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t(11;18)(q21;q21) in extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue in stomach: a study of 48 cases

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Gastric extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MZL-MALT) is speculated to be immune mediated and is notable for responding to treatment by *Helicobacter pylori* eradication. However, the gastric MZL-MALT with t(11;18)(q21;q21) has been shown to be resistant to treatment by *H. pylori* eradication. We studied the molecular, immunohistochemical, and histological aspects of 48 cases of gastric MZL-MALT and used a reverse transcription real-time PCR assay to assess the presence of a t(11;18)(q21;q21) in formalin-fixed, paraffin-embedded tissue. Florescence *in situ* hybridization for t(11:18)(q21;q21) was used to confirm the real-time PCR results. Three distinct morphological subtypes were recognized: monocytoid, small lymphocytic, and plasmacytoid. Morphology, immunophenotype, and immunoglobulin heavy chain (*IgH*) gene rearrangement were correlated with the results of the t(11:18)(q21;q21) assay. Of the 48 analyzed cases, 15 (31%) were positive for t(11;18)(q21;q21) and 33 (69%) were monoclonal for IgH gene rearrangement. Of the 15, 13 (87%) cases with t(11;18)(q21;q21) translocation showed *IgH* gene rearrangement by PCR. Of the 33 t(11;18)(q21;q21)-negative cases tested, 20 cases (61%) showed *IgH* gene rearrangement. The 15 t(11;18)(q21;q21) translocation-positive cases had either monocytoid (12 of 15) or small lymphocytic morphology (3 of 15). Aberrant expression of CD43 was observed in 8 of 15 (53%) t(11;18)(q21;q21)-positive cases and 21 of 31 (68%) t(11;18)(q21;q21)-negative cases. Our data show that t(11;18)(q21;q21)-positive MZL-MALTs frequently show monocytoid morphology, less often small lymphocytic morphology, and not purely plasmacytoid morphology. Identification of a t(11;18)(q21;q21) by reverse transcription real-time PCR is highly specific for MZL-MALT and helps in the diagnosis of MZL-MALT. Studying the correlation between this translocation and morphological features may increase our understanding of the role of this translocation in the pathogenesis and the clinical behavior of gastric MZL-MALT.
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Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MZL-MALT) is an indolent type of B-cell non-Hodgkin's lymphoma, first recognized as a specific type of lymphoma in 1983.[1] It consists 7–8% of all B-cell lymphomas and occurs most frequently in the stomach, accounting for at least 50% of primary gastric lymphomas.[2] Although MZL-MALT can arise at sites at which mucosa-associated lymphoid tissue normally is found, such as in the terminal ileum, most MZL-MALT arise from mucosa-associated lymphoid tissue acquired as a result of chronic inflammation. The stomach acquires MALT as a result of the chronic inflammation associated with *Helicobacter pylori*.[3,4] Chromosomal translocations, including t(11;18)(q21;q21)/API2/MALT1, t(1;14)/IGH-BCL-10, t(14;18)/IGH-MALT1, and t(3;14)/IGH-FOXP1, have

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been described in MZL-MALT occurring at various sites.[5–11] Among the above four translocations, t(11;18)(q21;q21) occurs specifically in extranodal MZL-MALT and not in closely related nodal or splenic marginal zone B-cell lymphoma, or other non-Hodgkin's lymphomas, with the exception in rare cases of diffuse large B-cell lymphoma. The t(11;18)(q21;q21) translocation has been associated with 24–48% of gastric MZL-MALT.[8,12–15] The gastric MZL-MALTs with t(11;18)(q21;q21) and a subset of MZL-MALT with large cell change have been shown to be resistant to treatment for *H. pylori* eradication. Otherwise eradication of *H. pylori* will lead to complete regression in about 70% of gastric MZL-MALT.[12,16] However, the role of t(11;18)(q21;q21) molecular testing in a clinical setting is not well defined. In this report, we studied the molecular, immunohistochemical, and histological aspects of 48 cases of gastric MZL-MALT with the aim of further understanding the role of detection of t(11;18)(q21;q21) translocation in the diagnosis of gastric MZL-MALT.

