



Significance of Bcl10 gene mutations in the clinical diagnosis of MALT-type ocular adnexal lymphoma in the Chinese population

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ABSTRACT. We investigated the expression of Bcl10 gene mutations in mucosa-associated lymphoid tissue-type ocular adnexal lymphoma (OAL), atypical lymphoid hyperplasia (ALH), and reactive lymphoid hyperplasia (RLH) in the Chinese population and its role in clinical diagnosis and pathogenesis. Forty-three samples were collected during patient surgeries. Pathological diagnosis confirmed OAL in 23 cases, ALH in 10 cases, and RLH in 10 cases. Normal peripheral lymph tissues from 12 cases were used as negative controls. Bcl10 gene expression was examined using molecular biological methods, and DNA sequences and mutations were compared with published data. The protein expression of Bcl10 and nuclear factor kappaB (NF- κ B) were detected with immunohistological and immunofluorescence colocalization. Bcl10 gene expression was detected in 15 OAL cases. Novel mutations were found in 11 cases. Notably, 1 mutation, which matched a published mutation, was detected in 1 ALH case; 1 novel mutation was found in 1 RLH case; and no Bcl10 gene mutation was found in controls. Most novel mutations were

truncation mutations, resulting in a truncated protein product of 99 amino acids (compared to the full-length 233 amino acids; GenBank accession No. EF189176). Results of tests for abnormal Bcl10 gene expression in nuclei or cytoplasm were consistent with changes in NF- κ B translocation. This report is the first of newly discovered mutations in the Bcl10 gene in the Chinese population. The distribution of the mutations is consistent with and more sensitive than that of the pathological diagnosis. These mutations can be used to identify the stage and clinical characteristics even when morphological changes are absent.

Key words: Bcl10 gene; Eye tumor; Lymphoma; Diagnosis; Mucosa-associated lymphatic tissue

INTRODUCTION

Mucosa-associated lymphoid tissue (MALT)-type ocular adnexal lymphoma (OAL) frequently occurs outside of the lymph nodes of marginal zone MALT lymphoma, accounting for 15% of all orbital solid tumors (Thieblemont et al., 1997). It is the most common type of OAL and is similar to lymphoproliferative lesions such as reactive lymphoid hyperplasia (RLH). During clinical diagnosis, determination of the course and type of disease using solely clinical manifestations is often difficult (Misdraji et al., 2011). Pathologically, the morphology is difficult to identify microscopically in some borderline atypical lesions, which affects therapy and prognosis. In 1999, Willis et al. first reported a t(1;14)(p22;q32) translocation that led to Bcl10 gene cloning and expression of various Bcl10 mutations and descriptions of their biological significance in MALT lymphoma and other types of tumors. The structure of wild-type Bcl10 and its tumor-suppressive functions have also been described in detail. The mutations and corresponding changes in biological behavior have been attributed to a signal transduction pathway that suppresses apoptosis of tumor cells (Rosebeck et al., 2011). A study of low-grade gastric MALT lymphoma by Du et al. (2000) found mutations of Bcl10 in 3 of 11 tumors that were unresponsive to *Helicobacter pylori* eradication therapy, but none was found in 22 tumors that regressed completely after the same treatment. However, the significance of Bcl10 in OAL, differences in the mutation in Chinese people, and its significance and association related to clinical diagnosis have not been reported. In this study, we first detected the existence of Bcl10 in Chinese patients with OAL, found novel mutation sites, identified novel mutations in the Bcl10 gene, and investigated their relationships with the activation of nuclear factor-kappaB (NF- κ B). Our aim was to assess the value of novel Bcl10 gene mutations in the diagnosis of MALT lymphoma and to provide new evidence for the role of the Bcl10-activated NF- κ B pathway in the development of MALT lymphoma.

MATERIAL AND METHODS

Subjects

Informed consent was obtained from all subjects, and the study was approved by the Ethics Committee of China. All subjects were recruited between 2008 and 2011 from the Ophthalmology Department at the First Affiliated Hospital of Chinese PLA General Hospital

and Changzheng Hospital, Second Military Medical University. Included in the study were 23 patients with OAL, 10 with atypical lymphoid hyperplasia (ALH), 10 with RLH, and 12 individuals with normal peripheral lymph tissue. The diagnoses were confirmed by 2 independent pathologists using a double-blind method according to the World Health Organization classification of myeloid tumors in the hematologic system (Rohatiner et al., 1994). All samples were obtained during surgery and stored in liquid nitrogen.

