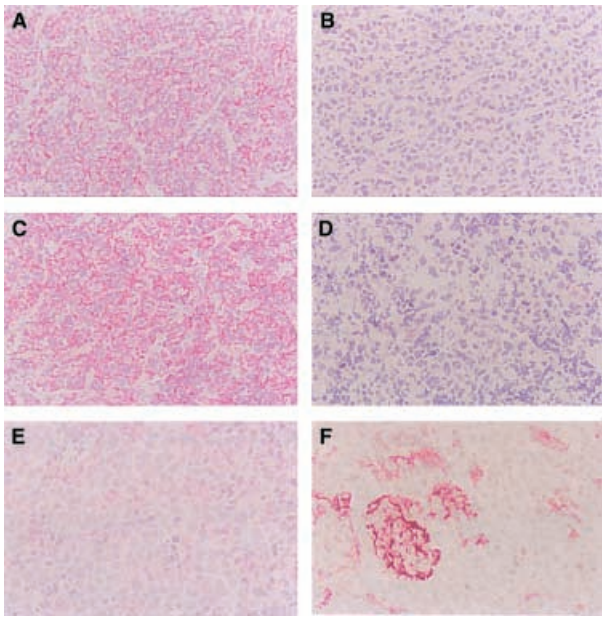

Fig 1. Immunohistochemical features of CD21S$^+$ diffuse large B-cell lymphoma (DLBCL). (A) CD21S$^+$ DLBCL (specimen 2475, lymph node). Most cells reacted with anti-CR_2. (B) The same case as in (A). None of the cells reacted with R4/23, which recognizes CD21L expressed specifically in follicular dendritic cells (FDCs). Lymphoma cells in this case were evaluated as CD21S$^+$. (C) CD21S$^+$ DLBCL (specimen 2611, paranasal tumour). Most cells reacted with anti-CR_2. (D) The same case as in (C). None of the cells reacted with R4/23, so that the cells were evaluated as CD21S$^+$. (E) CD21S$^-$ DLBCL (specimen 2556, lymph node). (F) CD21S$^-$ DLBCL (specimen 2535, lymph node). Lymphoma cells in neither case reacted with anti-CR_2. Scattered FDCs reacted with anti-CR_2. Original magnification ×200 for all panels.

and all relevant data were analysed with the SAS system (SAS Institute, Inc., Cary, NC, USA). *P*-values <0·05 were considered significant.

Results

Clinical features of CD21S$^+$ DLBCL and CD21S$^-$ DLBCL patients

Table III summarizes the patients' clinical features at presentation. The CD21S$^+$ DLBCL group had a median age of 65 years, ranging from 17 to 84 years, and comprised 42 men and 45 women. This group showed favourable general characteristics compared with the CD21S$^-$ DLBCL group: 12 patients with a performance status (PS) of more than 1 (14%, $P = 0{\cdot}033$), 25 with stages III/IV disease at diagnosis (29%, $P = 0{\cdot}0022$), 12 with more than one extranodal site (14%, $P = 0{\cdot}0084$) and 15 with B symptoms (17%, $P = 0{\cdot}004$). The International Prognostic Index (IPI) score (The International Non-Hodgkin's Lymphoma Prognostic Factors Project, 1993) was significantly lower for patients with CD21S$^+$ DLBCL than for those with CD21S$^-$ DLBCL ($P = 0{\cdot}0069$), with 67 (77%) of the former group categorized in the IPI low or low–intermediate risk group.

Table III. Clinical features.

	CD21S$^+$ DLBCL ($n = 87$) [n (%)]	CD21S$^-$ DLBCL ($n = 153$) [n (%)]	*P*-value
Age at diagnosis (years)			0·69
Median	65	65	
Range	17–84	18–92	
Over 60 years old	58 (67)	95 (62)	0·48
Sex (male/female)	42/45	83/70	0·37
PS more than 1	12 (14)	39 (25)	0·033
Serum LDH level above normal	33 (38)	74 (48)	0·12
Stage			0·014
I	24 (27)	30 (20)	
II	38 (44)	48 (31)	
III	11 (13)	40 (26)	
IV	14 (16)	35 (23)	
III/IV	25 (29)	75 (49)	0·0022
Extranodal involvement			
Any site	67 (77)	119 (78)	0·89
More than one site	12 (14)	44 (29)	0·0084
International Prognostic Index			0·0069
Low	50 (57)	65 (42)	
Low–intermediate	17 (20)	25 (16)	
High–intermediate	11 (13)	25 (16)	
High	9 (10)	38 (25)	
B symptoms present	15 (17)	53 (35)	0·004

LDH, lactate dehydrogenase; DLBCL, diffuse large B-cell lymphoma.