Materials and methods

Case Selection

A total of 67 cases diagnosed as gastric MZL-MALT between 1997 and 2007 were retrieved from the repository of the Armed Forces Institute of Pathology (AFIP). The cases were evaluated according to WHO histological criteria.[4] Cases without tissue blocks and/or unstained slides for molecular studies and immunohistochemistry were excluded, leaving 48 cases, which consisted the current study. Clinical and demographic findings were compiled from the medical record.

Immunohistochemistry

Immunophenotypic analysis was performed according to the standardized avidin–biotin complex method. The antibodies are listed in Table 1. When predigestion was required, 0.04% Pepsin (Sigma Chemical Co., St Louis, MO, USA) was used for 20 min in 0.1 M HCl (pH 2.0) at 40–42°C.

Positive staining for CD20 with negative CD3 was used to confirm a B-cell immunophenotype. Co-expression of CD43 was suggestive of a diagnosis of malignancy within the context of the atypical lymphoid infiltrates. Restriction (κ or λ), when found, also supported a diagnosis of malignancy. Positive staining for CD20 with negative CD5, CD10, or CD 23 was used to exclude the possibility of other small B-cell lymphomas including small lymphocytic lymphoma/chronic lymphocytic leukemia, mantle cell lymphoma, and follicular lymphoma.

Table 1 Immunohistochemical antibody panel

Antibody	*Clone*[a]	*Source*[b]	*Antigen retrieval*	*Dilution*
CD3	Polyclonal	Cell Marque	Steam[c]	1:1000
CD5	4C7	Novocastra	Steam[c]	1:1000
CD10	56C6	Novocastra	Steam[c]	1:100
CD20	L26	Dako	Steam[c]	1:800
CD23	BU38	The Binding Site	Steam	1:200
CD43	MT-1	Zymed	Steam[c]	1:200
Bcl-2	124	Dako	Steam[c]	1:100

[a]All antibodies are monoclonal unless otherwise indicated.

[b]Dako, Dako, Carpinteria, CA, USA; Cell Marque, Cell Marque, Hot Springs, AK, USA; Novocastra, Novocastra Lab Ltd, Newcastle upon Tyne, UK; The Binding Site, The Binding Site Limited, Birmingham, UK; Zymed, Zymed, South San Francisco, CA, USA.

[c]Steam antigen retrieval for 20 min in 1 mM EDTA.

Gene Rearrangement Studies

Immunoglobulin heavy chain (*IgH*) gene rearrangement studies were performed by PCR from formalin-fixed paraffin-embedded tissue using primers to the V_H-FRIII/J_H region as previously described[17] and amplified products were separated by capillary electrophoresis with ABI 3100 (Applied Biosystems, Foster City, CA, USA).

Reverse Transcription Real-Time PCR for t(11;18)(q21;q21)

Real-time RT-PCR for t(11;18)(q21;q21) was performed on 48 cases. Primers and probes for real-time PCR were designed using the Primer Express software, version 1.5 (Applied Biosystems) based on the published sequence in the GenBank database (U75698) available on the National Center for Biotechnology Information. Three sets of primers and probes were designed to detect three different transcripts that cover 96% of the variants of translocation occurring in MZL-MALT.[14,18] The detection of β-2-microglobulin RNA was used as a control to assess mRNA quality. All three probes for t(11;18)(q21;q21) translocation were labeled with the reporter dye 6-FAM (6-carboxyfluorescein) with MGB quencher; β-2-microglobulin probe was labeled with the reporter dye VIC and Tamara as quencher. RNA extraction and reverse transcription were performed as described previously.[19] Real-time PCR cycles were 2 min at 50°C, followed by 10 min at 95°C and 40 cycles of 15 s at 95°C, and 1 min at 60°C. Synthetic DNA oligonucleotides (Integrated DNA Technologies, Coralville, IA, USA) with three known fusion-transcript sequences were used as positive controls. The real-time PCR was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems). All samples were tested in duplicate. Oligonucleotide sequences of three sets of primers, probes, and synthetic DNA controls are shown in Table 2.