Histopathological assay and immunostaining

The histological material fixed in 10% formalin and embedded in paraffin was cut into 4- μ m sections. After deparaffinization, the sections underwent hematoxylin and eosin staining for histopathological assay. The avidin-biotin-immunoperoxidase technique was used as outlined in a previous study (Willis et al., 1999). Antigen retrieval was performed after hydration and endogenous peroxidase blocking with 3% hydrogen peroxide at 90°C for 30 min. The sections were then incubated for 30 min at room temperature with anti-CD45RO (1:200) and anti-CD3 (1:100) for T-cell labeling, with anti-CD20 and anti-CD79a (1:100) for B-cell labeling, with anti-immunoglobulin κ and λ light-chain (1:200) for discriminating benign lymphoid hyperplasia from MALT lymphoma, with anti-bcl2 and Ki-67 (1:50) to determine proliferation, and with anti-Bcl10 to identify Bcl10 gene expression products.

The evaluation of immunostaining was as follows: CD20, CD79a, and CD45RO stained the membrane yellow-brown; CD3, κ , and λ stained cytoplasm in a diffuse yellow-brown; and Bcl-2 and Ki-67 stained the nucleus yellow-brown. The positive staining index was the percentage of positive cells among 1000 cancer cells under 5 high-power visual fields. The staining was scored as follows: positive cells <5% (-), 5-24% (+), 25-49% (++), 50-74% (+++), and \geq 75% (++++).

Immunofluorescence colocalization

We used 4- μ m-thick optical sections for immunofluorescence colocalization. After blocking, the sections were exposed to fluorescein isothiocyanate-labeled Bcl10 antibody (1:100 green), CY3-labeled I κ α antibody (red), and 4',6-diamidino-2-phenylindole (blue) for staining nuclei (1:50). The sections were examined using a Leica TCS SP2 microscope (Leica Co., Ltd., German) and the LSM 510 Image Browser software (Leica Co., Ltd.).

DNA sequencing and mutation detection

Total RNA was isolated from frozen tumor tissues using a phenol/chloroform method, and RNA purity was assessed with spectrophotometry ($A_{260}/A_{280} > 1.8$). Complementary DNA was synthesized from total RNA ranging from 50 to 500 ng in a 10- μ L reaction mixture using a reverse transcription-polymerase chain reaction (RT-PCR) kit (Beijing TransGen Biotech Co. Ltd., Beijing, China) according to manufacturer instructions. Twenty microliters of PCR mixture contained 1 μ L RT product as a template, PCR buffer, 2 mM MgCl₂, 1.25 U/50 mL DNA polymerase, and 0.15 mM primers. The primer sequences were designed according to the Bcl10 gene and synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). We amplified β -actin as an internal control. The PCR conditions were an initial denaturing at 95°C for 5 min; followed by 35 cycles of 94°C for 40 s, 60°C for 40 s, and 72°C for 60 s; a final extension at 72°C

for 7 min; and a final hold on ice. These conditions produced the initial amount of total RNA used in the RT reaction. PCR products were examined on a 1% agarose gel. A 700-800-bp Bcl10 fragment was confirmed under ultraviolet light after ethidium bromide staining.

Sequence analysis

The purified PCR products were ligated into a PMD18-T vector, and the resultant ligation products were transformed into BL21 *Escherichia coli* competent cells. The positive clones were confirmed with restriction enzymes, sequenced using a PRISM™ 3700 DNA automatic sequencer (ABI Prism™ BigDye™ Terminator Cycles Sequencing Ready Reaction Kit), and analyzed with the BLAST software (Invitrogen, Shanghai).

Statistical analysis

The SPSS10.0 statistical package (SPSS Incorporated, Chicago, USA) was used for all analyses. The variables were examined using the Fisher exact test. A P value of <0.05 was considered to be statistically significant.