Lymphomatous involvement in terms of CD21S expression is shown in Fig 2. The most frequent site of lymphomatous involvement other than the lymph nodes in CD21S$^+$ DLBCL

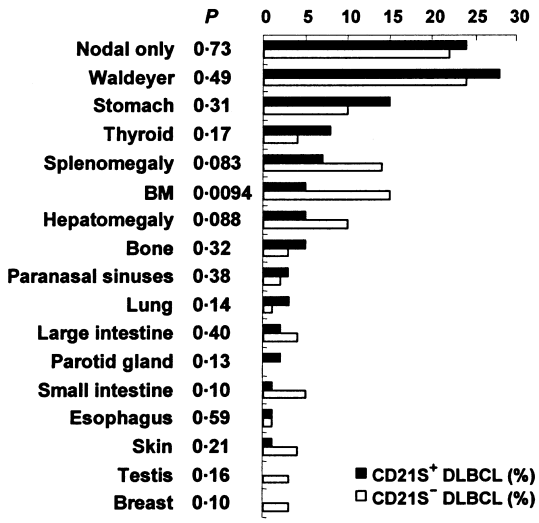

Fig 2. Lymphomatous involvement of CD21S$^+$ diffuse large B-cell lymphoma (DLBCL) and CD21S$^-$ DLBCL. The incidence of bone marrow involvement was lower for CD21S$^+$ DLBCL than CD21S$^-$ DLBCL. Both hepatomegaly and splenomegaly occurred less frequently in CD21S$^+$ DLBCL than in CD21S$^-$ DLBCL. BM, bone marrow.

Table IV. Immunophenotypic features.

	$CD21S^+$ DLBCL [$n = 87$ (%)]	$CD21S^-$ DLBCL [$n = 153$ (%)]	*P*-value
CD5	5/87* (6)	19/153 (12)	0·098
CD10	7/79 (9)	9/148 (6)	0·44
CD19	78/81 (96)	131/145 (90)	0·082
CD20	81/83 (98)	148/148 (100)	0·13
CD21	87/87 (100)	0/153 (0)	
IgM	41/76 (54)	86/140 (61)	0·29
IgD	19/77 (25)	28/144 (19)	0·37
κ chain	31/69 (45)	60/125 (48)	0·68
λ chain	27/69 (39)	35/125 (28)	0·11

*Positive/examined cases.

was Waldeyer's ring ($n = 24$: 28%; Fig 2). A comparison with $CD21S^-$ DLBCL demonstrated that the incidence of bone marrow involvement was lower ($P = 0·0094$), and involvement of spleen and liver tended to be less frequent. There were no significant differences in the incidence of involvement of other sites between the two groups (Fig 2).

Phenotypic features

Immunophenotypic features are summarized in Table IV. According to the definition adopted for this study (see Materials and Methods), all cases were positive for B-cell markers (CD19 and/or CD20). $CD21S^+$ DLBCL expressed CD5 less frequently and CD19 more frequently than $CD21S^-$ DLBCL, although there were no significant differences.

Therapeutic response and prognosis

The treatment consisted of chemotherapeutic regimens including anthracycline for 204 patients and without anthracycline for seven. Twenty-four patients with localized diseases were treated with radiotherapy or surgical resection alone as the first-line therapy. One patient with $CD21S^+$ DLBCL and four patients with $CD21S^-$ DLBCL did not receive any treatment because of their poor PS, and all five died of the disease. None of the patients was treated with Rituximab. The patients with $CD21S^+$ DLBCL who attained complete remission (CR) with the initial therapy accounted for 88% (76 of 86), and the corresponding patients with $CD21S^-$ DLBCL for 64% (95 of 149) ($P = 0·00004$, chi-square test). $CD21S^+$ DLBCL thus showed a significantly better survival than $CD21S^-$ DLBCL ($P = 0·00001$, Fig 3), with a 5-year survival rate for $CD21S^+$ DLBCL cases of 70%.