Table 2 Primers, probes, and control templates used for the detection of t(11;18) translocation by reverse-transcription real-time PCR

Targeted transcript	*Size*[a]	*Sequences of primers, probes, and control templates*
814[b]	65 bp	
Forward primer		5′-GGAAGAGGAGAGAGAAAGAGCAACT-3′
Reverse primer		5′-TGGATTCAGAGACGCCATCA-3′
Probe		5′-FAM-AGGAAAAAGAATCAAGAAGTG-MGB-3′
Control template		5′-GGAAGAGGAGAGAGA AAGAGCAACTGAGGAAAAAGAATC AAGAAGTGTTGATGGCGTCTCTGAATCCA-3′
1123[c]	80 bp	
Forward primer		5′-CAACTGAGGAAAAAGAATCAAATGAA-3′
Reverse primer		5′-AAAGGCTGGTCAGTTGTTTGCT-3′
Probe		5′-FAM-TAATCTTGGTCATCCTGATAAT-MGB-3′
Control template		5′-CAACTGAGGAAAAAGAATCAAATGAATTAAATAATCTTGGT CATCCTGATAATAAAGAGCAAACAACTGACCAGCCTTT-3′
1150[d]	94 bp	
Forward primer		5′-GCAACTGAGGAAAAAGAATCAAATAA-3′
Reverse primer		5′-CCTTGTCCTTCGCCAAAGG-3′
Probe		5′-FAM-AAGAGCAAACAACTGACCA-MGB-3′
Control template		5′-GCAACTGAGGAAAAAGAATCAAATAATAAAG AGCAAAACT GACCAGCCTTTGGCGAAGGACAAGGTTG-3′
B-2-Microglobulin	84 bp	
Forward primer		5′-TGACTTTGTCACAGCCCAAGATA-3′
Reverse primer		5′-AATCCAAATGCGGCATCTTC-3′
Probe		5′-VIC-TGATGCTGCTTACATGTCTCGATCCCA-TAMRA-3′

[a]Size of PCR product.
[b]Fusion transcript of *API2* gene at nucleotide position 1446 with *MALT1* gene at nucleotide position 814.
[c]Fusion transcript of *API2* gene at nucleotide position 1446 with *MALT1* gene at nucleotide position 1123.
[d]Fusion transcript of *API2* gene at nucleotide position 1446 with *MALT1* gene at nucleotide position 1150.

Table 3 Main characteristics of t(11;18)(q21;q21)-positive and -negative cases

Features	*t(11;18) Positive*	*t(11;18) Negative*	*Total*
Total	15	33	48
Mean age (years)	66.5	67	66.5
Median age (years)	63 (range 45–85)	69 (range 37–90)	68
Male	11/15 (73%)	24/33 (73%)	35/48 (73%)
Female	4/15 (27%)	9/33 (27%)	13/48 (27%)
Monoclonal IgH	13/15 (87%)	20/33 (61%)	33 /48 (69%)
Polyclonal IgH	2/15 (13%)	13/33 (39%)	15/48 (31%)
Monocytoid	12/15 (80%)	17/33 (52%)	29/48 (60.4%)
Small lymphocyte	3/15 (20%)	12/33 (36%)	15/48 (31.3%)
Plasmacytoid	0/15 (0%)	4/33 (12%)	4/48 (8.3%)
CD43 Positive	8/15 (53%)	21/31 (68%)	29/46 (63%)
CD43 Negative	7/15 (47%)	10/31 (32%)	17/46 (37%)