RESULTS

Nucleotide sequence mutations and functional protein domain mutants

Of the 43 cases studied, 21 displayed Bcl10 gene expression. Compared with the published mutation sequences, sequences from 12 cases contained novel mutations (Table 1). After analysis and comparison using the BLAST software, the significant mutations were as follows: 129-bp deletion at nucleotide 294 in the exon, resulting in a truncated protein product of 99 amino acids compared with the full-length protein of 233 amino acids. We registered this mutation in GenBank (accession No. EF189176). Four additional specimens had the same mutation, i.e., a G→A transversion at nucleotide 638 in the exon, changing Gly 213 to Glu. This point mutation was also detected in 1 RLH case. A T→C point mutation at nucleotide 308 in the exon changed Val 103 to Ala in the protein-coding region; a T→C point mutation at nucleotide 368 in the exon changed Glu 123 to Gly; an A→G point mutation at nucleotide 374 in the exon changed Ben 125 to Cyp; a T→C point mutation at nucleotide 5 in the exon changed Glu 2 to Gly; and a T→C point mutation at nucleotide 412 in the exon changed Val 103 to Ala. An A→G point mutation at nucleotide 145 in the exon has been reported previously (Willis et al., 1999).

Four of the 10 atypical ALH specimens were positive for Bcl10: a 33-bp deletion at nucleotide 346 in exon truncated the protein from 126 to 116 amino acids. This case presented a different spliceosome of messenger RNA from other specimens, and these mutations had been reported previously. Bcl10 gene expression was detected in 2 cases of RLH, 1 of which displayed the same mutations found in 4 cases described above in ALH cases. No Bcl10 gene expression was detected in the 12 normal peripheral lymph tissues. A significant difference was found between Bcl10 expression rates in the experimental group and that in the control group ($P < 0.05$). The Bcl10 mutation rate varied significantly in lesions with different degrees of malignancy ($P < 0.05$). No significant difference was found in Bcl10 mutation rates between different locations and different clinical stages ($P > 0.05$).

Table 1. Type of Bcl10 gene mutations of ocular adnexal MALT lymphoma in Chinese population.

Tumor	Case No.	Alteration	Codon	Mutation type	Predicted protein size (amino acids)	Published type
MALT lymphoma	6-2-9-1	308 T→C	103	Val→Ala	233	Novel mutation
	3-2-9-5	638 G→A	213	Gly→Glu	233	Novel mutation
	1-12	638 G→A	213	Gly→Glu	233	Novel mutation
	2-28	638 G→A	213	Gly→Glu	233	Novel mutation
	6-10	638 G→A	213	Gly→Glu	233	Novel mutation
	10-2	294del129	99	Truncation	109	Registration
	4-20	368 T→C	123	Glu→Gly	233	Novel mutation
	5-14	145 A→G	49	Arg→Gly	157	Published*
	6-25	374 A→G	125	Phe→Ser	233	Novel mutation
	7-13	5 T→C	2	Glu→Gly	233	Nonsense mutation
	8-20	412 T→C	103	Val→Ala	233	Novel mutation
	3-21	186 A→G	62	Arg→Arg	233	Nonsense mutation
	A1-11-29	Normal	Full-length amino acids	None	233	
	L-C	Normal	Full-length amino acids	None	233	
	8-11	Normal	Full-length amino acids	None	233	
	Reactive lymphoid hyperplasia	10-25	638 G→A	213	Gly→Glu	233
11-5		Normal	Full-length amino acids	None	233	
Atypical lymphoid hyperplasia	10-5	346del33	116-126	Splice aberration	157	Published*
	Xu-1	Normal	Full-length amino acids	None	233	
	4-20	Normal	Full-length amino acids	None	233	
	5-11	Normal	Full-length amino acids	None	233	

*Other several types.

Pathological features and immunophenotype of OAL

Of 23 patients with MALT lymphoma, 12 had lymphoid follicles. Compared with normal tissue, the residual germinal center in these cases was small and the cap was absent. Monotonous infiltration formed by a few centrocyte-like cells and immune blood cells was seen. The tumor cells displayed diffuse distribution with small cell volume, wide cytoplasm, and nuclei with cell-like or monocyte-like germinal center differentiation. No lymphoepithelial lesions were found. Three cases displayed combined infiltrated vessels and fat tissues. One case of ALH consisted mainly of mature lymphocytes, and the cells showed diversity. Sporadic plasmacytes, histiocytes, and immune blood cells were also seen. Of the 10 RLH cases, 1 featured normal follicle structure, small lymphocyte proliferation, and plasmacyte infiltration. Immunohistochemical analysis revealed CD20 (+), CD79a (+); CD3 (-), CD45RO (-). Five cases were immunoglobulin κ light-chain positive, and 2 cases were λ light-chain positive. Four cases were Bcl-2 positive (++); 3 cases, Bcl-2 positive (+); and 1 case, Bcl-2 negative (-). The Ki-67 proliferation rate was >10% in 4 cases.

