Splenic Marginal Zone Lymphomas Appear to Originate from Different B Cell Types

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Splenic marginal zone lymphomas (SMZLs) have been proposed to originate from postgerminal center memory B cells that usually have mutated immunoglobulin heavy-chain variable (VH) genes. However, the majority of SMZLs are thought to express both IgD and IgM, which is more typical of naïve B cells that have unmutated VH genes. To better define the SMZL cell of origin and pathogenesis, we studied the histological and immunophenotypic features of eight cases and also sequenced their rearranged VH genes. Half of the cases had unmutated VH genes consistent with a naïve B-cell origin and half had mutated VH genes consistent with a memory B-cell origin. Most of the unmutated cases (three of four) were positive for IgD, which further supports a naïve B-cell origin, whereas the others were negative. In addition, VH gene segment use seems to be nonrandom because seven of eight cases used genes from the VH1 or VH4 families and repetitive use of the V1-2, V1-69, and V4-34 gene segments was observed. Our results suggest there are two types of SMZLs, one that originates from naïve marginal zone B cells in addition to one that originates from memory marginal zone B cells, and that antigen selection may be occurring during lymphomagenesis. (Am J Pathol 2002, 161:81–88)

Although splenic marginal zone lymphoma (SMZL) is recognized as a distinct clinical and pathological entity, the normal B-cell counterpart of this neoplasm is uncertain.^{1,2} In the white pulp, SMZLs typically manifest a dimorphic growth pattern in which marginal zone-type-appearing cells (small- to medium-sized lymphocytes with slightly dispersed chromatin and moderately abundant cytoplasm) merge with a more central zone of small lymphocytes that are also part of the neoplastic clone.¹ The small neoplastic lymphocytes surround and frequently replace more centrally located reactive germinal centers and mantle zones are usually absent or very attenuated. Originally, the growth of SMZLs was thought to be primarily restricted to the marginal zone, which suggested a marginal zone cell derivation.³ However, the

lack of mantle zones, which mark the inner marginal zone boundary and was not initially appreciated, later raised uncertainty about whether the SMZL infiltrate was ever confined to the marginal zone and originated from marginal zone B cells.⁴ Moreover, the growth of SMZLs in lymph nodes contrasts with nodal or extranodal marginal zone lymphomas that are typically located external to well-defined mantle zones.² SMZL is also thought to differ phenotypically from other types of marginal zone lymphomas by usually showing co-expression of IgM and IgD.^{1,2} Interestingly, this phenotypic feature also appears to differ from the normal splenic marginal zone where approximately half of these B cells appear to be IgD-negative.^{5,6} Otherwise, SMZL immunophenotypically resembles normal splenic marginal zone B cells and other types of marginal zone lymphomas by usually lacking CD5 and CD10.¹

Analysis of immunoglobulin heavy chain variable (VH) genes can also provide important information regarding a lymphoma cell of origin.⁷ Although relatively few cases have been studied, most reports concerning SMZL have suggested the cell of origin is a postgerminal center memory B cell because the expressed VH genes have typically demonstrated numerous point mutations from their germline counterparts.^{8–10} Because of these data, the recent World Health Organization lymphoma classification proposed that SMZL originates from a postgerminal center B cell.¹ However, a recent study by Camacho and colleagues¹¹ of SMZL that progressed to large-cell lymphoma found four of five cases studied to have unmutated VH genes. These data suggest that at least a subset of SMZLs has unmutated VH genes and may originate from naïve pregerminal center B cells.

In this study, we have analyzed the histological, immunophenotypic, and VH gene mutational features of eight SMZL cases to better define its cell of origin. The analysis of VH genes was also performed to investigate the potential role of antigen stimulation in the development of SMZL.^{12,13} Our results suggest that SMZL arises from both memory B cells that have mutated VH genes and lack readily detectable IgD as well as naïve B cells that have unmutated VH genes and express IgD. Because the

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normal splenic marginal zone compartment appears to consist of both naïve and memory B cells,⁵ our findings are consistent with SMZL originating from different types of splenic marginal zone B cells.

