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MUM1/IRF4 Expression Is an Unfavorable Prognostic Factor in B-Cell Chronic Lymphocytic Leukemia (CLL)/Small Lymphocytic Lymphoma (SLL)

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B-Cell chronic lymphocytic leukemia (B-CLL)/small lymphocytic lymphoma (SLL) consists of heterogeneous diseases that are distinguished by morphological, immunophenotypic and molecular features. *MUM1* (multiple myeloma oncogene 1) is a protooncogene that is deregulated as a result of (6;14)(p25;q32) chromosomal translocation in multiple myeloma, and is also expressed in a variety of malignant lymphoma entities. We examined the expression of MUM1 in B-CLL/SLL, and found that 2 of 4 B-CLL-derived cell lines and 14 of 29 patients' specimens expressed MUM1 by immunohistochemical analysis. MUM1 expression was not associated with CD38 expression, somatic hypermutation of immunoglobulin heavy chain gene variable region (IgV_H), or any other clinical characteristics of the patients. Interestingly, the patients who were positive for MUM1 showed shorter overall survival times than those who were negative for MUM1 (50% survival: 22 months vs. 82 months) (P=0.0008, log-rank test). Multivariate analysis by Cox's proportional-hazards regression model showed that MUM1 expression and unmutated IgV_H status were independent unfavorable prognostic factors in patients with B-CLL/SLL. These findings suggest that MUM1 expression is a useful prognostic factor in B-CLL/SLL. The biological role and mechanism of action of MUM1 in B-CLL/SLL need to be clarified for the development of therapies for patients with the poor prognostic subtype.

Key words: MUM1 — CLL/SLL — Prognostic factor — Somatic hypermutation — CD38

B-Cell chronic lymphocytic leukemia (B-CLL)/small lymphocytic lymphoma (SLL) is one of the most common leukemias in Western countries,[1,2] but its incidence is very low in Asia including Japan, where it accounts for just 2% of all leukemias.[1,3,4] The clinical course of patients with B-CLL/SLL is known to be heterogeneous.[5,6] B-CLL/SLL is generally recognized as an indolent disease; however, a certain percentage of patients who are diagnosed in the early clinical stage die from the disease within a short period of time. Hence, B-CLL/SLL is considered to comprise a multitude of heterogeneous diseases.[5,6] The clinical staging systems proposed by Rai *et al.*[7] and Binet *et al.*[8] are useful for predicting patients' survival and are currently being used. In spite of their convenience in routine clinical use, these staging systems can not accurately predict the risk of disease progression in a patient, or the survival time upon conventional treatment at the time of initial diagnosis. Therefore, it is important to identify definite markers that can detect those patients with unfavorable prognosis irrespective of the clinical stage. Recently, several investigators have reported that there are two distinct subtypes of B-CLL with distinct clinical courses.[9,10] These two subtypes are distinguished by the difference in B-cell origin, i.e., pre-germinal center (GC) or post-GC origin, which is determined by the absence or presence, respectively, of somatic hypermutation (SH) of the immunoglobulin heavy chain gene variable region (IgV_H).[9,10] The former subgroup with unmutated IgV_H sequences was reported to be associated with higher CD38 expression as detected by flow cytometric analysis.[9] Ibrahim *et al.*[11] later confirmed that CD38 expression was associated with poor prognosis using multivariate analysis. However, Hamblin *et al.*[12] reported a contradictory result, finding no correlation between the status of IgV_H SH and CD38 expression. Accordingly, it is still controversial whether the expression of CD38 or absence of IgV_H SH is an unfavorable prognostic factor in B-CLL. Although the genetic background of B-CLL remains unknown, patients with B-CLL carrying trisomy 12 have been reported to have

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shorter overall survival.[13, 14] This led us to hypothesize that the molecular profile, such as genetic aberration and gene expression, might reflect the variability of the natural history of B-CLL.