Twenty (86.96%) of the 23 MALT specimens were Bcl10 positive, including 13 cases (65.0%) positive in both the nucleus and the cytoplasm and 7 cases (35.0%) positive only in the cytoplasm (Figure 1). Five (50%) of the 10 atypical lymphatic hyperplasia specimens had diffuse Bcl10 expression in the cytoplasm. One case (10%) Bcl10 expression was positive in both the nucleus and the cytoplasm, and 3 (30%) of the 10 RLH specimens displayed Bcl10 expression in the cytoplasm. All control specimens were Bcl10 negative. The nuclear expression of the Bcl10 protein was significantly correlated with mutation of the Bcl10 gene ($P < 0.05$). The expression of the Bcl10 protein was not significantly different in lesions with various degrees of malignancy ($P > 0.05$). Eighteen (78.3%) of the 23 MALT specimens were

NF- κ B positive both in the nucleus and the cytoplasm, and 5 cases (21.7%) were positive only in the cytoplasm. Seven (70%) of the 10 ALH specimens had diffuse NF- κ B expression in the cytoplasm. One case (10%) was NF- κ B positive in both the nucleus and the cytoplasm, and 5 (50%) of the 10 RLH specimens displayed NF- κ B expression in the cytoplasm.

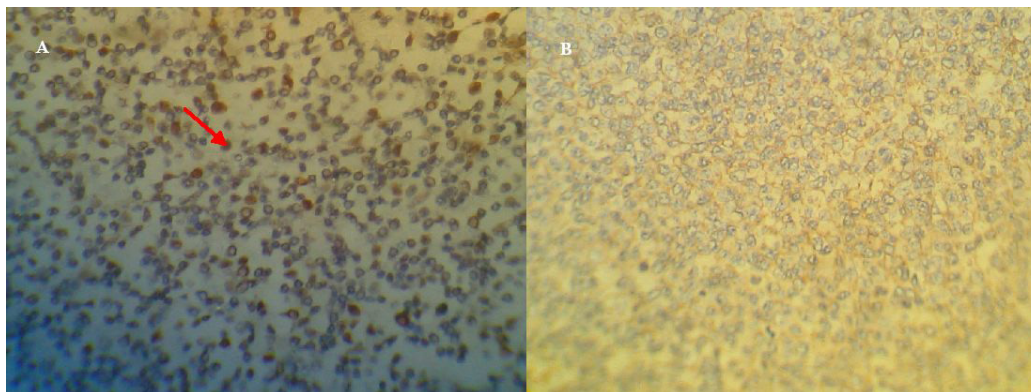


Figure 1. A. Bcl10 expression in tumor cell nuclei. B. Nuclear factor-kappaB expression in tumor cell nuclei and cytoplasm.

In the control group, all specimens had diffuse NF- κ B expression in the cytoplasm. The positive rates of the NF- κ B protein in MALT-type OAL, ALH, and RLH were significantly different ($P < 0.05$). The nuclear expression of the Bcl10 protein was significantly correlated with the expression of NF- κ B ($P < 0.05$).

Bcl10 gene mutation in MALT-type OAL, ALH, and RLH

Of 43 OAL cases, the Bcl10 gene cDNA was detected in 15 cases with MALT, and novel mutations were found in 11 cases. The mutation-positive rate was 73.33%. Bcl10 mutation was detected in 1 ALH case, and had been reported previously. A novel Bcl10 mutation was detected in 1 RLH case. The 12 cases with normal lymph tissues showed no Bcl10 expression (Table 2). The Fisher exact test showed that the differences in Bcl10 gene mutation were statistically significant among the cases with MALT lymphoma, ALH, RLH, and normal lymph tissue.

