BCL10 Expression in Normal and Neoplastic Lymphoid Tissue

Nuclear Localization in MALT Lymphoma

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BCL10 is an apoptotic regulatory molecule identified through its direct involvement in t(1;14)(p22;q32) of mucosa-associated lymphoid tissue (MALT) lymphoma. We examined BCL10 protein expression in various normal tissues and B-cell lymphomas by immunohistochemistry of formalin-fixed and paraffin-embedded tissues using mouse BCL10 monoclonal antibodies. BCL10 protein was expressed in lymphoid tissue but not in 21 various other tissues with the exception of breast. In normal B-cell follicles, the protein was expressed abundantly in the germinal center B cells, moderately in the marginal zone, but only weakly in the mantle zone B cells. Irrespective of their stage of B-cell maturation, BCL10 was predominantly expressed in the cytoplasm. In contrast, each of the four MALT lymphomas with t(1;14)(p22;q32) showed strong BCL10 expression in both the nucleus and cytoplasm. Twenty of 36 (55%) MALT lymphomas lacking the translocation exhibited BCL10 expression in both the nucleus and cytoplasm although at a much lower level, whereas the remaining 16 cases displayed only cytoplasmic BCL10. Unlike MALT lymphoma, both follicular and mantle cell lymphomas generally displayed BCL10 expression compatible to their normal cell counterparts. Our results show differential expression of BCL10 protein among various B-cell populations of the B-cell follicle, indicating its importance in B-cell maturation. The subcellular localization of BCL10 was frequently altered in MALT lymphoma in comparison with its normal cell counterparts, suggesting that ectopic BCL10 expression may be important in the development of this type of tumor. (*Am J Pathol* 2000, 157:1147–1154)

Chromosomal translocation (1;14)(p22;q32) is a rare but recurrent chromosomal aberration in mucosa-associated lymphoid tissue (MALT) lymphoma and has been found exclusively in this type of tumor.¹ MALT lymphoma cells with t(1;14)(p22;q32) grew spontaneously in culture in the absence of B-cell stimulants, whereas in contrast, those without the translocation died rapidly by apoptosis.² After stimulation with B-cell mitogens, the proliferative response of the tumor cells with t(1;14)(p22;q32) was 50 times higher than that of the tumor cells without the translocation.² These data suggest that t(1;14)(p22;q32) involves a critical gene, which promotes malignant B-cell survival and growth.

Molecular cloning of the breakpoint of t(1;14)(p22;q32) has allowed the identification of the involved gene.^{3,4} BCL10 encodes a protein of 233 amino acids with residues 13 to 101 forming a caspase recruitment domain (CARD), found in a number of apoptotic regulatory molecules.⁵ Wild-type BCL10 weakly promoted apoptosis in *in vitro* assays and activated nuclear factor κ B (NF- κ B),^{3,4,6–10} a transcription factor for several cell survival molecules.¹¹ In the rat embryonic fibroblast transformation assay, the wild-type molecule inhibited transformation induced by synergistic oncogenes.³ Truncated BCL10 isolated from cDNA clones of a gastric MALT lymphoma with t(1;14)(p22;q32) lost the pro-apoptotic activity but retained the ability of NF- κ B activation and moreover gained functional enhancement of malignant transformation.³ Thus, wild-type BCL10 may act as a tumor suppressor whereas mutation may result in BCL10 gaining oncogenic functions. Truncating BCL10 mutations have been found in ~5% of MALT and follicular lymphomas.^{12,13}

BCL10 mRNA is commonly expressed in normal tissues but most highly expressed in lymphoid tissues.^{3,4,6–9} In the B-cell follicle, BCL10 mRNA was expressed highly in the germinal center, moderately in the marginal zone, and weakly in the mantle zone B cells.³

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BCL10 mRNA was also highly expressed in MALT lymphomas with and without t(1;14)(p22;q32).³ However, expression of the protein and its cellular localization are unknown. We generated mouse BCL10 monoclonal antibodies and studied the protein expression in various normal tissues and different subtypes of B-cell lymphomas.

Materials and Methods

Materials

Formalin-fixed and paraffin-embedded tissue specimens from 31 normal and reactive lymphoid tissues including eight fetal thymus from 16 to 40 weeks of gestation, four appendices, eight tonsils, six lymph nodes, and five spleens, 116 lymphomas comprising 40 MALT, 20 mucosal diffuse large B-cell lymphomas (DLBCL) (14 with low-grade MALT lymphoma component), 21 follicular, 17 mantle cell, and 18 nodal diffuse large B-cell lymphomas, as well as 74 normal nonlymphoid tissues of 21 different types were retrieved from the surgical files of Department of Histopathology, Royal Free and University College Medical School. Of the MALT lymphomas, four cases previously showed t(1;14)(p22;q32) by cytogenetics and 34 of the 40 cases originated from the stomach. The histology of all lymphoma cases was reviewed by AD.