The *MUM1* (multiple myeloma oncogene 1) gene was identified as a protooncogene that is transcriptionally activated by (6;14)(p25;q32) chromosomal translocation in multiple myeloma.[15, 16] It has also been designated as *IRF4* (interferon regulatory factor 4),[17] as it works in cooperation with PU.1,[18] STAT6[19] and Blimp-1[20] as a transcriptional regulator in lymphoid cells, although its precise biological function remains unknown. We and others previously reported that a major proportion of mature lymphoid malignancies consists of activated neoplastic lymphocytes expressing MUM1 protein.[21, 22] Since MUM1 protein expression is strictly regulated during lymphoid differentiation in normal lymphoid tissues, aberrant expression of MUM1 could be added to the panel of phenotypic markers available for characterization of the histogenesis of B-cell lymphoma/leukemia.[21, 22] We also found that several lymphoma subtypes, i.e., B-CLL/SLL and diffuse large B-cell lymphoma (DLCL), showed heterogeneity in terms of the degree of MUM1 expression as detected by immunohistochemistry.[21] Similar results were reported in malignant B-cells using quantitative reverse transcription-polymerase chain reaction (RT-PCR)[23] and tissue microarrays.[24] As to DLCL, Alizadeh *et al.*[25] profiled gene expression using cDNA microarrays, and identified MUM1 as an important marker of DLCL of the activated B-cell phenotype that could predict poorer prognosis in comparison with the GC B-cell phenotype. In our previous study, we found that coexpression of MUM1 and CD38 was always observed in normal reactive lymph nodes by immunohistochemistry.[21] In this report we describe MUM1 expression in B-CLL/SLL cases with respect to prognostic significance and application in disease subtyping.

PATIENTS, MATERIALS AND METHODS

Patient characteristics Twenty-nine Japanese patients who were diagnosed as having B-CLL or SLL at four independent institutions (Nagoya City University, Aichi Cancer Center, Mie University and Fukushima Medical University) between 1981 and 2000 were included in this study. Specimens obtained at the time of initial diagnosis (lymph node and/or bone marrow biopsies) were used after the informed consent of the patients had been obtained. The diagnosis of B-CLL or SLL was confirmed based on cytological, histological and immunophenotypic analyses according to the World Health Organization Classification of Tumors.[26] A histological diagnosis was made independently by two hematopathologists (authors H. I. and S. N.), and those in agreement were included in this study. All tumors showed monoclonality, which was confirmed by the restricted expression of the immunoglobulin (Ig) light chain, and positivity for CD20 and CD5 antigens by flow cytometric analysis. Immunostaining for representative lymphoid markers including CD3, CD5, CD10, CD20, CD21, CD23 and cyclin D1 was performed and all 29 specimens examined were negative for cyclin D1 and CD10. Among the 29 patients, 22 cases were diagnosed as having B-CLL, and the remaining 7 cases as having SLL.

Cell lines derived from B-CLL patients Four cell lines that had been established from patients with B-CLL [WSU-CLL,[27] WaC3 CD5+,[28] MO1043[29] and MEC-2[30]] were subjected to immunoblot analysis for MUM1 expression.

Immunoblot analysis The cell pellet prepared from a cell line was resuspended in 1× Laemmli sample buffer and boiled for 5 min. Total protein lysate derived from 1×10^6 cells was fractionated on a 7.5% SDS acrylamide gel and transferred to a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The blocking reaction and incubation with M-17 anti-ICSAT/MUM1/IRF4 goat serum (Santa Cruz Biotechnology, Santa Cruz, CA), followed by incubation with anti-goat IgG horseradish peroxidase-linked antibody (Amersham, Buckinghamshire, UK), were performed as previously described.[21] Reactive bands were detected by using an ECL system (Amersham).

Double color fluorescence *in situ* hybridization (DCFISH) Chromosome preparation and DCFISH were performed as described previously.[16, 31] The probes labeled for FISH analysis were DNAs derived from MUM1-PACs120i21 or 61b14 and IgCγ-BAC417p24, as previously described.[16, 31]

Immunostaining analysis Formalin-fixed, paraffin-embedded lymph node or bone marrow tissues were subjected to immunostaining analysis using anti-ICSAT (M-17) goat serum, and MUM1 protein was detected with the streptavidin-biotin complex method (sABC) as described previously.[21] The following antibodies were also used in a series of immunostaining analyses: anti-CD38 (SPC32; Novocastra Laboratories, Newcastle, UK), anti-Ki-67 (MIB-1; DAKO A/S, Glostrup, Denmark) and anti-CD138/syndecan 1 (B-B4; Serotec, Oxford, UK) mouse serum. We defined MUM1 and Ki-67 positivity based on criteria according to the sample's morphological subtype as classified by the system of Lennert and Feller, i.e., the diffuse subtype (DS), tumor-forming subtype (TFS) or pseudofollicular subtype (PFS).[32] In DS and TFS, the tumor sample or cell line was considered to be positive for MUM1 or Ki-67 if more than 20% of the tumor cells were stained by the respective antibody.[21] In PFS, since MUM1-positive cells seemed to be limited to prolymphocytes and paraimmunoblasts in pseudofollicles as reported by Falini *et al.*,[22] the tumor sample or cell line was considered to be positive for MUM1 if more than 20% of the

tumor cells in the pseudofollicles were stained. This cut-off value of 20% was determined based on the mean+3 SD (standard deviation) of MUM1 positivity in the light zone area, which showed the most frequent positivity in reactive lymphoid tissues.[21] A tumor sample or cell line was considered to be positive for CD38 or CD138 if more than 20% of the tumor cells were stained by the respective antibody in any morphological subtype.