Colocalization of the Bcl10 gene and NF- κ B

The signals of a laser-scanning confocal microscope were subjected to 3-D reconstruction using the SFP option. Fluorescein isothiocyanate-labeled Bcl10 protein was distributed in the nucleus and cytoplasm in a dotted manner or in clusters of green fluorescence granules. Cy3-labeled NF- κ B protein was widely distributed in the cytoplasm. The reconstructed image showed colocalization of the yellow fluorescence of Bcl10 and NF- κ B, including 14 cases of Bcl10 and NF- κ B coexpression (Figure 2). The expression of Bcl10 was positively correlated with that of NF- κ B ($P < 0.05$).

Table 2. Type of Bcl10 gene mutations of ocular adnexal MALT lymphoma in Chinese population.

Tumor	Case No.	Alteration	Codon	Mutation type	Predicted protein size (amino acids)	Published type
MALT lymphoma	6-2-9-1	308 T→C	103	Val→Ala	233	Novel mutation
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	2-28	638 G→A	213	Gly→Glu	233	Novel mutation
	6-10	638 G→A	213	Gly→Glu	233	Novel mutation
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	4-20	368 T→C	123	Glu→Gly	233	Novel mutation
	5-14	145 A→G	49	Arg→Gly	157	Published*
	6-25	374 A→G	125	Phe→Ser	233	Novel mutation
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	3-21	186 A→G	62	Arg→Arg	233	Nonsense mutation
	A1-11-29	Normal	Full-length amino acids	None	233	
	L-C	Normal	Full-length amino acids	None	233	
	8-11	Normal	Full-length amino acids	None	233	
Reactive lymphoid hyperplasia	10-25	638 G→A	213	Gly→Glu	233	Novel mutation
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Atypical lymphoid hyperplasia	10-5	346del33	116-126	Splice aberration	157	Published*
	Xu-1	Normal	Full-length amino acids	None	233	
	4-20	Normal	Full-length amino acids	None	233	
	5-11	Normal	Full-length amino acids	None	233	

*Other several types.

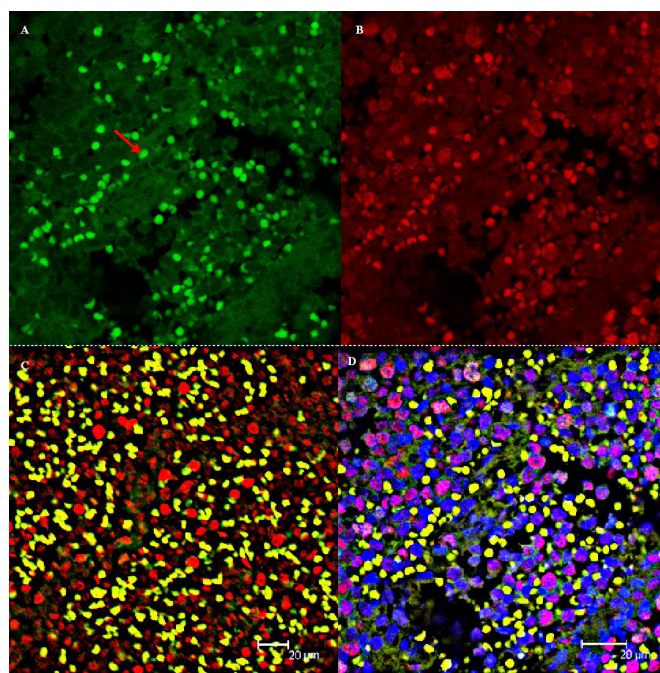


Figure 2. A. Bcl10 protein is indicated by punctate bright-green fluorescent particles distributed in the nucleus and cytoplasm. B. NF-kappaB protein is indicated by diffuse bright-red fluorescence widely distributed in the nucleus and cytoplasm. C. Overlapping images: visible yellow fluorescence for the double expression of Bcl10 green fluorescence and the red fluorescence of NF-kappaB. D. 4',6-Diamidino-2-phenylindole staining of nuclear superimposed images. The orange fluorescence indicates NF-kappaB expression in the nucleus.

DISCUSSION

Bcl10, an intracellular protein, is homologue of the equine herpesvirus-2 E10 gene. The 233-amino acid BCL10 protein promotes apoptosis under certain physiological conditions (Wu and Ashwell, 2008). It contains a single N-terminal caspase activation and recruitment domain (CARD) containing antiparallel α -helices similar to those seen in the “death domain” and a serine/threonine-rich C-terminus (Zhou et al., 2004). The Bcl10 gene has been cloned from the breakpoint region of t(1;14) (p22;q32) (Willis et al., 1999) and is considered an apoptosis-associated gene involved in NF- κ B signal pathway activation. The CARD of Bcl10-mediated self-oligomerization triggers the ubiquitin of mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) and phosphorylation of I κ B kinase, NF- κ B activation, and κ B translocation into the nucleus.

