Primary cutaneous marginal zone B-cell lymphoma: a molecular and clinicopathological study of cases from Asia, Germany, and the United States

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Primary cutaneous marginal zone B-cell lymphoma is considered the cutaneous counterpart of extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Although its molecular pathogenesis is currently unknown, an etiological link with *Borrelia burgdorferi* infection has been identified in European, but not in American or Asian cases. To better understand the pathogenesis and the geographical differences of cutaneous marginal zone B-cell lymphoma, 60 cases from the East Asia, Germany, and the United States at their initial presentation were subjected to the following analyses; (1) clinicopathological comparison between the geographical regions, (2) detection of *B. burgdorferi* DNA, (3) detection of the *API2-MALT1* fusion transcript, a gene alteration specific to mucosa-associated lymphoid tissue lymphoma, and (4) inactivation of tumor suppressor genes (death-associated protein kinase (*DAPK*), *p16^{INK4a}*, *p14^{ARF}*, *MGMT*, *TIMP3*, *CDH1*, and *RARB*) by hypermethylation of the CpG islands. Cases from the three geographical regions showed similar clinicopathological features. However, moderate/marked tissue eosinophilia was found in 9/25 Asian cases, but only 1/23 German cases ($P=0.011$) and 0/12 American cases ($P=0.015$). All 60 cases were negative for either *Borrelia* DNA or *API2-MALT1* fusion. Tumors from the three regions were highly methylated for *DAPK* (38–50% of the cases, mean 43%) and *p16^{INK4a}* (42–70%, mean 49%), and the positivities were significantly higher than those of nonneoplastic skin (8%, $P=0.0010$ and 14%, $P=0.0032$, respectively). Methylation of these genes had no significant association with progressive features of the tumor. Primary cutaneous marginal zone B-cell lymphomas from the three geographical regions have common clinicopathological features, however, moderate/marked tissue eosinophilia is a feature found almost exclusively in Asian cases. *Borrelia* infection and *API2-MALT1* fusion are not significant in this tumor. Methylation of *DAPK* and *p16^{INK4a}* genes is a frequent event in this lymphoma at its initial presentation, but may not be associated with tumor progression.

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Primary cutaneous marginal zone B-cell lymphoma is now considered a distinct entity in the World Health Organization (WHO)–European Organization Research and Treatment of Cancer (EORTC) classi-

fication of cutaneous lymphomas.^{1,2} This tumor is histologically characterized by small B cells including marginal zone (centrocyte-like), monocy-toid, and plasmacytoid lymphocytes, and plasma cells, and is considered part of the broad group of extranodal marginal zone B-cell lymphomas commonly involving mucosa-associated lymphoid tissue (MALT). This lymphoma is rare, often affects adults aged more than 40 years of age with no clear gender preponderance, and has a favorable prognosis.^{1–3}

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The etiology of cutaneous marginal zone B-cell lymphoma has not been well clarified. Evidence suggesting a link between this lymphoma and *Borrelia burgdorferi* infection has been reported from many European countries including Germany, and penicillin therapy may cure *B. burgdorferi*-associated cases.^{4–7} However, this association has not been evident in the United States or Asia.^{8,9} *API2-MALT1* fusion, resulting from t(11;18)(q21;q21) is specific to MALT lymphoma, and has been found in 10–20% of gastric cases and in up to 50% of pulmonary cases, but is rare in autoimmune-associated cases.¹⁰ This gene alteration has been reported in 0–7.8% of cutaneous marginal zone B-cell lymphomas,^{9,11–16} but overall its frequency is not clear.

As the skin is one of the most important immune defense systems, cutaneous lymphoproliferative disorders are considered to be associated with environmental or geographical factors, especially in marginal zone B-cell lymphoma, which shows a close association with chronic inflammation. The present international study with cutaneous marginal zone B-cell lymphoma from East Asia, Germany, and the United States was designed to investigate any possible geographical differences among these cases, and to detect underlying biologic or genetic abnormalities. We analyzed the clinicopathological features of 60 cases of primary cutaneous marginal zone B-cell lymphoma, and searched for *B. burgdorferi* infection and *API2-MALT1* fusion involvement. In addition, we studied inactivation of tumor suppressor genes by hypermethylation of the CpG islands, which is one of the important steps in the development of clinically overt tumors including malignant lymphoma.