Fluorescence *In Situ* Hybridization

A cross validation study of the real-time PCR for t(11;18)(q21;q21) with florescence *in situ* hybridization (FISH) was performed with Phenopath Laboratories (Seattle, WA, USA). The t(11;18) dual-color, dual-fusion assays were performed using two separate FISH probes (Abbott Molecular, Downer's Grove, IL, USA), the first probe, labeled with SpectrumGreen and specific for the *API2* gene on 11q21 (~1.0 Mb) and a second probe labeled with SpectrumOrange specific for the *MALT1* gene on 18q21 (~670 kb) according to the manufacturer's instructions with minor modifications. Automated quantitative morphometric image analysis was performed using the Metafer scanning system (Metasystems, Watertown, MA, USA) to enumerate and analyze the FISH signals. Each sample image is

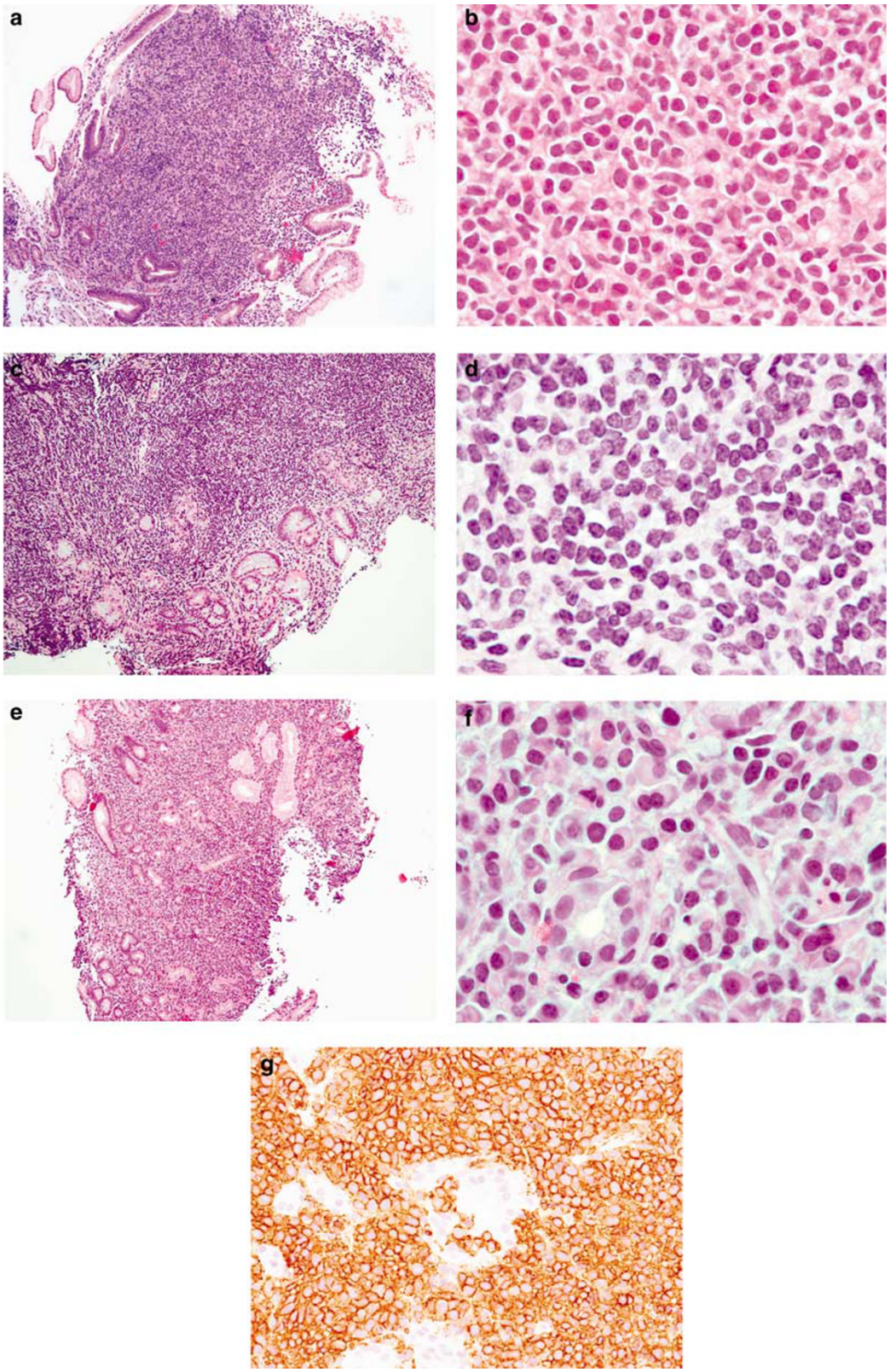

Figure 1 Examples of gastric biopsy findings in MZL-MALT. (**a**) Low magnification of gastric MALT lymphoma with monocytoid morphology. (**b**) Gastric MALT lymphoma with monocytoid morphology, 100 × . (**c**) Low magnification of gastric MALT lymphoma with small lymphocytic morphology. (**d**) Gastric MALT lymphoma with small lymphocytic morphology, 100 × . (**e**) Low magnification of gastric MALT lymphoma with plasmacytoid morphology. (**f**) Gastric MALT lymphoma with plasmacytoid morphology, 100 × . (**g**) CD20 and lymphoepithelial lesion.

