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Original contribution

# Analysis of *API2-MALT1* fusion, trisomies, and immunoglobulin *VH* genes in pulmonary mucosa-associated lymphoid tissue lymphoma☆

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**Summary** Pulmonary mucosa-associated lymphoid tissue lymphoma is unique in that chronic inflammation is rare and that *API2-MALT1* fusion, resulting from t(11;18)(q21;q21), occurs frequently. In this study, we examined 20 cases for *API2-MALT1* fusion using the multiplex reverse-transcription polymerase chain reaction and looked for trisomy 3, trisomy 18, and abnormalities of *MALT1* and *IGH* genes using fluorescence in situ hybridization. In addition, we analyzed *VH* genes by subcloning of the monoclonal polymerase chain reaction products. Of 20 cases studied, we detected gene abnormalities in 16: *API2-MALT1* fusion in 9, trisomy 3 in 5, trisomy 18 in 4, *MALT1* abnormality in 13, and *IGH* abnormality in 1. *MALT1* gene abnormalities were concordant with *API2-MALT1* fusion or trisomy 18. One case showed *API2-MALT1* fusion and trisomy 3. On detection of *API2-MALT1* fusion and trisomies, we were able to divide our cases into 3 groups, *API2-MALT1* positive, trisomy positive, and no detectable gene abnormality, suggesting that tumor development had processed along different genetic pathways. All 20 cases were analyzed for *VH* genes. Most of the *VH* genes selected by the lymphomas belonged to the *VH3* family, but there was no restriction to any particular *VH* fragment. Of interest, *VH* genes were unmutated in 7 cases, suggesting that T-cell–independent extrafollicular B-cell maturation may be important in the development of this lymphoma. In addition, both mutated and unmutated tumor cases were found to carry the *API2-MALT1* fusion and trisomy 3. This observation suggests that these gene abnormalities may occur in microenvironments found before or outside of follicular germinal centers.


## 1. Introduction

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is a low-grade tumor characterized by unique histopathologic manifestations including centrocyte-like or monocytoid neoplastic cells, lymphoepithelial lesions, and follicular colonization [1,2]. Chronic inflammation or autoimmune disease is often associated with the emergence of the lymphoma, and the etiologic link between gastric MALT lymphoma and *Helicobacter pylori* infection has been shown by regression in most patients treated with antibiotic therapy. Several chromosomal translocations specific to MALT lymphoma have been identified such as t(11;18)(q21;q21), t(1;14)

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(p22;q32), t(14;18)(q32;q21), and t(3;14)(p14.1;q32) [1,2]. The first translocation, which is the most frequent, creates the *API2-MALT1* fusion gene, and the latter 3, although rare, form a rearrangement between *IGH* gene and *BCL10*, *MALT1*, and *FoxP1* genes, respectively. In addition, trisomies 3 and 18 are frequently detected in MALT lymphomas [3]. These gene alterations are important in tumor development and may be associated with the distinctive clinicopathologic features of MALT lymphoma.

The immunoglobulin heavy chain gene (*IGH*) is formed by rearrangement of the variable (*VH*), diversity (*D*), and joining (*JH*) gene segments at the pre–B-cell stage, and there are approximately 50 functional *VH* fragments that are grouped into 7 structurally related families [4]. Analysis of *IGH* genes have provided invaluable information on the status of tumors formed during B-cell differentiation and have added another dimension to the classification of B-cell tumors at the molecular level. Somatic hypermutation of immunoglobulin genes is a consequence of the exposure of B cells to the germinal center of secondary lymphoid organs and is the hallmark of germinal center or postgerminal center B cells [5]. Most B-cell lymphomas have been thought to be derived from either mutated B cells or naive unmutated B cells [6]. However, recent studies have revealed a considerable molecular heterogeneity in some B-cell lymphoid malignancies.