PCR amplification of the immunoglobulin heavy chain gene Genomic DNA was extracted from tumor samples as described previously.[33] Semi-nested PCR was performed using the primer sets described previously with a slight modification.[34] In brief, the first PCR amplification from 100 ng of DNA as a template was performed using the upstream consensus V_H primer (FR2A: 400 ng) and downstream J_H primer (LJ_H: 100 ng). For the second PCR amplification, a 1% aliquot of the first PCR product was used as a template with the nested consensus primer (VLJ_H: 200 ng) and upstream primer (FR2A: 200 ng). The primer-annealing step consisted of five cycles at 63°C for 60 s and the remaining 35 cycles at 57°C. The conditions of denaturation (96°C for 30 s) and primer extension (72°C for 60 s) were kept the same in all cycle steps. After the last cycle, the elongation step was extended for 10 min. A stringent primer annealing condition at 63°C for 60 s was kept the same in all cycles of the second PCR (25 cycles). Ten microliters of each amplified product was subjected to 2.5% agarose gel electrophoresis and visualized with ethidium bromide staining.

DNA sequencing and validation of SH Each PCR product was retrieved from the agarose gel with the QIAquick Gel Extraction Kit (QIAGEN, Chatsworth, CA), ligated into the pGEMT-easy vector (Promega Corp., Madison, WI), and transfected into DH5α competent cells (GIBCO BRL, Tokyo). Using the blue-white selection with X-gal system (TaKaRa Biochem. Co., Kyoto), 8 to 12 white colonies were randomly picked and propagated overnight in Luria-Bertani (LB) medium. Plasmid DNA that had been purified by the alkaline lysis method was sequenced using the Big dye terminator kit (Applied Biosystems, Foster City, CA) with the aid of an automatic DNA sequencer (ABI3100, Applied Biosystems). The obtained sequences were compared with the germline sequences through the BLAST homology search program (Basic Local Alignment Search Tool; National Center for Biotechnology Information, Bethesda, MD) in order to determine the usage of V_H segments and to determine the somatic mutations by comparing the sequence with the most proximal germline sequence. Based on the number of somatic mutations detected in these genes, the IgV_H sequences were classified into two categories: "unmutated" or "mutated." As per current convention,[1, 9, 10] "unmutated" cases were defined as those with <2% difference from the most simi-

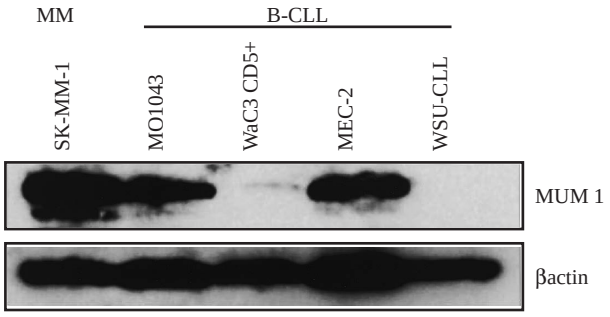

Fig. 1. Immunoblot analysis of the MUM1 protein in B-CLL cell lines. The MUM1 protein of approximately 50 kDa was detected in two out of the four B-CLL-derived cell lines, MO1043 and MEC-2. SK-MM-1 overexpressing MUM1 as a result of t(6;14)(p25;q32) was used as a positive control. MM, multiple myeloma; B-CLL, B-cell chronic lymphocytic leukemia.

Table I. Clinical Characteristics of the Patients with B-CLL/SLL

Age (year) (median, range)	64 (48–79)
Gender	
Male	18 (62%)
Female	11 (38%)
WBC ($\times 10^3/\mu l$) (median, range)	20.9 (2.9–68.9)
Lymphocytes ($\times 10^3/\mu l$)	12.9 (1.7–66.6)
Platelets ($\times 10^3/\mu l$)	213 (51–320)
Hemoglobin (g/dl)	13 (5.3–16.3)
LDH (IU)	221 (59–650)
Diagnosis (n=29)	
CLL	Total, 22
Binet	
A	5 (17%)
B	14 (48%)
C	3 (10%)
SLL	Total, 7
Stage	
I	0 (0%)
II	1 (3%)
III	5 (17%)
IV	1 (3%)
Hepatomegaly	
No	27 (93%)
Yes	2 (7%)
Splenomegaly	
No	21 (72%)
Yes	8 (28%)

lar germline sequence among the expressed V_H genes; "mutated" cases were defined as those in which the B-CLL cells displayed ≥2% difference in the expressed V_H gene. In our series, this analysis was performed in 21 cases whose genomic DNAs were available for the PCR and consequently unique PCR products were obtained.