Ye et al. (2005) reported that t(14;18)(q32;q21) is characterized by strong cytoplasmic MALT1 and Bcl10 expression, and t(1;14)(q22;q32) is characterized by weak cytoplasmic MALT1 and strong nuclear Bcl10 expression. However, the nuclear expression of Bcl10 is closely related to the API2-MALT1 fusion protein induced by t(11;18)(q21;q21) (Sagaert et al., 2007) and clinical disease stage (Coupland, 2004). Therefore, Bcl10 expression is closely related to the genesis and development of MALT lymphoma and plays a key role in chromosomal variation. Bcl10 is considered a marker protein in MALT lymphoma (Marcus, 2007). It can specifically link with lymphocyte receptors through CARD11/lipid rafts, subsequently adjusting the oligomerization of MALT1 to form a complex via oligomerization of the N-terminal CARD without upstream signaling (Sun and Yang, 2010). It activates downstream NF- κ B signaling via unknown molecular mechanisms, causing abnormal NF- κ B translocation in the nucleus that regulates cell apoptosis and changes in lymphocyte proliferation (Hamoudi et al., 2010). Therefore, Bcl10 plays a very important role in the pathogenesis of MALT lymphoma.

The Bcl10 gene mutation leads to the insertion, deletion, and substitution of single nucleotides presenting as frameshift mutations of the open reading frame, predicting positions of stop codons, and truncating the C-terminal region of CARD. Although Bcl10 gene mutation can activate the NF- κ B pathway, it loses the capability to adjust apoptosis and gains carcinogenic capabilities, resulting in the formation and proliferation of MALT lymphoma cells (Kingeter and Schaefer, 2010). The mutation and nuclear expression of Bcl10 activate the NF- κ B signal pathway and play a central role in tumorigenesis of MALT lymphoma (Du, 2011). The activation of the NF- κ B signal pathway by Bcl10 leads to the degradation of I κ B phosphorylation, translocation into the nucleus, and changes in gene transcription - important mechanisms in MALT lymphoma.

In this study, samples were harvested during surgery and frozen in liquid nitrogen. The pathological diagnoses were MALT-type OAL, ALH, and RLH. In clinical diagnosis, distinguishing MALT-type OAL from orbital lymphoid tissue nonspecific hyperplasia, inflammatory pseudotumors, and other orbital disease is challenging because their clinical presentations are similar; however, their therapy methods and patient prognoses are quite different. Therefore, accurate and significant differential diagnosis is extremely important.

A palpable mass was the most obvious characteristic among the 23 cases of pathologically diagnosed MALT lymphoma, 10 ALH cases, and 10 RLH cases. No significant difference in clinical indexes was found among the cases. The clinical presentation served only as a reference, and the diagnosis depended mainly on imaging features obtained before surgery

and the report on pathological morphology that followed. However, pathological diagnosis involves mainly the identification of morphology, and the naked eye cannot discern or diagnose atypical boundary diseases or fine changes at early disease stages, especially in cells with immature cell morphology that have not developed typical MALT lymphoma characteristics, such as follicle colonization, lymphoepithelial lesions, and so on (McKelvie, 2010).

In the 23 cases of MALT lymphoma, only 12 presented as typical follicle colonization microscopically. Center cell-like cell infiltration and cap disappearance were seen, normal and colonized follicles coexisted in 5 cases, and diffusely distributed tumor cells without follicle-like structure were seen in 6 cases. With only clinical manifestations and pathological data, diagnoses are easily missed. This study implemented RT-PCR to amplify the Bcl10 gene and detect mutations in each sample. These data are more sensitive than pathological information in determining tumor processes, identifying early boundary diseases and their malignant degrees, and predicting prognoses. In the 10 RLH cases, follicle structure and mature lymphocyte infiltration were seen under a light microscope, but only 1 case displayed novel Bcl10 mutation on RT-PCR and sequencing analysis, indicating that this case had boundary changes from RLH to MALT lymphoma. The biological behavior revealed functional changes at the genetic level, but these changes could not be identified by the naked eye in pathological morphology to make a final diagnosis. This case should be treated as a malignant tumor; surgery should be performed to incise the tumor and part of the surrounding tissue; and local radiotherapy and chemotherapy should be administered for better treatment effects and refined prognoses.