Materials and methods

Case Selection

Asian cases of primary cutaneous marginal zone B-cell lymphoma were retrieved from the files of Department of Pathology, Chang Gung University, Taiwan, and Department of Pathology, Nagoya City University, Japan. Cases from Germany and the United States were retrieved from the files of the Department of Pathology, University of Wuerzburg, and the Department of Pathology, University of Pittsburgh, respectively. A total of 105 cases suspected of cutaneous marginal zone B-cell lymphoma were reviewed by hematopathologists. Of them, 55 cases were excluded either because they did not fulfill the criteria of the criteria for a cutaneous marginal zone B-cell lymphoma according to the criteria of WHO–EORTC classification for cutaneous lymphomas,^{1,2} they were recurrences, or they lacked demonstrable monoclonality either based on immunoglobulin light chain restriction or *IGH* gene rearrangement.⁹ Clinical data (age, sex, primary site, treatment, response, and follow-up) were obtained.

Clinical data concerning gross appearance and number of lesions (solitary or multiple) were available in Asian cases. In all cases, there was no history of extracutaneous lymphoma, either previous or concurrent, and no evidence of extracutaneous lymphoma for 6 months after the appearance of the cutaneous lesion.

All cases were within the morphological boundaries of primary cutaneous marginal zone B-cell lymphoma, and exhibited the following immunophenotype: CD20(+), CD79a(+), CD3(–), CD45RO(–), CD5(–), CD10(–), CD23(–), BCL2(+), BCL6(–), and cyclin D1(–). The following pathological/phenotypic factors were analyzed in the cases from three geographical regions: proliferation pattern, increased lymphoid follicles, marked plasma cells, increased large cells, tissue eosinophilia, and nuclear BCL10 expression. When most lymphoma cells showed intense nuclear BCL10 expression, the case was considered as positive. Cases were considered to have increased large cells when more than 5% and less than 10% large cells were observed without solid or sheet-like proliferations.¹⁷ Tissue eosinophilia was semi-quantified into three groups: absent or mild, less than 50 eosinophils/10 high power fields (HPFs); moderate, 50–200 eosinophils/10 HPFs; and marked, more than 200 eosinophils/10 HPFs. Local ethical guidelines were followed for the use of archival materials for research, and such use was approved by the ethics committees of the authors' institutions where required. As a control, 29 nonneoplastic skin biopsies were obtained from Japanese patients. All specimens were fixed in formalin, and embedded in paraffin. Some of the tumors from Asian cases in this series were included in our previous study.⁹

Extraction of Genomic DNA and Total RNA

Genomic DNA and total RNA were extracted from paraffin sections in digestion buffer containing proteinase K as previously described.¹⁸ For lymphoma cases, only lymphomatous areas were scraped with examination of tissue sections under a dissecting microscope, using a serial H&E section as a guide. For control skin, only the dermal layer was scraped. We confirmed that all cases had a sufficient quality of DNA and RNA by polymerase chain reaction (PCR) for β -globin gene and reverse transcription (RT)–PCR for β -actin mRNA, respectively.

PCR Detection of *Borrelia* DNA

As reported previously, we performed PCR using consensus primers for *B. burgdorferi hbb* gene, which encodes a protein of histone-like family. The PCR product is expected to be 184 bp in length, and well suited to DNA extraction from paraffin tissue materials.⁹ This PCR assay should cover

39 *Borrelia* strains. DNA extracted from heat-treated *B. burgdorferi* (Kirkegaard and Perry Lab, Gathersburg, MD, USA) was used as a positive control. The sequence analysis of the PCR product of the positive control showed 98% homology with the reference *B. burgdorferi* strain, B31.⁹ Normal human DNA extracted from various paraffin-embedded materials, including the skin, spleen, and lymph nodes, yielded no positive bands in this PCR assay.