divided using a tiling algorithm that permits localization of the nuclei. Signals within tiles are stratified according to proximity and those tiles containing a fusion signal defined as a red and green signal separated by two or less image pixels are considered positive. The percentage of tiles is calculated for each specimen with a minimum of 500 tiles counted for each specimen. The threshold for positivity is established from a group of reactive and neoplastic cases that do not contain a t(11;18). A positive case was defined as a case in which the percentage of positive tiles detected is greater than 3 standard deviations above the mean of the negative control group (18.6%).

Statistical Analysis

Statistical analysis was performed with JMP Statistical Discovery software, Version 5.1.1 (SAS Institute, Cary, NC, USA). The χ^2-test was applied to identify significant difference between two groups of data. *P*-values of <0.05 were considered as statistically significant.

Results

Clinical Findings

Cases included in this study had an age range of 37–90 years; the mean age was 66.5 years and median 68 years. A male predominance with male to female ratio of 35:13 (2.7:1.0) was observed in this study (Table 3).

Histopathological Features

Three morphological groups of gastric MZL-MALT with different appearances of neoplastic lymphocytes were observed. The first group, monocytoid, which accounted for 60.4% (29 of 48) of cases in this study, was characterized by neoplastic cells having increased pale cytoplasm with small- to medium-sized, centrally located nuclei containing moderately dispersed chromatin and inconspicuous nucleoli. Scattered large cells were accepted in this group but not more than 20%. The second group, small lymphocytic, accounting for 31.3% (15 of 48)