Materials and Methods

Patient Selection

All cases were selected from the pathology files of University of Pittsburgh Medical Center-Presbyterian Hospital. Initially, all patients diagnosed within a 3-year period with B-cell lymphoma from a splenectomy specimen were identified. Cases were excluded from further study if diagnoses of follicular lymphoma, small lymphocytic lymphoma, mantle cell lymphoma, Burkitt's lymphoma, or diffuse large B-cell lymphoma could be rendered using the criteria of the World Health Organization.¹ Also excluded was a case of lymphoplasmacytic lymphoma presented at the European Association for Hematopathology Meeting slide workshop on the spleen, in 1996 in Paris, France, and a case of unclassifiable polymorphic lymphoma with increased transformed cells.

Immunophenotypic Studies

For three-color flow cytometry analysis, splenic tissue was processed into single cell suspensions and stained with a variety of directly labeled monoclonal antibodies. Antibodies were purchased from Becton-Dickinson, San Jose, CA, unless otherwise indicated and included those against kappa/TB28-2, lambda/1-155-2, CD3/Leu4, CD5/Leu1, CD10/SS236 (DAKO, Carpinteria, CA), CD19/Leu12, CD20/Leu16, and CD103/B-ly7. After fixation, cellular fluorescence was evaluated on viable lymphocyte populations with a FacsCaliber flow cytometer (Becton-Dickinson). Immunoperoxidase staining was performed on 4-mm deparaffinized tissue sections using a manual method or the Ventana automated immunostainer (Ventana Medical Systems, Tucson, AZ). All antibodies were purchased from DAKO unless indicated otherwise and included those against cyclin D1 (H295), CD43 (DF-T1), CD23 (Binding Site Unlimited, Birmingham, UK), Bcl-2 (124), Bcl-6 (PG-B6p; Professor B. Falini, University of Perugia, Perugia, Italy), IgM (A0426), IgD (A0093), IgG (A0423), and IgA (A0092). Before staining with the anti-Ig antibodies, slides were pretreated by pressure cooking for 15 minutes in citrate buffer.

Cloning and Sequencing of Polymerase Chain Reaction Products

Rearranged VH genes were amplified for 37 cycles using *Taq* polymerase (Perkin Elmer, Norwalk CT) from fresh splenic tissue specimen DNA using 5' VH leader primers and 3' JH primers as described.¹⁴ Resultant polymerase chain reaction products were purified using Wizard DNA preps (Promega, Madison, WI) and cloned using the pCR-Script kit (Stratagene, La Jolla, CA) following the

manufacturer's directions. Plasmid DNA was isolated from overnight cultures of randomly selected colonies using Wizard Minipreps (Promega). Manual dideoxy sequencing was performed in both directions using Sequenase (US Biochemical, Cleveland, OH) following the supplied protocol for double-stranded plasmid templates with M13 forward and reverse primers.

Sequence Analysis

VH sequences were analyzed using MacVector software (IBI, New Haven, CT) and the VBASE database.¹⁵ Germ-line D segments were not assigned unless 10 or more consecutive nucleotides of identity were present as recommended by Corbett and colleagues.¹⁶ Expected numbers of replacement (R) and silent (S) mutations in the complementarity-determining regions (CDRs) and framework regions were determined by considering all possible mutations as described by Chang and Casali.¹⁷ The VH sequences described in this paper are available from the GenBank database (accession numbers AF491921 to AF491928).

Results

Clinical Features

Clinical information related to the eight selected patients is presented in Table 1. The average age was 68 years (range, 61 to 83 years) with equal numbers of males and females. All of the patients had splenomegaly with spleen weights ranging from 460 to 1300 g and did not have reported adenopathy by physical examination. Before splenectomy, some of the patients were known to have B-cell lymphoproliferative disorders based on earlier morphological and flow cytometric analysis of the peripheral blood and/or bone marrow. For patient 2, the clinical diagnosis was prolymphocytic leukemia, whereas patient 4 was thought to have hairy cell leukemia and patient 7 low-grade B-cell lymphoma. Patient 3 was thought to have a B-cell lymphoproliferative disorder based on the presence of monoclonal paraproteins.