Multiplex RT-PCR for the *API2-MALT1* Fusion Transcript

The *API2-MALT1* fusion transcript was detected according to the method we previously described.¹⁹ All eight variant transcripts known to date can be detected with this assay. Briefly, total RNA was subjected to first-round multiplex one-tube RT-PCR, then to second-round nested multiplex PCRs (three in parallel). This RT-PCR assay using paraffin tissues is highly efficient and has 94% of the sensitivity and 100% of the specificity obtained with RT-PCR using frozen materials.²⁰

Methylation-Specific PCR

As described elsewhere, DNA from the specimens was first subjected to bisulfite treatment.²¹ Denatured DNA was modified by reaction with sodium bisulfite under conditions that convert all unmethylated cytosine residues to uracil by deamination. Modified DNA was purified, and modification was completed by NaOH treatment, followed by ethanol precipitation. Bisulfite-modified DNA was then subjected to PCR amplification using primers designed to anneal specifically to the methylated and unmethylated DNA within a given gene. In this study, we analyzed the hypermethylation status in the promotor CpG islands of following seven tumor suppressor genes: death-associated protein kinase (*DAPK*), *p16^{INK4a}* (*p16*), *p14^{ARF}* (*p14*), *O*-6-methylguanine-DNA methyltransferase (*MGMT*), tissue inhibitor of metalloproteinase 3 (*TIMP3*), E-cadherin (*CDH1*), and retinoic acid receptor- β (*RARB*). The primer sequences are shown in Supplementary data 1. PCR was performed for 35 cycles, and the annealing temperature ranged from 55 to 63°C depending on the primer pairs used.

Statistical Analysis

Statistical comparisons were performed using Fischer's exact test and χ^2 -test. To identify the parameters significantly associated with an overall survival, the statistical difference was estimated using the log-rank and Wilcoxon tests. All analyses were two tailed. A value of $P < 0.05$ for each test was regarded as statistically significant. All of the

analyses were performed using the statistical package JMP (SAS Institute Inc., Cary, NC, USA).

Results

Clinicopathological Findings

All 60 cases of primary cutaneous marginal zone B-cell lymphoma occurred in adults (mean age of 56 years, median 57, range 26–87), with no differences in age between patients from the three regions (Asia, Germany, and the United States; Table 1). No gender preponderance was noted. In 25 Asian cases, tumors were found in the head and neck, trunk, and upper extremities in 10 (40%), 8 (32%), and 7 (28%) cases, respectively. Tumors were frequently found on the trunk (9/23, 39%) and upper extremities (6/23, 26%) in German cases, and mostly on the trunk (9/12, 75%) in the cases from the United States. The cases from the three geographical regions showed similar histological findings (Figure 1a–d). The dermis showed a nonepidermotropic diffuse and nodular proliferation of small- to medium-sized lymphoid cells with reactive lymphoid follicles. The lymphoid cells had centrocyte-like or monocytoid appearance with pale cytoplasm, slightly irregular nuclei, and inconspicuous nucleoli. Marked plasmacytic differentiation was found in 19/60 (32%) cases, and increased large cells in 12/60 (20%) cases. Moderate or marked tissue eosinophilia in the tumor tissue was detected frequently in 9 (36%) of 25 Asian cases (Figure 1e), but in only 1 (4%) of 23 German, and none of 12 American cases ($P = 0.011$, Asia vs Germany; $P = 0.015$, Asia vs the United States; Table 1). There were no significant differences between Asian cases with and without tissue eosinophilia with regard to the following clinicopathological factors: age, sex, primary site, gross appearance, solitary or multiple, proliferation pattern, increased lymphoid follicles, marked plasma cells, and nuclear BCL10 expression. Nuclear BCL10 expression was detected in 30–40% of the cases, and there were no regional differences (Table 1).

Follow-up data were retrieved from 27 cases (Table 2). The follow-up period ranged from 7 to 180 months (median 36 months, mean 57 months). After a variety of treatments including surgical excision, topical steroid, psoralen and ultraviolet A phototherapy, and chemotherapy, 17 patients achieved a complete remission and 3 patients showed a partial remission, whereas 2 patients showed no change, and 3 showed progressive disease. Response to treatment was unknown in two cases. Of 17 patients with a complete remission, 5 relapsed. One case, which was observed with no treatment, showed spontaneous regression and no recurrence during the follow-up (Case 2, Table 2). Another case, which had no treatment, showed partial tumor regression but with persistent tumor 60 months after its initial

Table 1 Comparison of clinical and histological findings between primary cutaneous marginal zone B-cell lymphoma cases from Asia, Germany, and the United States