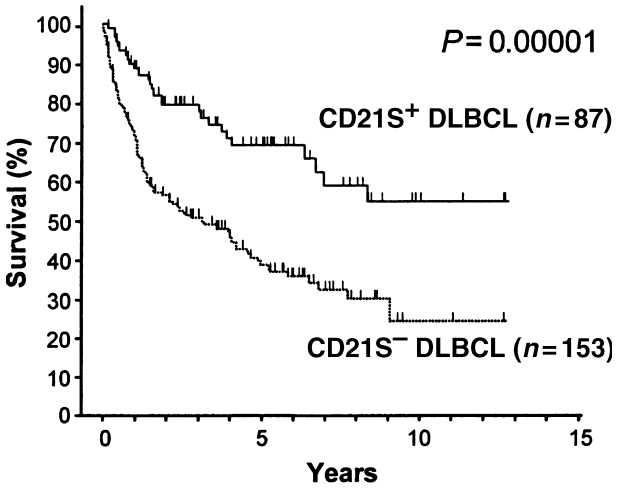

Fig 3. Overall survival for patients with $CD21S^+$ diffuse large B-cell lymphoma (DLBCL) and with $CD21S^-$ DLBCL. $CD21S^+$ DLBCL showed a significantly better survival than $CD21S^-$ DLBCL.

Univariate Cox analysis identified the following prognostic factors for the 240 patients with DLBCL: CD21S expression in tumour cells, age, PS, serum lactase dehydrogenase (LDH) level, clinical stage, multiple site extranodal involvement, and IPI category (Table V). Multivariate analysis of these six parameters showed that $CD21S^-$ expression, age over 60, PS > 1, high-LDH level, and advanced stages (III or IV) were significant prognostic factors (Table V), but multiple site extranodal involvement was not. Multivariate analysis for CD21S expression and IPI category demonstrated that both

Table V. Prognostic factors affecting overall survival.

Variables (unfavourable factors)	Univariate		Multivariate (final model)	
	Relative risk (CI)	*P*-value	Relative risk (CI)	*P*-value
In relation to IPI factors				
CD21S status (negative)	2·54 (1·62–3·99)	<0·0001	2·43 (1·53–3·86)	0·0002
Age (>60 years)	1·57 (1·03–2·40)	0·037	1·89 (1·22–2·94)	0·0045
PS (2–4)	4·10 (2·71–6·20)	<0·0001	2·25 (1·42–3·57)	0·0006
LDH (greater than normal)	3·28 (2·21–4·88)	<0·0001	2·03 (1·30–3·18)	0·0020
Stage (III/IV)	3·00 (2·03–4·42)	<0·0001	1·73 (1·24–2·39)	0·0011
Extranodal disease (>1 site)	2·46 (1·63–3·72)	<0·0001	–	–
In relation to IPI category				
CD21S status (negative)	2·54 (1·62–3·99)	<0·0001	2·30 (1·46–3·62)	0·0003
IPI (HI/H)	4·31 (2·92–6·36)	<0·0001	4·08 (2·76–6·03)	<0·0001

CI, 95% confidence interval; IPI, International Prognostic Index.

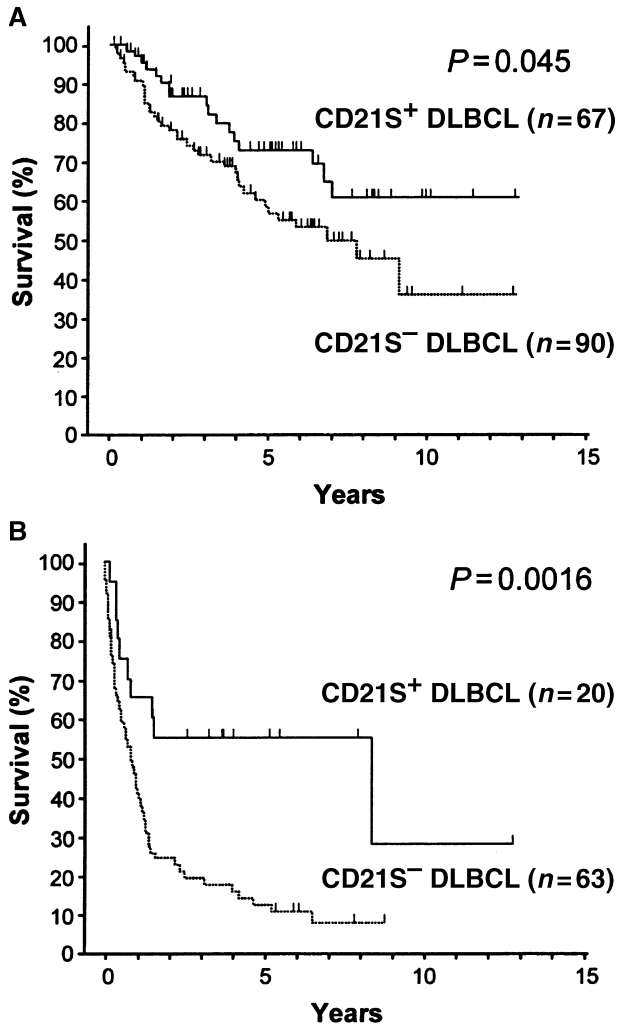

Fig 4. Overall survival for patients with $CD21S^+$ diffuse large B-cell lymphoma (DLBCL) and with $CD21S^-$ DLBCL after stratification for International Prognostic Index (IPI). (A) low or low–intermediate IPI. (B) High–intermediate or high IPI.