Histological Findings

The morphological features of the splenectomy specimens were all consistent with SMZL (Table 2). All showed expansions of splenic white pulp as well as lymphocyte infiltration of the red pulp with marginal zone-type cells that have moderately abundant cytoplasm. In all cases except cases 4 and 7, white pulp nodules could be identified that had a dimorphic appearance consisting of a central darker core of smaller lymphocytes surrounded by a peripheral rim of marginal zone-type cells (Figure 1). The dimorphic nodules had absent or very attenuated mantle zones and sometimes surrounded central reactive germinal centers. In case 2, there was evidence of histological transformation because of the increased numbers of transformed cells observed in the expanded marginal zones. As mentioned above, the white pulp nodules

Table 1. Patient Information

Case	Age/sex	Clinical diagnosis/history	Spleen weight (gs)	Peripheral blood lymphocytes/ul	Bone marrow involvement
1	62/F	Splenomegaly	990	780	Probable
2	83/M	Prolymphocytic leukemia (from bone marrow and PB)	460	85750	Present
3	61/F	Waldenstrom's macroglobulinemia	750	740	Unknown
4	62/M	Hairy cell leukemia (from bone marrow)	Unknown	5540	Present
5	70/F	Splenomegaly	930	1600	Present
6	62/F	Splenomegaly	490	2600	Unknown
7	65/M	Pancytopenia with lymphoma (from bone marrow)	1300	1300	Present
8	76/M	None (splenectomy related to traumatic injury)	1240	20650	Unknown

in cases 4 and 7 did not demonstrate distinct central zones of small lymphocytes but were composed mainly of cells that resembled marginal zone cells similar to SMZL described by others¹⁸ (Figure 1). Three of the cases (cases 3, 5, and 6) showed plasmacytic differentiation with monoclonal plasma cells that could be easily identified by immunoperoxidase light chain staining.

Immunophenotypic Studies

All of the cases were evaluated by flow cytometry and were positive for CD20, CD19, monoclonal surface light-chain expression but not CD5 or CD10 (Table 3). One case (patient 4) was found to be positive for CD103, a marker typically associated with hairy cell leukemia but also seen on some splenic lymphomas.¹⁹ Additional immunohistochemistry studies revealed that all of the cases showed positive staining for bcl-2 but were negative for bcl-6, cyclin D1, CD23, and CD43. All but one of the cases showed positive staining for IgM and all were negative IgG and IgA. Two of the cases showed positive IgD staining (cases 3 and 4) by immunohistochemistry as did the one case examined for IgD expression by flow cytometry (case 2).

Use of VH, D, and JH Gene Segments

Rearranged VH genes were amplified from DNA obtained from fresh splenic tissue, cloned, and the VH genes from at least three randomly selected clones were sequenced in each case. All of the VH clones sequenced from a

given case were clonally related to one another based on identical CDR3 sequences except for case 7 in which one of the four sequenced clones contained a nonrelated VH gene. The rearranged VH genes identified for each case seemed to represent functional rearrangements because no stop codons or crippling mutations were identified. Comparing the VH genes to reported germline sequences revealed that four of the eight SMZL cases used VH1 family gene segments (cases 1, 3, 6, and 7), three used VH4 family VH gene segments (cases 4, 5, and 8), and only one case (case 2) used a gene segment from the largest VH3 family (Table 4). The VH1 family genes V1-69 and V1-2 were each used by two cases, and the VH4 family gene V4-34 was also used by two cases. The majority of cases used J4 or J6 joining segments, which are the most frequently used J segments by normal B cells.²⁰ Similarly, the majority of cases used D segments from the D3 and D6 families, which are also frequently used by normal B cells.¹⁶ In two cases D segments could not be assigned most likely because of somatic mutations or excessive trimming during rearrangement because both had relatively large CDR3 areas of greater than 30 nucleotides after excluding the J segment contribution. No associations were apparent between the use of particular D and J segments with different VH gene segments.

VH Gene Mutations

Many of the SMZL VH genes contained single point mutations from the most closely related germline se-

Table 2. Spleen Morphologic Features

Case	Histology	Dimorphic nodules	Plasmacytic differentiation	Additional observations
1	SMZL*	Yes	No	—
2	SMZL	Yes	No	Increased transformed cells
3	SMZL	Yes	Yes	—
4	SMZL	No	No	—
5	SMZL	Yes	Yes	—
6	SMZL	Yes	Yes	Red pulp nodules associated with epithelioid histiocyte clusters
7	SMZL	No	No	—
8	SMZL	Yes	No	Predominance of small lymphocytes

*SMZL, splenic marginal zone lymphoma.