Statistical analysis The patients' characteristics were compared using Fisher's exact test, Student's *t* test and the Mann-Whitney *U* test. Differences between subgroups were evaluated by the Mann-Whitney *U* test (nonparametric analysis), and $P<0.05$ was taken as significant. The overall survival time was defined as the period between the time of diagnosis and the time of death. Survival analysis was performed according to the Kaplan-Meier method. The statistical significance of differences in survival was determined by the log-rank and generalized Wilcoxon's tests. Univariate and multivariate analyses of the prognostic factors were performed using Cox's proportional-hazards regression model, and variables were selected with the stepwise increased method. Age was defined as a constitutive factor. All calculations were performed with the aid of StatView software (SAS Institute version 5.0, Cary, NC).

RESULTS

MUM1 expression in cell lines and patient specimens Two out of the four B-CLL cell lines, MO1043 and MEC-2, expressed an approximately 50-kDa MUM1 protein, whereas the remaining two cell lines, WSU-CLL and WaC3 CD5+, did not (Fig. 1). On DCFISH analysis of the two B-CLL cell lines expressing MUM1 protein, neither a fusion signal between *MUM1* and *IgH* loci nor amplification at the 6p25 locus was detected (data not shown). The clinical characteristics of the patients enrolled in this study are summarized in Table I. Seven patients were diagnosed