were significant and independent prognostic factors (Table V). Finally, the Kaplan–Meier method was used to compare overall survival for $CD21S^+$ DLBCL with that for $CD21S^-$ DLBCL after stratification for low/low–intermediate (Fig 4A) or high/high–intermediate (Fig 4B) IPI. The results indicated that $CD21S^+$ DLBCL had a better overall survival than $CD21S^-$ DLBCL for both stratifications ($P = 0{\cdot}045$ and $P = 0{\cdot}0016$ respectively).

Discussion

The study presented here demonstrated the clinical significance of CD21S expression in the tumour cells of DLBCL, with $CD21S^+$ DLBCL cases accounting for 36% of all DLBCLs. Moreover, a multivariate analysis of the overall survival of all 240 patients with DLBCL identified CD21S expression of the tumour cells as an independent prognostic factor.

The CD21 is a representative pan-B-cell antigen. While the expression of CD21antigen in mature B-cell neoplasms has been the subject of investigations since the 1970s, the clinical significance of CD21 in malignant lymphoma has yet to be determined. One of the main reasons for this is that CD21 expression on neoplastic B cells is usually too weak to be detected immunohistochemically using routinely processed paraffin sections. Moreover, it is difficult to eliminate the $CD21^+$ FDCs because both FDCs and B cells express CD21. In the study reported here, we resolved this problem by means of immunohistochemistry and using frozen sections with two kinds of anti-CD21 antibodies. B cells express a short CD21 lacking exon 10a (CD21S), while FDCs selectively express CD21L (Liu *et al*, 1997) (Table I). Anti-CR_2 recognizes both CD21S and CD21L, whereas R4/23, 7D6, DRC-1 and KiM4 recognize CD21L. Thus, $CD21^+$ B cells react to anti-CR_2 but do not react to R4/23, whereas FDCs react to both anti-CR_2 and R4/23 (Table II). We used these characteristics to examine CD21S expression of the tumour cells in the largest series of DLBCL cases reported to date, and were able to confirm previous results that indicated favourable outcomes for $CD21S^+$ DLBCL (Schuurman *et al*, 1988).

In the study presented here, $CD21S^+$ DLBCL showed some clinical features in common with extranodal MZBCL of mucosa-associated lymphoid tissue (MALT) type, such as female predominance, good PS and localized disease (The International Non-Hodgkin's Lymphoma Prognostic Factors Project, 1993; The Non-Hodgkin's Lymphoma Classification Project, 1997). Indeed, CD21 is regarded as a marginal zone B-cell antigen. As for immunophenotype, the frequency of the expression of CD19 in $CD21S^+$ DLBCL tended to be higher than that in $CD21S^-$ DLBCL. CD21S forms a complex with CD19 on the membrane of B cells, and this CD19/CD21S complex plays important roles in the innate immune recognition of microbial antigens by the complement system to the activation of B cells (Fearon & Carroll, 2000). Frequent expression of CD19 in $CD21S^+$ DLBCL may thus reflect characteristics of the normal counterpart cells of $CD21S^+$ DLBCL. This indicates that the molecular and cellular features of $CD21S^+$ DLBCL obviously deserve to be clarified in future studies.

The reason for a favourable prognosis for $CD21S^+$ DLBCL remains unknown. $CD21S^-$ DLBCL can be regarded as a highly aggressive lymphoma because its 5-year survival rate was only 38%. The fact that CD21 expression is lost from B cells during the early stages of activation and is absent from activated B cells (Timens *et al*, 1989a; Fearon & Carroll, 2000) suggests that $CD21S^-$ DLBCL might be an activated B cell-like DLBCL, which was identified in gene expression profiling using cDNA microarrays (Alizadeh *et al*, 2000). Further study is needed to test this notion, because the absence of CD21S does not necessarily identify the phenotype, although the CD21S status is lost with the activation of B cells.

To summarize, our study confirmed the favourable outcome of DLBCL expressing CD21S on the tumour cells. Since

immunohistochemical staining using frozen sections has not been widely performed in routine diagnostic analyses of lymphoma, flow cytometric studies are needed to confirm whether this finding indeed facilitates establishing a prognosis for DLBCL.

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