Table 3. Immunophenotypic Results

Case	CD20*	CD10	CD5	Light chain	CD103	IgM	IgD
1	+	—	—	κ	NA	+	—
2	+	—	—	κ	—	+	+
3	+	—	—	λ	—	+	+
4	+	—	—	κ	+	+	+
5	+	—	—	κ	NA	+	—
6	+	—	—	λ	—	+	—
7	+	—	—	κ	NA	+	—
8	+	—	—	κ	—	—	—

*All cases were also positive for CD19 and bcl-2 but negative for bcl-6, cyclin D1, CD23, CD43, IgA, and IgG.

quences. Based on the number of nucleotide differences from germline, the eight SMZL cases could be separated into two groups, unmutated and mutated. Those cases with VH genes showing greater than 98% homology to germline (cases 1 to 4) comprised the unmutated group (Table 4), whereas cases with less than 97% homology to germline fell into the mutated group (cases 5 to 8). Most of the mutated cases also showed evidence of ongoing mutation because intraclonal VH gene diversity was identified in three of four of these cases. Only confirmed mutations, those that are shared among some but not all of the VH clones from a given case, were used as evidence for intraclonal diversity or ongoing mutation because they are a more reliable indicator and less likely to result from polymerase error.²¹ However, the presence of ongoing mutation in each of these cases was also supported by finding VH clones with additional unique non-common mutations not shared between different clones at frequencies in excess of our expected polymerase error rate of 0.03%.¹⁴

The locations (framework region or CDR) and type (R or S) of VH gene mutations in the mutated SMZL cases are given in Table 5. None of the SMZL VH genes appears to have ratios of R to S mutations in CDR1 and CDR2 greater than expected by chance alone that would be suggestive of positive selection.^{12,22} Indeed, the relatively low ratios of R to S mutations in cases 5 and 6 are more consistent with selection against R mutations in the CDRs, or negative selection. The ratios of R to S mutations in the framework region appear to be less than those expected by chance alone for all of these VH genes. This finding is a common feature for VH genes expressed by normal B cells and suggests there is also selection for maintaining expression of a functional immunoglobulin molecule in SMZL.^{12,22}

Discussion

This study suggests that SMZL can be divided into two different types based on VH gene mutational status, one with unmutated VH genes (all had >98% homology to

germline VH gene segment) and the other with mutated VH genes (all had <97% homology to germline). Although some of the unmutated cases had occasional single nucleotide differences from germline, VH genes with 98% or greater homology to published germline counterparts are typically considered unmutated because VH gene polymorphisms, which are very common, can account for that degree of difference.²³ In normal B cells, VH gene hypermutation is restricted to the germinal center stage of development and plays a role in the affinity maturation process.²² For the most part, VH gene mutation patterns in malignant B cells appear to parallel normal B-cell development in that neoplasms of naïve pregerminal center B cells have unmutated VH genes whereas neoplasms of germinal center and postgerminal center B cells display mutated VH genes.^{7,22} Our findings are therefore consistent with the proposal that the unmutated type of SMZL originates from naïve pregerminal center B cells and the mutated type of SMZL originates from postgerminal center memory B cells.

It is interesting that normal splenic marginal zone B cells also appear to be heterogeneous consisting of both memory and naïve B cells.⁵ Analysis of VH genes expressed by normal splenic marginal zone B cells revealed that many are mutated consistent with these representing memory B cells.^{24,25} However, normal splenic marginal zone B cells without VH gene mutations or only occasional mutations were also found in these studies suggesting that some marginal zone B cells represent naïve B cells. Additional support for a population of naïve splenic marginal zone B cells comes from evidence suggesting the splenic marginal zone is enriched for B cells that respond to thymus-independent type II antigens.⁵ Our proposal that SMZL originates from both naïve and memory B cells is, therefore, in line with the apparent heterogeneity of normal splenic marginal zone B cells.