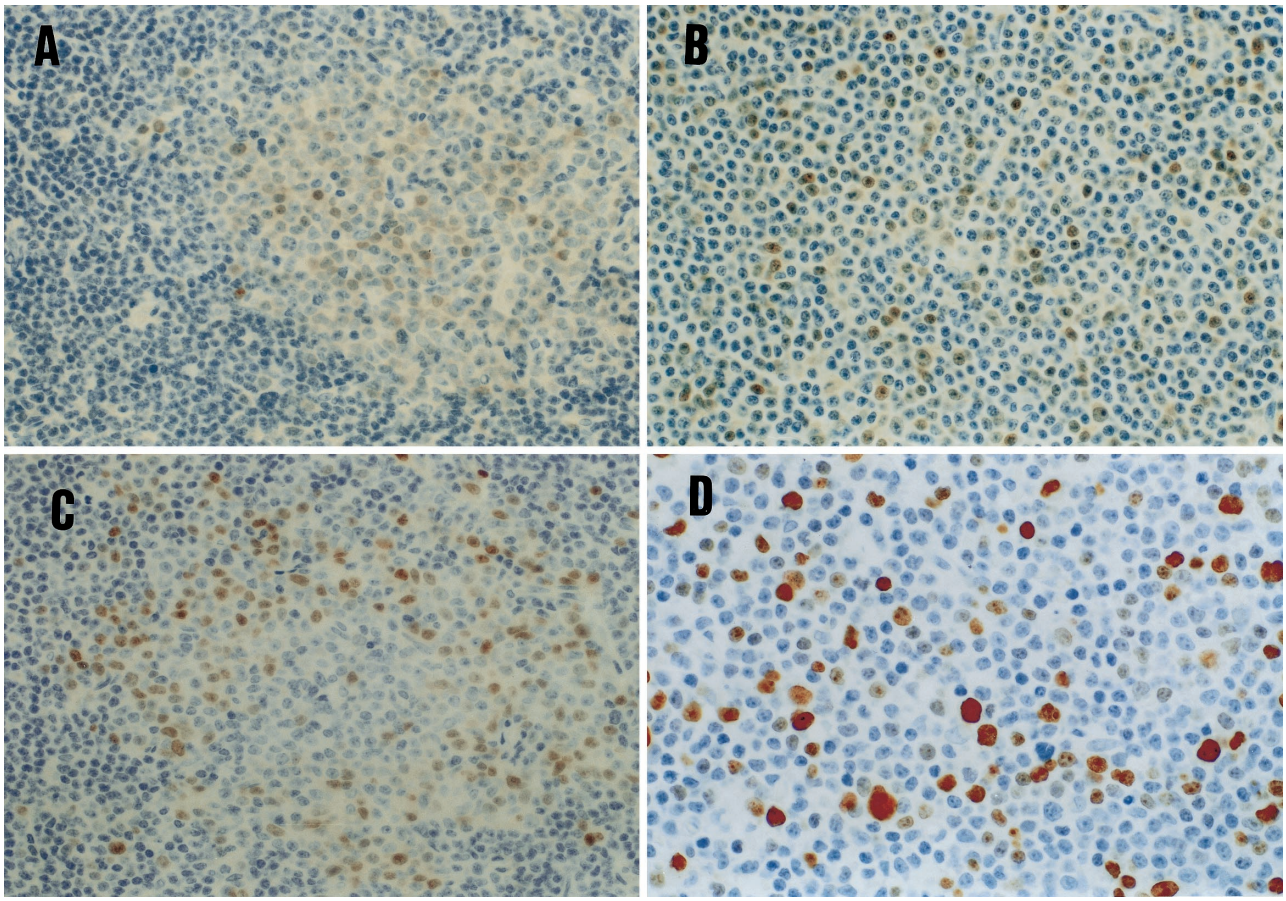

Fig. 2. Immunostaining analysis for MUM1 protein or Ki-67 in lymph node samples obtained from representative patients with B-CLL/SLL. (A) A case showing the pseudofollicular subtype. There is moderate staining of MUM1 in prolymphocytes and paraimmunoblasts in a pseudofollicle (×400). (B) A case showing the diffuse subtype with positivity for MUM1 (×400). (C and D) Immunostaining analysis of samples with the same morphological subtypes as (A) and (B), respectively, with Ki-67 antibody (C, ×400; D, ×400).

Table II. Comparison of the Patients with B-CLL/SLL According to the MUM1 Expression Status

Characteristics	MUM1+ n=14 (48%)	MUM1− n=15 (52%)	P
Clinical stage			
Early stage	9 (64%)	11 (73%)	0.70[a]
Advanced stage	5 (36%)	4 (27%)	
Splenomegaly			0.68[a]
Yes	3 (21%)	5 (33%)	
No	11 (79%)	10 (67%)	
WBC ($\times 10^3/\mu$l)	26.0 (5.9–68.9)	16.5 (5.9–39.5)	0.12[b]
Hemoglobin (g/dl)	12.4 (6.2–15.1)	12.1 (5.3–16.3)	0.84[b]
Platelets ($\times 10^3/\mu$l)	214 (51–320)	178 (92–251)	0.28[b]
LDH (IU)	262 (59–650)	246 (179–429)	0.21[b]
Gender			0.13[a]
Male	11 (79%)	7 (47%)	
Female	3 (21%)	8 (53%)	
Age (year)	63 (48–77)	64 (54–79)	0.51[c]

Early stage includes stage I and II of SLL, and Binet's stage A and B of CLL.
Advanced stage includes stage III and IV of SLL, and Binet's stage C of CLL.
a) Fisher's exact test.
b) Student's *t* test.
c) Mann-Whitney *U* test (non-parametric test).

as having SLL because their clinical features did not meet the criteria for B-CLL. The 22 B-CLL patients were classified in the staging system defined by Binet, and over 50% (14/22 cases) of the patients were categorized into Binet's stage B. However, the features of B-CLL or SLL in the 29 patients did not deviate much from the typical features of B-CLL or SLL, respectively. Overall, the rate of MUM1 positivity as detected by immunohistochemical analysis in the B-CLL/SLL samples was 48% (14/29 cases) (Fig. 2, A and B). The clinical features at the time of initial diagnosis were compared between the MUM1-positive and -negative cases (Table II). No significant differences were found between these two groups in terms of the prevalence of splenomegaly, age, leukocyte count, hemoglobin level and platelet count. The MUM1-positive cases tended to be male and to have an elevated leukocyte count, although the differences were not statistically significant (P=0.13 and P=0.12, respectively). In this study, as cytogenetic data were not available except for three cases carrying 47,XX,+12; 48,XY,+12,+19 and 47,XY,+12, we omitted these data from further statistical analysis. However, all of these three cases harboring trisomy 12 were negative for MUM1 immunostaining.

MUM1 expression and its relationship with other phenotypic markers Fifteen B-CLL/SLL samples were positive for Ki-67. The B-CLL/SLL samples were stained heterogeneously by Ki-67, an antibody that can recognize an antigen expressed in all phases of the cell cycle except G0 (Fig. 2, C and D). The distribution of cells positive for MUM1 or Ki-67 in the MUM1-positive lymph node samples were similar, although the same tumor cells did not always show double positivity. Also, the MUM1-positive samples were not always positive for Ki-67 and *vice versa*. The overall positivity for CD38 among the B-CLL/SLL samples was 34% (10/29 cases) on immunohistochemical analysis; however, CD38 positivity was not associated with positivity for MUM1 (7 and 3 samples showing CD38 expression were MUM1-positive and MUM1-negative, respectively) (Table IIIA). On the other hand, all specimens were negative for CD138.