Factor	All regions (n = 60)	Asia (n = 25)	Germany (n = 23)	USA (n = 12)	P		
					Asia vs Germany	Asia vs USA	Germany vs USA
Clinical findings							
Age (years)							
> 60	26	7	12	7	NS	NS	NS
< 60	34	18	11	5			
Sex							
Male	31	12	15	4	NS	NS	NS
Female	29	13	8	8			
Primary site (n = 56)							
Head and neck	15	10	2	3	NS	0.028	NS
Trunk	26	8	9	9			
Upper extremities	13	7	6	0			
Others	2	0	2	0			
Histological findings							
Proliferation pattern							
Nodular	25	14	7	4	NS	NS	NS
Nodular and diffuse	35	11	16	8			
Increased lymphoid follicles							
Yes	32	17	11	4	NS	NS	NS
No	28	8	12	8			
Marked plasma cells							
Yes	19	5	9	5	NS	NS	NS
No	41	20	14	7			
Increased large cells							
Yes	12	4	6	2	NS	NS	NS
No	48	21	17	10			
Tissue eosinophilia							
Moderate or marked	10	9	1	0	0.011	0.015	NS
Absent or mild	50	16	22	12			
Nuclear BCL10							
Positive	21	10	7	4	NS	NS	NS
Negative	39	15	16	8			

NS, not significant.

presentation (Case 13, Table 2). None of the patients died of the lymphoma.

***Borrelia* DNA and the *API2-MALT1* Fusion Transcript**

Satisfactory preservation of DNA was verified in all 60 cases by successful amplification of the β -globin DNA fragments. *Borrelia* DNA was not found in any case from Asia, Germany, or the United States (Figure 2). This negative result was confirmed by at least two separate experiments whereas the positive control for *Borrelia* DNA constantly showed a distinct PCR band. The *API2-MALT1* fusion transcript was examined using multiplex RT-PCR. A sufficient quality of RNA was preserved in all 60 cases as evidenced by amplification of reference β -actin mRNA. No case showed the fusion transcript, a finding that was confirmed by repeat

assays (Figure 3). Positive controls yielded definite bands.

Gene Methylation Status

As shown in Figure 4, the promoter regions of seven tumor suppressor genes (*DAPK*, *p16*, *p14*, *MGMT*, *TIMP3*, *CDH1*, and *RARB*) were analyzed for gene methylation in 89 skin biopsies (60 tumor cases and 29 cases of nonneoplastic skin). As summarized in Figure 5, methylation status was successfully evaluated for the former five genes in more than 88% of the cases, although the evaluation was somewhat lower in the latter two with mean detection rates of 77 and 73%, respectively. *DAPK* gene methylation was detected in 9/24 (38%), 6/12 (50%), and 10/22 (45%) of tumor cases from Asia, Germany, and the United States, respectively (differences not significant), with an average of 25/58 (43%) cases. *DAPK*

was methylated in 2/26 (8%) nonneoplastic control cases. *DAPK* methylation ratios for each geographical region and that of total cases were significantly higher than that of nonneoplastic skin (Table 3). *p16*

gene methylation was frequently detected in cutaneous marginal zone B-cell lymphoma cases, and the detection was similar between the three regions (42, 47, and 60% in Asia, Germany, and the United States, respectively) with an average of 47% (25/53 cases). *p16* gene methylation in tumor cases was significantly higher than control cases (4/28, 14%; Table 4). The other five genes (*p14*, *MGMT*, *TIMP3*, *CDH1*, and *RARB*) were differentially methylated in 47, 7, 15, 31, and 14% of the tumor cases, respectively. These methylation ratios were not statistically different from those of the control skin.

When association of gene hypermethylation with clinicopathological factors was analyzed, none of the following factors showed a statistically significant association with the methylation status of either *DAPK* or *p16* gene (Table 4): age, sex, primary site, gross appearance, proliferation pattern, increased lymphoid follicles, marked plasmacytic differentiation, increased large-cell element, tissue eosinophilia, and *BCL10* expression. Prognostic analysis showed that *p16* and *DAPK* hypermethylation had no impact on the disease-free survival of the patients (data not shown).