Although primary pulmonary lymphoma is rare, representing 1% to 3.6% of extranodal lymphomas, most tumors (70%-90%) consists of MALT lymphomas, with the lung being one of the most preferential sites [7]. Unlike MALT lymphomas at other sites, most pulmonary MALT lymphomas develop in the absence of chronic inflammation that facilitates tumor development [2,7,8]. To date, no MALT lymphoma–associated microorganisms corresponding to *H pylori* in gastric MALT lymphoma have been reported. Although infrequent, chronic inflammation induced by an autoimmune disease is the most critical of known risk factors for developing MALT lymphoma [9]. Interestingly, the presence of *API2-MALT1* fusion is particularly high in pulmonary MALT lymphomas as compared with those at other sites [3,10]. In the present study, we investigated pulmonary MALT lymphomas for the *API2-MALT1* fusion, trisomy 3, trisomy 18, and alterations of *MALT1* and *IGH* genes using the reverse-transcription (RT) polymerase chain reaction (PCR) or fluorescence in situ hybridization (FISH). In addition, we performed *VH* gene analysis and evaluated somatic hypermutation in these tumors and its association with gene abnormalities.

## 2. Materials and methods

### 2.1. Case selection

Pulmonary MALT lymphoma cases (n = 20) were retrieved from the pathology files of Nagoya City University Graduate School of Medical Sciences. Specimens were obtained at the initial presentation of the patients, fixed in formalin, and embedded in paraffin. All cases were reviewed according to the criteria of the World Health Organization classification for malignant lymphoma [1]. All of the cases were within the morphologic boundaries of MALT lymphoma and exhibited the following immunophenotype: $CD20^+$, $CD79a^+$, $CD3^-$, $CD45RO^-$, $CD5^-$, $CD10^-$, $CD23^-$, and cyclin $D1^-$. In cases with plasmacytic differentiation, IgG4 immunostain was performed to detect a possible association with IgG4-related disorders. Eleven cases in this series were included in our previous study [10]. Informed consent was obtained, and the study was approved by the institutional review board of Nagoya City University.

### 2.2. Immunohistochemistry for BCL10

Tissue sections were deparaffinized and rehydrated. After antigen retrieval by heat treatment, immunohistochemistry was performed using an automated immunostainer with a monoclonal antibody against BCL10 (clone 151; Zymed, San Francisco, CA). MALT lymphomas carrying *BCL10* gene rearrangement and normal lymph nodes were used as positive and negative controls, respectively. When more than 90% of the tumor cells showed nuclear BCL10 expression, the case was considered as positive [10].

### 2.3. Multiplex RT-PCR for the *API2-MALT1* fusion transcript

Total RNA was extracted from the paraffin sections after proteinase K digestion of the tissues. RNA was subjected to first-round multiplex 1-tube RT-PCR, then to second-round nested multiplex PCRs (3 parallels), as we previously described [11]. RNA samples known to possess the *API2-MALT1* fusion were used as positive controls. As an internal RNA quality control, the $\beta$-actin mRNA fragment was amplified.

### 2.4. FISH analysis

The tissue FISH procedure was performed using formalin-fixed, paraffin-embedded sections. To detect trisomies 3 and 18, we used CEP 3 and 18 Spectrum Orange Probes, which hybridize to the centrometric regions of the chromosomes (Vysis, Downers Grove, IL). To detect *MALT1*- and *IGH*-associated gene alterations, FISH was carried out using LSI MALT1 (Vysis) and IGH (Kreatech Diagnostics, Amsterdam, the Netherlands) Dual Color, Break Apart Rearrangement Probes, respectively. When fusion partners were unknown in cases with *IGH* gene split, FISH was further performed using BCL10 Split Signal FISH DNA probe (DAKO, Tokyo, Japan). For a negative control, we analyzed 10 reactive lymph nodes, and the signal frequency threshold was determined for each FISH probe by counting the number of signals or fusions in 1 nucleus per more than 100 cells. The thresholds were determined as the mean plus 3 SDs. The