Status of SH of the IgV_H gene Of the 21 cases, 12 cases

Table III. Relationship between IgV_H Status, MUM1 and CD38 Expression

A)

		MUM1 +	MUM1 −	Total
CD38	+	7	3	10
	−	7	12	19
	Total	14	15	29

NS[a]

B)

		CD38 +	CD38 −	Total
SH	+	3	9	12
	−	4	5	9
	Total	7	14	21

NS[a]

C)

		MUM1 +	MUM1 −	Total
SH	+	7	5	12
	−	5	4	9
	Total	12	9	21

NS[a]

a) Fisher's exact test.
NS: no significance.
PCR amplification and sequencing of the IgV_H gene, and determination of the presence or absence of SH, were performed in 21 cases.

harbored SH in their IgV_H gene and the other 9 cases had an IgV_H gene with a germline sequence. The nucleotide sequences of clones of the IgV_H gene of representative B-CLL/SLL cases with or without IgV_H SH are shown in Fig. 3. No sample showed ongoing mutation in our series. In contrast to several previous reports,[9, 11] CD38 expression as detected by our immunostaining procedure was not significantly associated with the status of the IgV_H gene (Table IIIB). There was no significant relationship between the status of the IgV_H gene and MUM1 expression (Table IIIC).

Overall survival of B-CLL/SLL patients according to MUM1 expression and IgV_H status The patients who were positive for MUM1 showed significantly shorter overall survival than those who were negative for MUM1 (50% survival±SE [standard error]: 22±16 months vs. 82±35 months) as shown in Fig. 4A (log-rank test, P=0.0008; Wilcoxon's test, P=0.0008). Among the MUM1-positive patients, 3 (21%) and 6 (43%) patients were categorized into an early stage of A or B, respectively, according to Binet's staging system. The patients who did not have IgV_H SH showed significantly shorter overall survival than those with SH (50% survival±SE: 20±24 months vs. 64±10 months) (Fig. 4B). Survival analysis based on the CD38 expression status did not show any significant difference between the CD38-positive and -negative groups (data not shown).

Prognostic factors for B-CLL/SLL Univariate analysis using Cox's proportional-hazards regression model identified the following unfavorable prognostic factors for overall survival: increased age, elevated white blood cell (WBC) count, low hemoglobin concentration, high serum

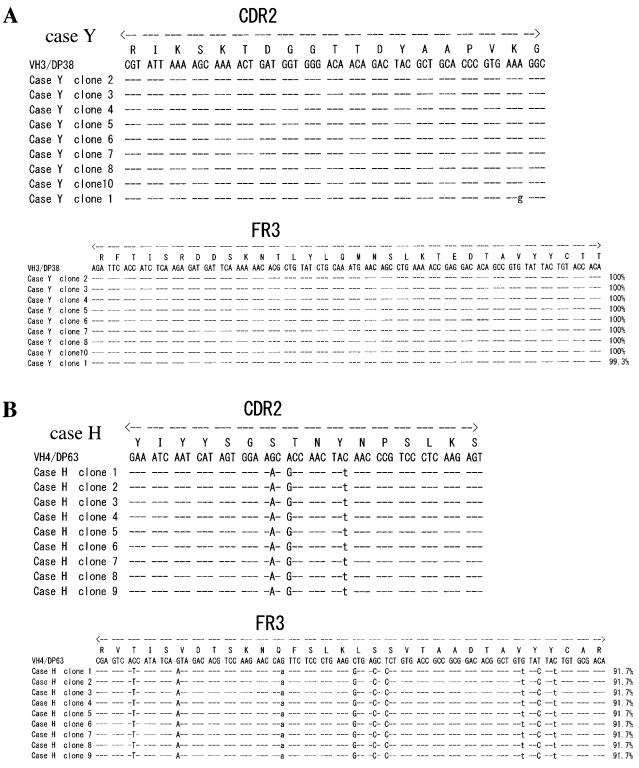

Fig. 3. IgV_H gene status in representative cases with B-CLL/SLL. (A) Nucleotide sequence of the IgV_H gene of 10 clones from a representative B-CLL case without IgV_H SH. The sequences of the clones are compared with that of VH3/DP38, a germline sequence. The deduced amino acid sequence is shown on the top. Nucleotide substitutions resulting in nonsense and missense mutations are indicated by small letters and large letters, respectively. (B) Nucleotide sequence of the IgV_H gene of 9 clones from a representative B-CLL case with IgV_H SH. The sequences of the clones are compared with that of VH4/DP63, a germline sequence.