Discussion

In the present study, we examined 60 cases of primary cutaneous marginal zone B-cell lymphoma from Asia, Germany, and the United States. These cases generally showed clinicopathological features similar to those that have been described. However, a predominant localization to the trunk and upper extremities^{1,2} was not shown in this study. The prognosis of the patients was excellent with no tumor-related deaths recorded in this study. However, the therapeutic response was not very satisfactory. A complete remission was achieved in 17 (68%) of 25 cases with known responses to treatment. Among these 17 patients, 5 cases showed tumor recurrence but without any lymphoma-related deaths. In total, 13/25 (52%) patients were alive with disease at the last follow-up. This finding confirmed previous observations that primary cutaneous marginal zone B-cell lymphoma is an indolent tumor, but often shows resistance to or recurrence after treatment of the tumor.^{3,22}

Of note, we found that moderate or marked tissue eosinophilia was observed frequently in Asian

Figure 1 Histopathological findings in primary cutaneous marginal zone B-cell lymphoma. (a) Nodular proliferation of dense lymphoid cells mainly in the dermis. H&E staining, original magnification $\times 40$. (b) Lymphoid and plasmacytic cells with frequent intranuclear pseudoinclusions (Dutcher bodies, arrows), H&E staining, original magnification $\times 400$. (c, d) Tumor cells showing immunoglobulin light chain restriction (c, κ -type; d, λ -type), hematoxylin counterstain, original magnification $\times 400$. (e) A representative case of tissue eosinophilia (an Asian case). This case is graded as 'moderate'. H&E staining, original magnification $\times 200$.

Table 2 Treatment, response, and follow-up of primary cutaneous marginal zone B-cell lymphoma

Case	Region	Age	Sex	Treatment	Response	Follow-up (months)	Comments
1	Asia	71	M	Surgical excision	PD	12	Recurrence in other sites
2	Asia	27	F	None	CR (spontaneous)	24	No recurrence
3	Asia	49	M	Surgical excision	CR	24	No recurrence
4	Asia	70	F	Topical steroid	NC	24	Tumor persisting
5	Asia	65	M	Surgical excision	CR	32	No recurrence
6	Asia	57	F	Chemotherapy	CR	36	No recurrence
7	Asia	44	M	Surgical excision	CR	168	Recurrence in other sites
8	Asia	69	M	Topical steroid	PD	96	Recurrence in other sites
9	Asia	40	F	Surgical excision	CR	36	No recurrence
10	Asia	54	F	None	PD	8	Tumor persisting
11	Germany	75	F	Unknown	PR	120	Tumor persisting
12	Germany	52	F	Surgical excision	CR	36	No recurrence
13	Germany	65	F	None	PR (spontaneous)	60	Tumor persisting
14	Germany	45	F	Radiation	CR	48	No recurrence
15	Germany	50	F	Radiation	CR	48	No recurrence
16	Germany	40	M	Unknown	CR	7	Recurrence
17	Germany	35	F	Surgical excision and radiation	CR	180	Recurrence
18	Germany	78	M	Chemotherapy	PR	60	Tumor persisting
19	Germany	70	M	Radiation and topical steroid	CR	72	Recurrence
20	Germany	74	M	Radiation	CR	96	No recurrence
21	USA	58	M	Surgical excision	CR	53	No recurrence
22	USA	68	F	Unknown	Unknown	96	Alive and well
23	USA	56	M	Surgical excision	CR	32	No recurrence
24	USA	72	F	Unknown	NC	9	Tumor persisting
25	USA	77	M	Unknown	Unknown	108	Alive and well
26	USA	53	F	Radiation	CR	33	No recurrence
27	USA	67	F	Surgical excision	CR	19	No recurrence

CR, complete remission; PR, partial remission; NC, no change; PD progressive disease; M, male; F, female.

Figure 2 Detection of *Borrelia* DNA by PCR amplifying a fragment of conserved *hbb* gene. N, negative control; P, positive control; lanes 1–7, tumor cases showing no detectable PCR products. bp, base pair.