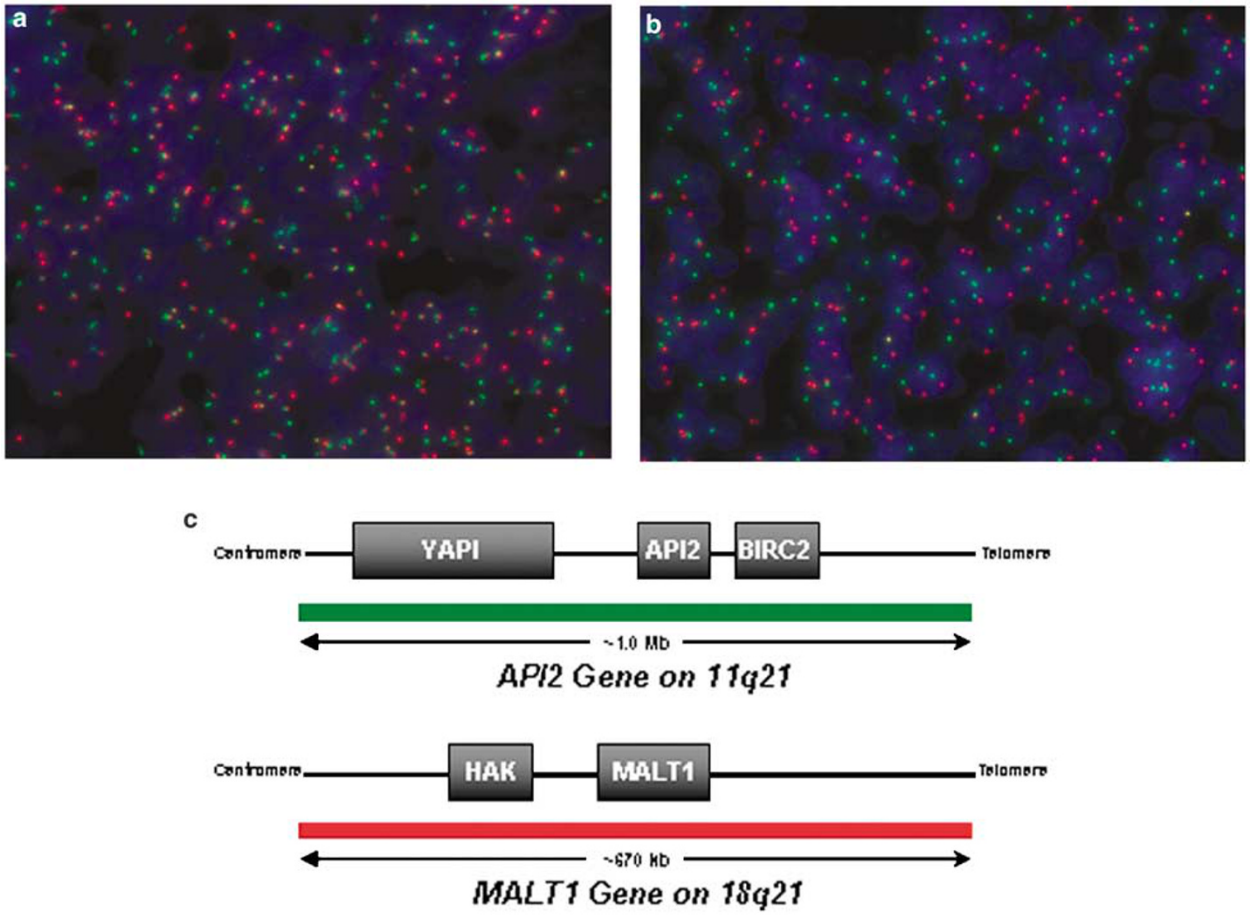

Figure 2 FISH images of hybridization of the API2/MALT1 t(11;18)(q21;q21) Dual Color, Dual Fusion translocation probe with a t(11;18)(q21;q21) PCR positive gastric MZL-MALT case showing fusion of the green and orange signals well above individual green and orange signals (**a**) and a t(11;18)(q21;q21) PCR negative gastric MZL-MALT case showing single green and orange signals (**b**). Schematic illustration of FISH probes for t(11;18)(q21;q21) (**c**).

of cases, was consisted of small lymphocytic cells with scant cytoplasm, but meeting the criteria for MZL-MALT. They had no monocytoid changes but focal plasmacytoid cells were acceptable. The third group, plasmacytoid, accounting for 8.3% (4 of 48) of cases, was characterized by greater than 50% of the neoplastic cells having a range in differentiation from lymphoplasmacytoid to the appearance of mature plasma cells, such as increased eosinophilic cytoplasm, a perinuclear hof, eccentric nuclei, Dutcher bodies, and only focal or minimal monocytoid changes (Figure 1).