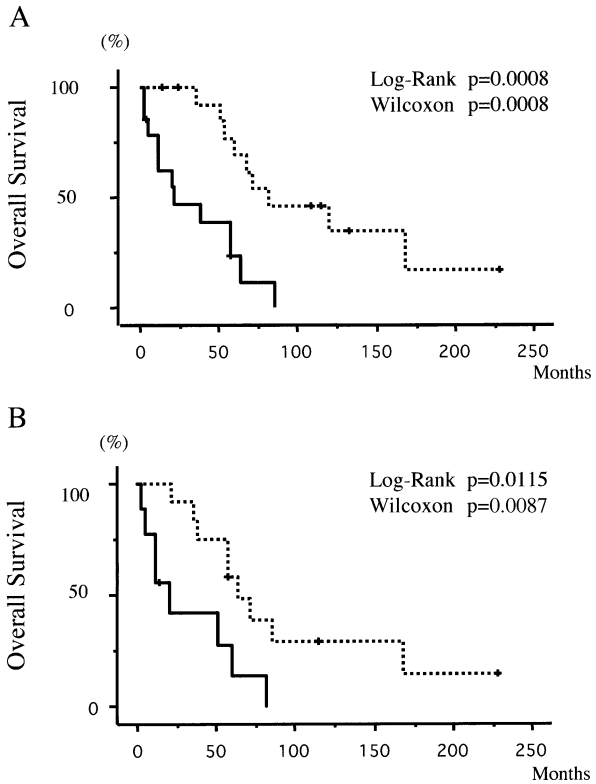

Fig. 4. Comparison of the survival curves of B-CLL/SLL cases according to the Kaplan-Meier method. (A) Comparison of the overall survival curves of the B-CLL/SLL patients based on the MUM1 expression status. Dashed and solid lines respectively indicate survival curves of the patients whose specimen show MUM1-negativity (n=15) and MUM1-positivity (n=14). (B) Comparison of the overall survival curves of the B-CLL/SLL patients based on the presence or absence of IgV_H SH. Dashed and solid lines respectively indicate survival curves of the patients who have mutated (SH^+: n=12) and unmutated (SH^-: n=9) IgV_H phenotype.

Table IV. Prognostic Factors Affecting Overall Survival

Category n=20	Univariate[a]		Multivariate[b]	
	Hazards ratio (CI)	P value	Hazards ratio (CI)	P value
Age (1-year increment)	1.08 (1.01–1.16)	0.022	1.16 (1.04–1.29)	0.0070
WBC (1000/μl increment)	1.05 (1.01–1.10)	0.014	—	
Hemoglobin (1g/dl increment)	0.749 (0.572–0.981)	0.036	—	
LDH (10 IU/liter increment)	1.05 (1.00–1.11)	0.049	—	
Clinical stage (advanced/early)	6.23 (1.46–26.5)	0.013	—	
MUM1 (positive/negative)	3.43 (1.13–10.4)	0.030	44.3 (5.09–386)	0.0006
IgV_H SH (absence/presence)	1.38 (1.34–11.7)	0.013	16.1 (2.45–106)	0.0038

CI indicates confidence interval.
n indicates number of analyzed patients, data on all analyzed parameters could not be obtained in 9 patients.
a) Other categories that did not show statistical significance with respect to prognosis by univariate Cox proportional hazard model are: gender (male/female), platelets (1000/μl increment), splenomegaly (presence/absence), CD38 (positive/negative) and Ki-67 (positive/negative).
b) Final model.

lactate dehydrogenase (LDH), advanced clinical stage, MUM1 expression, and absence of IgV_H SH (Table IV). Multivariate analysis including 20 cases with all of the above-mentioned variables demonstrated age, absence of IgV_H SH and MUM1 expression as being significant and independent unfavorable prognostic factors (Table IV).

DISCUSSION

Various parameters have been reported thus far as biological prognostic indicators, and they include positivity for CD38,[9, 11] absence of IgV_H SH,[9, 10] FcμR constellation,[35] p53 abnormality,[36–39] high Ki-67 index[40] and trisomy 12.[13, 14] In this study, we identified that MUM1 expression was an unfavorable prognostic factor in B-CLL/SLL. Among the MUM1-positive patients, 9 out of 11 B-CLL patients were diagnosed as belonging to stage A or B in Binet's staging system. Hence, analysis of MUM1 expression could extract patients whose prognosis was unfavorable irrespective of the clinical stage. Absence of IgV_H SH was another independent prognostic factor in our series. This result was consistent with several previous reports from Western countries,[9, 10] suggesting that B-CLL of post-GC cell origin is associated with better prognosis than that of pre-GC cell origin. An intriguing result was that MUM1 expression was not associated with the cell origin of the B-CLL/SLL cells and was determined to be an independent prognostic factor. In addition, only 2 out of the 4 B-CLL cell lines expressed MUM1, indicating that the expression pattern of MUM1 is heterogeneous even in the cell lines. This suggested to us that MUM1 expression was not merely associated with disease progression in patients, but that it is a distinct biological marker that can be used to categorize B-CLL patients at the time of diagnosis.