Figure 3 Detection of *API2-MALT1* fusion transcript by multiplex RT-PCR (second PCR-B). N, negative control; P1–P4, four variants of *API2-MALT1* fusion used as positive controls (note that PCR bands vary in size); lanes 1–7, tumor cases negative for the fusion transcript.

cases, which has not been noted before. In contrast, this finding was almost absent in Germany and the United States cases. Tissue eosinophilia has been reported in 4 of 15 American cases,²³ although the degree of eosinophilia was not specified. In malignant lymphoma, tissue eosinophilia is often associated in Hodgkin's lymphoma and T-cell lymphoma.^{24,25} This phenomenon may be associated with production of certain cytokines by the tumor cells, and may have a prognostic significance.²⁶ In B-cell lymphomas, however, tissue eosinophilia is

Figure 4 Methylation-specific PCR for methylation status of seven tumor suppressor genes, *DAPK*, *p16*, *p14*, *MGMT*, *TIMP3*, *CDH1*, and *RARB*. Cases 1–8, tumor cases; M, methylated; U, unmethylated. The PCR product sizes are shown in Supplementary data 1.

rare, and its significance is unknown.²⁷ In the present series, we found no significant differences in the clinicopathological features between those with and without tissue eosinophilia. We speculate that frequent tissue eosinophilia may be associated with ethnic or geographical factors. One such example is Kimura's disease, which is characterized by marked eosinophilia.^{28,29} This benign disorder, involving the skin as well as the lymph nodes, is known to be comparatively frequent in Asian countries.

Region	Case	DAPK	p16	p14	MGMT	TIMP-3	CDH1	RARB
Asia	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
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Figure 5 Methylation profile of seven tumor suppressor genes in 60 cases of primary cutaneous marginal zone B-cell lymphoma and 29 cases of the control skin. Filled boxes indicate methylated sites, open boxes indicate unmethylated sites, and gray boxes indicate that the methylation status is not determined.

Table 3 Methylation of *DAPK* and *p16* genes between geographical regions

Case	Gene methylation (%)			
	DAPK	P ^a	p16	P ^a
Asia	9/24 (38)	0.016	10/24 (42)	0.033
Germany	10/22 (45)	0.0059	9/19 (47)	0.020
USA	6/12 (50)	0.0066	7/10 (70)	0.0022
All regions	25/58 (43)	0.0010	26/53 (49)	0.0032
Control	2/26 (8)		4/28 (14)	

^aComparison with control.

A subset of primary cutaneous marginal zone B-cell lymphoma cases has been associated with *B. burgdorferi*, however, there appears to be geographic variation. *Borrelia* infection has been documented serologically, and *Borrelia*-specific DNA has been detected by PCR and Southern blot analysis.⁴⁻⁷ In addition, antibiotic therapy has been effective in some cases that were positive for *B. burgdorferi*.^{6,7} Interestingly, this association has been reported mainly from Europe, but rarely from the United States.^{8,30} We previously reported that such an association is also rare in Asian cases.⁹ In this international study including cases from Asia, Germany, and the United States, we found that none of the cases was positive for *B. burgdorferi* DNA. Although this negative PCR result does not exclude the possibility of a link between the tumor and *B. burgdorferi* infection, the microorganism is not significant in the development or pathogenesis of the cases of primary cutaneous marginal zone B-cell lymphoma.

The molecular pathogenesis of cutaneous marginal zone B-cell lymphoma is poorly understood with the translocations associated with other MALT lymphomas reportedly found in few cases. Streubel *et al*¹² reported *API2-MALT1* translocation in 4 of 51 cases, but others, including the cases reported here, did not detect any cases with this abnormality.^{9,11,13-16} The *IGH-MALT1* translocation has been reported in 7/51 cases¹² and 3/12 cases.¹⁵ However, it was absent in several other studies.^{13,14} The explanation for these variable results remains unknown. In MALT lymphomas, an association between nuclear BCL10 expression and the presence of *API2-MALT1* fusion has been suggested.^{10,31} However, such an association was not evident in our series where frequent nuclear BCL10 expression in tumor cells was shown in the absence of *API2-MALT1* fusion, as has been reported previously.^{9,16} In cutaneous marginal zone B-cell lymphoma, nuclear BCL10 expression has not been associated with *BCL10* gene mutation⁹ or other *MALT1* gene translocations.¹⁶ These molecular data indicate that some other mechanisms exist for nuclear BCL10 expression in cutaneous marginal zone B-cell lymphoma.