Immunohistochemical Findings

Immunohistochemical studies showed that the neoplastic lymphocytes were reactive with CD20 and negative for CD3 in all cases. All cases were negative for CD5, CD10, and CD23 in which the stains were performed. CD43 expression on B cells was present in 63% (29 of 46) cases (Table 3). Bcl-2 was present in 95% (20 of 21) cases tested. κ and λ were restricted in a small number of cases.

Gene Rearrangements Studies

IgH gene rearrangement studies showed a dominant monoclonal band in 69% (33 of 48) of gastric MZL-MALT. Of the 15, 13 (87%) cases with t(11;18)(q21;q21) translocation showed a monoclonal *IgH* gene rearrangement. Of the 33 t(11;18)(q21;q21)-negative cases tested, 20 cases (61%) showed a monoclonal *IgH* gene rearrangement. The difference between detection rate of monoclonal *IgH* gene rearrangement in t(11;18)(q21;q21)-positive cases (87%) and in the t(11;18)(q21;q21)-negative cases (61%) is not statistically significant ($P=0.0710$) (Table 3).

t(11;18)(q21;q21) Transcripts Detection

The analytic sensitivity studies made use of synthetic DNA control templates designed according to the sequences of three fusion transcripts (Table 2). The analytic sensitivity of the assay was characterized by making serial dilutions of the synthetic DNA control templates. In dilution studies, the assay yielded a positive signal from less than 10 copies of control templates. A total of 48 gastric MZL-MALT samples of formalin-fixed paraffin-embedded tissues from the AFIP repository were tested by reverse transcription real-time PCR for t(11;18)(21;q21). Of the 48 analyzed cases, 15 (31%) were positive for t(11;18)(q21;q21).

Fluorescence *In situ* Hybridization Results

Eighteen cases, nine t(11;18)(q21;q21) positive and nine negative by real-time RT-PCR, were tested by FISH. All nine PCR-positive cases were confirmed by FISH; one PCR-negative case was borderline positive by FISH; one PCR-negative case did not show any signal in FISH study; and the remainder of the PCR-negative cases were negative by FISH (Figure 2).

Correlation of Histology and Molecular Findings

Of 15 t(11;18)(q21;q21) translocation-positive cases, 80% (12 of 15) had monocytoid morphology and 20% (3 of 15) had small lymphocytic morphology. The t(11;18)(q21;q21) was not detected in MZL-MALT cases with plasmacytoid morphology. The difference of the detection of t(11;18)(q21;q21) among gastric MZL-MALT with different morphology was statistically significant ($P=0.0123$). Aberrant expression of CD43 was observed in 8 of 15 (53.3%) of t(11;18)(q21;q21)-positive cases and 21 of 31 (68%) of t(11;18)(q21;q21)-negative cases (Table 3).

Discussion

The diagnosis of gastric MZL-MALT usually requires immunohistochemical studies as well as histology. Histopathological features include the following: neoplastic B lymphocytes (with heterogeneous histological features), follicular colonization (malignant lymphocytes infiltrating reactive germinal centers), lymphoepithelial lesions, Dutcher bodies, and infiltration of the muscularis mucosae by neoplastic lymphocytes. Not all of these features need to be seen in any one particular case of gastric MZL-MALT. Positive staining for CD20 without expression of CD3 is used to confirm a B-cell immunophenotype. Co-expression of CD43 in B cells is supportive for a diagnosis of lymphoid malignancy. Lack of expression of CD5, CD10, or CD 23 is used to exclude the possibility of other small B-cell lymphomas, such as small lymphocytic lymphoma/chronic lymphocytic leukemia, mantle cell lymphoma, and follicular lymphoma. Restriction (κ or λ), when found, also supports a diagnosis of malignancy.[4,20,21] However, establishing a diagnosis of MZL-MALT is not always straightforward, particularly in biopsy specimens with scant tissue and uncertain immunophenotype. In many instances, it can be difficult to distinguish a reactive gastric lymphoid infiltrate tissue from an MZL-MALT.[20] Previous studies show that molecular assays, *IgH* gene rearrangement, and t(11;18)(q21;q21) in particular, had value in establishing the diagnosis for MZL-MALT.[14,22–24]