It remains unknown why MUM1 is expressed in only a percentage of B-CLL/SLL cases. We, therefore, examined whether MUM1 expression in B-CLL cell lines was due to deregulated expression as a result of t(6;14)(p25;q32), as is the case with multiple myeloma, since Cuneo *et al.*[41] recently reported that the 6p24–25 locus was occasionally involved in non-random rearrangements in B-CLL cases. However, this hypothesis was refuted by the results of the DCFISH analysis of two B-CLL cell lines expressing MUM1. We and other investigators have previously reported that MUM1 expression in B-cells is induced either by lymphocyte activation or by differentiation into plasma cells.[21, 22] As none of our B-CLL/SLL samples was positive for the plasma cell marker, CD138/syndecan-1 antigen, it is speculated that MUM1 expression was induced by some sort of lymphocyte activation signal(s) that is constitutively active in a proportion of B-CLL/SLL cases. Unexpectedly, CD38 expression was not associated with MUM1 expression in our study, which was contradictory to our previous data showing coexpression of these two antigens in normal B-lymphocytes.[21] Although many investigators have recently reported that a high level of CD38 expression as detected by flow cytometric analysis (FACS) in B-CLL cells is associated with poor prognosis,[9, 11] the CD38-positive subgroup did not show a poorer prognosis in our series of patients. This discrepancy might be explained by the following two reasons. One is the detection method for CD38 expression. We used immunohistochemistry, which might have different sensitivity from flow cytometric analysis. Another possibility is that Japanese B-CLL may differ from that in Caucasians. Besides the difference in its incidence, several morphologic differences between Western and Japanese B-CLL have been reported.[4] B-CLL in patients in Western countries generally shows proliferation of well-differenti-

ated small lymphocytes,[42, 43] while the B-CLL in Japanese patients occasionally shows a greater percentage of large lymphocytes,[4] although immunophenotypic and karyotypic analyses have revealed similar results between B-CLL in Japan and that in Western countries.[44, 45]

The reason why MUM1-positive B-CLL patients show significantly unfavorable outcome in survival analysis remains uncertain. Transforming activity of MUM1 has been recognized in a fibroblast cell line, Rat-1, and primary chicken embryonic fibroblasts.[15, 46] Thus, MUM1 expression may induce the growth of leukemic cells and may result in aggressive clinical behavior. Additionally, Bechter *et al.*[47] reported that the telomere length and telomerase activity were correlated with survival among patients with B-CLL. Since MUM1 has been suggested to extend the lifespan of chicken primary embryonic fibroblasts,[46] its expression could delay senescence of tumor cells. The relationship between MUM1 and telomerase activity needs to be further investigated. Another interesting point from cDNA microarray analysis was that most of the genes whose expression level was correlated with the survival of B-CLL patients, encoded cell adhesion-related molecules.[48, 49] Since MUM1 principally acts as a transcriptional factor that is indispensable for B- and T-cell activation and differentiation,[50] the potential association between MUM1 expression with such downstream gene expression needs to be clarified. Similarly, MUM1/IRF4 was reported to be one of the important components that define the activated B-cell-like subtype of DLCL,[25] suggesting that the *MUM1* gene is one of the genes simply related to the proliferating potential of tumor cells. In our series, Ki-67 expression, which was reported as a poor prognostic factor in low-grade and high-grade non-Hodgkin's lymphomas,[40, 51] was not associated with MUM1 expression and was not correlated with prognosis (data not shown). The lack of a relationship between the expression of MUM1 and Ki-67 suggests that MUM1 expression does not simply represent proliferative activity.

In conclusion, our study demonstrated the usefulness of MUM1 staining on formalin-fixed specimens as a prognostic factor in B-CLL/SLL and for extracting a distinct population of B-CLL/SLL patients who are likely to show a poorer prognosis. This would be followed by designing risk-adapted treatment strategies for individual patients. Further prospective studies to confirm the significance of MUM1 in B-CLL/SLL are warranted, as well as basic research to explore the biological role of MUM1 in B-CLL cells.

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