Similarly, in 1 ALH case, pathological assay could not exclude abnormal lymphoplasia. Microscopic examination showed cells exhibiting diversity with infiltration of scattered plasma cells, histiocytes, and immunoblasts. Genomic tests found a deletion mutation, which has been reported earlier. This finding also suggested that pathologically diagnosed ALH already has malignant properties, indicating that it should be treated as such to refine prognosis. Statistically, the differences in Bcl10 gene mutation were significant among subjects with MALT lymphoma, ALH, RLH, and normal peripheral lymph nodes.

This study found that Bcl10 had different novel mutations in different types of disease and that the distribution of the mutations in MALT lymphoma, ALH, and RLH was consistent with pathological type. This information may help clinicians make earlier and more sensitive estimations of disease course at the genetic level. In the 11 cases with new mutations, the mutant in 5 cases was a G→A point mutation at nucleotide 638 in the exon, changing Gly 213 to Glu in the protein coding region with a 45% mutation rate. The next was a 129-bp deletion at nucleotide 294 in the exon, which truncated the 233-amino acid protein product to 99 amino acids (GenBank accession No. EF189176). A T→C point mutation at nucleotides 308, 368, and 5 in the exon changed Val 103 to Ala, Glu 123 to Gly, and Glu 2 to Gly, respectively. An A→G point mutation at nucleotides 115, 374, and 186 changed Cys 58 to Arg, Ben 125 to Cyp, and Arg 62 to Ala.

Compared with the mutation types published by Willis et al. (1999), the mutations in MALT-type OAL in Chinese people are mainly point mutations. New mutations were also found in ALH and RLH, suggesting that the mutation of the wild-type Bcl10 plays an important role in the progression of ALH and RLH to MALT lymphoma. Further study is needed to investigate the mutations that might lead to changes in biological behavior in tumor cells and their roles in the activation of the NF-κB intracellular signal pathway. Examination of fluorescence expression of Bcl10 in this study showed that in MALT lymphoma, the NF-κB response factor of Iκα displays

diffuse expression in the cytoplasm, whereas Bcl10 might not be expressed in samples that express $\text{I}\kappa\alpha$. Both Bcl10 and NF- κ B were expressed in 14 cases of MALT lymphoma.

Currently, activation of the NF- κ B signal pathway caused by the mutation and nuclear expression of Bcl10 is believed to play an important role in the tumorigenic mechanism of MALT lymphoma (Hosokawa and Seto, 2004). The activation of the NF- κ B pathway by Bcl10, leading to the degradation of $\text{I}\kappa\text{B}$ phosphorylation and κB translocation to the nucleus, in turn triggering transcription, is an important mechanism of tumorigenesis. Few studies about local ocular lesions in systemic MALT lymphoma have been published, and most reports focus on retrospective investigations of clinical parameters and prognosis from clinical data and follow-up. Only minor attention has been paid to pathogenesis with respect to molecular pathology, biology, and intracellular signal transduction pathways (Garrison et al., 2009). The abnormal expression and mutants of Bcl10 in MALT-type OAL are closely related to various anomalies of karyological chromosomes and are usually the last targeted object of each chromosomal karyotypic abnormality. Bcl10 expression can be found in various chromosomal anomalies, but the mechanism through which Bcl10 mutation leads MALT1 oligomerization and activates the NF- κ B essential modulator in the NF- κ B pathway, as well as the specific signaling pathway of the transcription changes induced by κB translocation into the nucleus, remain unclear (Prasad et al., 2010).

This study is the first to detect and confirm Bcl10 expression in OAL, ALH, and RLH completely in Chinese people. We uncovered novel mutations and determined the relationship between Bcl10 and NF- κ B expression using laser confocal fluorescence. This study provides new evidence and reference for the role of the NF- κ B pathway activated by Bcl10 mutation and abnormal expression in the pathogenesis of MALT lymphoma and its clinical and differential diagnosis. The results also suggest that the detection of target gene expression and mutation using molecular biological methods may provide a more accurate and sensitive index for clear clinical diagnosis.

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