In our study, 60.4% of gastric MZL-MALT cases had monocytoid morphology; 31.3% had small lymphocytic features; and about 8.3% had plasmacytoid differentiation (Table 3). The t(11;18)(q21;q21) translocation was present in 41.4% (12 of 29) of cases with monocytoid mor-

phology and 20% (3 of 15) of cases with small lymphocytic morphology. This translocation was not seen in cases with plasmacytoid morphology (Table 3). Thus, cases with monocytoid change are more likely to have t(11;18)(q21;q21) translocation. However, the presence of t(11;18)(q21;q21) cannot be assumed solely by histology, and should be substantiated by performing the molecular assay for t(11;18)(q21;q21).

Another unresolved issue is whether the presence of t(11;18)(q21;q21) can be used as a diagnostic criteria for malignancy. In our initial validation studies (data not shown), in addition to 48 MZL MALT cases, 62 other examples of benign and neoplastic processes were tested for t(11;18)(q21;q21). These included 18 chronic active gastritis, 22 diffuse large B-cell lymphoma, 5 small B-cell lymphoma, 3 anaplastic large T-cell lymphoma, and 14 nonhematopoietic neoplasms. None of these cases was positive for t(11;18) translocation, yielding a specificity of 100%. Thus, the presence of t(11;18)(q21;q21) in an atypical lymphoid infiltrate in the stomach, based on previous studies[15,25–27] and in our hands, is highly specific for the diagnosis of gastric MZL-MALT. Therefore, it may help to confirm a diagnosis of MZL-MALT in problematic cases such as cases with limited tissue, borderline morphology, or unclear immunophenotype.

Cases with plasmacytoid morphology constituted less than 10% of gastric MZL-MALT cases in our study, a finding similar to that of Wöhrer *et al*, but much less than that published by others.[4,20,28] Wöhrer *et al* also showed that plasmacytoid morphology is mainly found in extragastric MZL-MALTs. As this study has shown, none of four cases of gastric MALT lymphomas with plasmacytoid morphology had t(11;18)(q21;q21) translocations. However, the significance of the plasmacytoid morphology in gastric MZL MALT is largely unknown.

Similar to the t(11;18)(q21;q21) translocation, there is no consensus on the role of *IgH* gene rearrangement testing in routine diagnosis of MZL-MALT. Monoclonal *IgH* gene rearrangement was present in 69% (33 of 48) of gastric MZL-MALT cases overall in our study. There was a slightly higher detection rate of monoclonal *IgH* gene rearrangement in t(11;18)(q21;q21)-positive cases (87%) than in the t(11;18)(q21;q21)-negative cases (61%), but it is not statistically significant ($P = 0.0710$). This study supports other studies that have shown that one out of three of gastric MALT lymphomas does not have detectable monoclonal *IgH* gene rearrangement.[29–32] Moreover, in about 5–10% of active gastritis cases, lymphoid tissue had monoclonal *IgH* gene rearrangement.[29–32] It is our opinion that *IgH* gene rearrangement testing should not be used alone. However, it is still useful in our experience in difficult cases in conjunction with morphology and immunohistochemistry as an adjunct test to reveal the clonality of the lymphocytes.

In conclusion, t(11;18)(q21;q21) is most frequently associated with monocytoid morphology in gastric marginal zone B-cell lymphoma of MALT type, and much less commonly in MZL-MALT with small lymphocytic morphology, and infrequently, if ever, in MZL-MALT with plasmacytoid morphology. More in-depth studies on the correlation between genotype and phenotype may further our understanding of the role of this translocation in the pathogenesis, clinical behavior, and diagnosis of gastric MZL-MALT.

Disclaimer

The opinions and assertions herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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