Our finding significant numbers of SMZLs with unmutated VH genes differs from most earlier studies.^{8–10} However, these earlier studies only examined a small number of cases and occasional SMZLs with VH genes that would be considered unmutated were identified. In contrast to these earlier studies, the recent report by Camacho and colleagues¹¹ found a majority of their SMZL cases studied (four of five) to have unmutated VH genes. It may be important that the SMZLs in this study were restricted to those that had progressed to large cell lymphomas and may therefore represent a subset with a more aggressive clinical course. The possibility that SMZLs with unmutated VH genes may have a more aggressive clinical course than SMZLs with mutated VH genes parallels chronic lymphocytic leukemia (CLL), in which CLL with unmutated VH genes are more aggressive clinically than CLL cases with mutated VH genes.^{23,26} Our proposal that VH gene mutational status may be used to differentiate two types of SMZLs, there-

Figure 1. Histological features of SMZL cases. **A:** Low magnification of case 2 that has dimorphic white pulp nodules typical of SMZL. **B:** High magnification of case 2 showing the transition between the central small lymphocyte core and outer marginal zone that in this case has increased transformed cells. **C:** Low magnification of case 4 that did not have well-defined dimorphic white pulp nodules. **D:** High magnification of a white pulp nodule of case 4 illustrating that most of the cells morphologically resemble marginal zone cells.

Table 4. VH Gene Analysis

Case	Germline Gene Segments			% Homology	VH clones analyzed, no.	Intraclonal diversity*
	V	D	J			
1	V1-69	D3-22	J4	99.3	3	No
2	V3-43	D3-9	J6	99.0	5	No
3	V1-2	D3-22	J4	98.5	4	No
4	V4-34	D5-5	J6	100	6	No
5	V4-31	NA [†]	J2	91.8	3	Yes
6	V1-2	D3-3	J3	95.2	3	Yes
7	V1-69	NA	J4	95.6	3	Yes
8	V4-34	D6-19	J6	96.2	3	No

*Only variable or noncommon mutations that were shared between different VH clones were counted (case 5 had two single nucleotide mutations shared between two clones, case 6 had four, and case 7 had one).

[†]Germline D segments could not be assigned in cases 5 and 7 using the criteria of 10 or more consecutive matching nucleotides.

fore, is not without precedent and similar to what has already been proposed for CLL.

The World Health Organization lymphoma classification as well as other recent reports suggest that most cases of SMZL should be positive for both IgD and IgM.^{1,2} Therefore, finding five of eight SMZL cases in our study lacking IgD seems to be atypical and could raise doubts regarding our diagnoses even though all of the other characteristic histological and immunophenotypic features were present. One potential factor contributing to finding fewer than the expected number of IgD-positive cases may be related to a low sensitivity of our immunoperoxidase detection technique even though it clearly detects normal mantle zones. Indeed, this possibility is supported by our finding one SMZL case that appeared negative by immunoperoxidase to be IgD-positive by flow cytometry. Similar to our findings, however, other studies of SMZL have also reported a significant proportion of cases to be negative for IgD.^{8,10,18,27} It is possible, therefore, that there are more IgD-negative SMLZ cases than now generally appreciated. This idea is also more consistent with frozen tissue studies of normal splenic marginal zone B cells that demonstrate that approximately half are negative for IgD whereas the remaining are weakly positive and a few are strongly IgD-positive.^{5,6}

Recent studies of normal B cells have indicated that high levels of surface IgD and IgM expression are more typically found on naïve B cells with unmutated VH genes.²⁸⁻³⁰ This is consistent with our SMZL findings and suggests that high IgD expression may also mark some naïve B cells in the splenic marginal zone. However, co-expression of IgD and IgM is not a specific marker for naïve B cells because a small percentage of peripheral blood memory B cells with mutated VH genes and CD27

expression have this phenotype.³⁰ Moreover, CD27 and CD148 appear to identify memory B cells in the spleen some of which appear to express low intensity IgD with IgM.³¹ In addition, co-expression of IgM and IgD may be less restricted in B-cell neoplasms as suggested by follicular lymphomas often being positive for both IgD and IgM.^{32,33} Therefore, the correlation we have observed between IgD expression and unmutated VH genes may have to be modified somewhat after additional cases and quantitative levels of IgD are studied.

Finding evidence of ongoing mutation in most of the SMZL cases with mutated VH genes raises the possibility that these neoplasms originate in germinal center B cells rather than postgerminal center memory B cells.^{12,22} Furthermore, it is well recognized that follicular lymphomas involving the spleen can sometimes morphologically resemble SMZL.³⁴ However, none of the four mutated SMZL cases resembled follicular lymphoma, none expressed CD10, and none of the three tested cases harbored a (14;18) translocation. It is also possible the initial transforming event occurs in a germinal center B cell that could theoretically result in sustained activation of the VH gene mutator even if the final transformation event occurs in a postgerminal center B cell. An alternative explanation for the observed low-level ongoing mutation is that SMZL cells may sometimes re-enter the permissive germinal center,³⁵ which has been described for normal marginal zone B cells.³⁶ Therefore, finding low-level on-going VH gene mutation does not necessary imply an origin from germinal center B cells.