Inactivation of tumor suppressor genes by hypermethylation of the CpG islands is one of the

Table 4 *DAPK* and *p16* gene methylation and clinicopathological factors

Factor	DAPK <i>gene</i>			p16 <i>gene</i>		
	Methylated	Unmethylated	P	Methylated	Unmethylated	P
<i>Clinical</i>						
Age (years)						
>60	8	17	NS	12	10	NS
<60	17	16		13	18	
Sex						
Male	12	18	NS	17	11	NS
Female	13	15		8	17	
Primary site						
Head and neck	5	10	NS	5	8	NS
Others	20	23		20	20	
Gross appearance ^a						
Plaque or papule	3	4	NS	2	5	NS
Nodule	6	11		8	9	
No. of lesions ^a						
Solitary	3	11	NS	5	9	NS
Multiple	5	4		5	4	
<i>Histological</i>						
Proliferation pattern						
Nodular	8	15	NS	9	12	NS
Nodular and diffuse	17	18		17	15	
Increased lymphoid follicles						
Yes	15	17	NS	14	16	NS
No	10	16		12	11	
Marked plasma cells						
Yes	8	9	NS	9	7	NS
No	17	24		17	20	
Increased large cells						
Yes	6	6	NS	6	5	NS
No	19	27		20	22	
Tissue eosinophilia						
Absent or mild	22	26	NS	22	22	NS
Moderate or marked	3	7		4	5	
Nuclear BCL10						
Positive	9	11	NS	11	8	NS
Negative	16	22		15	19	

NS, not significant.

^aData from Asian cases.

important processes in the development of clinically overt tumors. To the best of our knowledge, this epigenetic abnormality has not been studied in primary cutaneous marginal zone B-cell lymphoma. We therefore analyzed methylation of seven tumor suppressor genes, and found that *DAPK* and *p16* genes were highly methylated in tumor cases whereas nonneoplastic skin tissues (from Asian individuals) were rarely methylated. A similar low methylation of the two genes in nonneoplastic skin has been reported from the United States.³²

DAPK is a novel calcium/calmodulin-dependent and cytoskeletal-associated serine/threonine kinase with death-inducing functions.^{33,34} The *DAPK* gene

was initially isolated as a positive mediator of apoptosis induced by interferon- γ . Overexpression of *DAPK* in tumor cells led to cell death in the absence of any external stimuli. Recent studies showed that this molecule also participates in tumor necrosis factor- α and Fas-induced apoptosis.^{35,36} The *p16* gene encodes for nuclear protein that can block cell-cycle progression from the G₁ to the S phase by effectively inhibiting the kinase activity of CDK4/6, thereby exerting a negative control on cell proliferation.^{37–39} Loss of *p16* expression gives a remarkable growth advantage in cells. These observations highlight a potential role of *DAPK* and *p16* as tumor suppressor genes. We showed that both *DAPK*

and *p16* genes were frequently methylated in tumor cases at their initial presentation (approximately 45% of the cases) and that these epigenetic alterations had no impact on clinicopathological and prognostic features of this lymphoma. These findings suggest that methylation of these genes is a molecular event that may contribute more to the development of the tumor than to its progression. In other B-cell malignancies, *DAPK* and *p16* gene methylation has been associated with tumor progression.^{40–42} However, recent studies have shown that methylation of these genes may be an early event in lymphomagenesis: *DAPK* and *p16* gene methylation is frequently found in premalignant and low-grade lymphoid disorders, not associated with tumor progression, and their methylation status is maintained in higher-grade or advanced lymphoid neoplasms.^{43–48} In line with these findings, methylation of *DAPK* and *p16* is a common, early epigenetic phenomenon that allows tumor cells to promote cell-cycle progression and escape the normal apoptosis of a non-antigen-stimulated expanded B-cell population. Unlike base substitutions or gene deletions, changes in DNA methylation are potentially reversible. A hypomethylating agent, 5-aza-2'-deoxycytidine (decitabine) has been clinically useful in hematopoietic malignancies.^{49,50} The role of the agent in the tumor therapy remains to be determined or will have to be the subject of future studies.

In summary, we showed primary cutaneous marginal zone B-cell lymphomas from three geographical regions have common clinicopathological features as have been previously described. However, moderate or marked tissue eosinophilia was significantly frequent in the Asian cases. *Borrelia* infection and *API2-MALT1* fusion, both of which have been implicated in the development of this tumor, were not detected in our series. Finally, we showed that methylation of *DAPK* and *p16* genes was frequent in primary cutaneous marginal zone B-cell lymphoma cases at their initial presentation, and had no impact on progressive features of the tumor cases.

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Disclosure/conflict of interest

All the authors declared no competing interests.

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