The IgD-positive SMZL cases with unmutated VH genes raise the possibility of a mantle cell origin for this subset because normal mantle cells and mantle cell lymphomas typically demonstrate strong IgD positivity and

Table 5. Analysis of VH Gene Mutations in Mutated SMZL Cases

Case	CDR 1 and 2				FWR 1, 2 and 3			
	R*	S	R/S	R/S (random)	R	S	R/S	R/S (random)
5	6	4	1.5	4.1	8	6	1.3	2.7
6	3	3	1.0	4.3	2	6	0.3	3.0
7	5	2	2.5	3.6	2	4	0.5	3.0
8	3	0	3	4.5	5	3	1.7	2.7

*Mutations listed are those common to all VH clones from a given case and are classified as either replacement (R), those leading to amino acid changes, or silent (S). The random R/S ratio values represents the ratios of R to S mutations expected by chance alone (without selection).

have unmutated VH genes.³⁷ However, none of these cases had the expected CD5 or cyclin D1 positivity indicative of mantle cell lymphoma. The possibility that these represent a previously undescribed lymphoma derived from mantle zone cells that differentiate to cells resembling marginal zone cells could still be considered.³⁸ However, because it appears naïve B cells with unmutated VH genes are also present in normal splenic marginal zones, we favor a marginal cell origin for the unmutated SMZL cases.

This study also suggests that SMZL shows biased or nonrandom use of VH gene segments with overrepresentation of the VH1 and VH4 families (seven of eight cases) and underrepresentation of the largest VH3 family (one of eight cases) that contains ~50% of the functional VH gene segments.^{15,20} Although the number of cases is small, finding repeat use of VH4-34 (two cases), V1-69 (two cases), and V1-2 (two cases) further suggests there may be preferential use of specific gene segments from the VH1 and VH4 families. Additional support for this possibility comes from the study of Dunn-Walters and colleagues,⁸ in which two of four SMZL cases analyzed appeared to express V4-34, and the study of Miranda and colleagues,⁹ in which one of four cases used V4-34 and two of four used a V1-2 gene segment. Although none of the SMZL cases studied by Tierens and colleagues¹⁰ used V4-34 or V1-2, three of four cases used other VH gene segments from the VH1 and VH4 families. Preferential use of specific VH gene segments or families suggests that selection by a limited number of antigens may be playing a role in the development of SMZL.¹³ This is also supported by analyses of VH gene mutations in the mutated SMZL cases, which suggested there was selection for expression of functional immunoglobulin molecules.

Although two types of SMZL can be distinguished by VH gene mutational status, differences in the use of specific VH segments between the two types were not apparent because both types had representative cases using V1-69, VH4-34, and V1-2. Furthermore, differences in histological features were also not apparent as a similar number of cases showing plasmacytic features or having well-defined dimorphic white pulp nodules were also represented in both groups. Finding similar histological features and patterns of VH gene use among the two SMZL types is consistent with the proposal that both originate from splenic marginal zone B cells.

In conclusion this study suggests that SMZLs can originate from pregerminal center naïve B cells that have unmutated VH genes as well as from postgerminal center memory B cells that have mutated VH genes. Because the normal splenic marginal zone appears to contain both memory B cells and naïve B cells,⁵ we propose that the mutated and unmutated types of SMZL originate from different types of splenic marginal zone B cells. Even though differences between the proposed two types of SMZL were not identified other than IgD expression, additional studies looking at more parameters are needed to adequately address this issue. In particular, it will be important to obtain longer term clinical data to see if there may be differences in SMZL patient survival similar to

what has been described for the recently proposed two types of CLL that are also distinguished by VH gene mutational status.^{23,26}

After this study was submitted for publication, an article examining VH genes using 35 cases of SMZL was published.³⁹ Similar to our findings, approximately half of these SMZL cases had unmutated VH genes, and there seemed to be preferential use of VH1 family gene segments, especially V1-2, among both unmutated and mutated types. Survival data from this article indicated that SMZL with unmutated VH genes may indeed have a more aggressive clinical course as suggested above.

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