Expression and Purification of Recombinant BCL10 Protein

The full length (amino acids 1 to 233) and amino terminus (amino acids 1 to 122) of BCL10 were polymerase chain reaction-amplified from a BCL10 cDNA clone using a forward primer containing *Nco*I site (5'ATCCATGGAGC-CCACCGCACCAGTCC3') and a reverse primer containing *Not*I site (5'ATGCGGCCGCTTGTCGTGAAACAGTACGTGA3' for the full length; 5'ATGCGGCCGCACAACTGCTACATTTAGTC3' for the amino terminus). The polymerase chain reaction products were cloned into the TA cloning vector pGEM-T, subcloned into PUC119/Myc-His at the *Nco*I and *Not*I sites and then transformed into *Escherichia coli* HB2151. Colonies were screened using polymerase chain reaction with vector primers (M13 forward and reverse) and positive clones were further sequenced to check for correct sequence and reading frame. Ten positive clones were induced to express BCL10 protein in a 5-ml culture with 1 mmol/L isopropyl- β -thiogalactopyranoside (IPTG) at 28°C for 10 to 16 hours and their BCL10 expression was assessed by Western blotting with 9E10 antibody (Sigma, Poole, UK), which recognizes the *c-myc* tag. The clone expressing the highest level was subjected to induction in a 2-L culture under the same conditions. BCL10 was purified using Ni-NTA (QIAGEN, Crawley, UK) affinity chromatography under denaturing conditions with 8 mol/L of urea according to the manufacturer's instructions. Purified BCL10 was dialyzed against 30 mmol/L Tris-HCl (pH 8.0) and concentrated using Centrplus concentrators (Amicon, Beverly, MA). The purity and yield were checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Generation of Monoclonal Antibody

BALB-c strain mice (Harlan, London, UK) were primed with 50- μ g full-length recombinant BCL10 protein in complete Freund's adjuvant followed by two boost injections with 50- μ g BCL10 protein in incomplete Freund's adjuvant. Three days after the second boost injection, the splenic cells of the immunized mice were fused with the myeloma cell line NSO as described previously.¹⁴ A total of 941 clones were screened using an enzyme-linked immunosorbent assay with full-length recombinant BCL10 protein and 128 positive clones were obtained. To identify clones that might be suitable for immunohistochemistry of formalin-fixed paraffin-embedded tissue materials at an early stage of the cloning process, the enzyme-linked immunosorbent assay-positive clones were also screened by immunohistochemical staining of paraffin sections from MALT lymphoma with t(1;14)(p22;q32). The positive clones were expanded in 24-well culture plates for 4 to 8 days. Eleven positive clones were sustained as shown by enzyme-linked immunosorbent assay and were subjected to single-cell cloning. A total of eight enzyme-linked immunosorbent assay-positive single clones were finally obtained.

Generation of BCL10 Stable Expression Cell Lines

HEK 293 cells were separately transfected with pcDNA3.1/myc-His (Invitrogen, Carlsbad, CA) containing either a full-length BCL10, mutant 106 (amino acids 1 to 168), carboxyl terminus (amino acids 101 to 233), or no insert using the calcium-phosphate method.³ Colonies were selected with 1 mg/ml of G418, ring-cloned, and screened for exogenous BCL10 expression. The stable cell lines were maintained in high-glucose Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 100 g/ml of penicillin, and 100 μ g/ml of streptomycin supplemented with 1 mg/ml of G418.

Western Blotting Analysis

Frozen tissue sections or cell pellets were homogenized in 50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 0.02% sodium azide, 0.1% sodium dodecyl sulfate, 1% Nonidet P-40, 0.5% sodium deoxycholate, 100 mg/ml phenylmethylsulfonyl fluoride, and 1 mg/ml leupeptin. Protein extracts were mixed with sodium dodecyl sulfate gel loading buffer, denatured, separated on 12% polyacrylamide gels, and electrotransferred onto nitrocellulose membranes. The membranes were sequentially incubated with a BCL10 monoclonal antibody (1/2,000 dilution of culture supernatant from hybridoma clones), biotinylated rabbit anti-mouse Ig (DAKO, High Wycombe, UK), alkaline phosphatase-conjugated avidin and were finally visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

Immunohistochemistry

Culture supernatant from hybridoma clones was directly used for immunostaining. For formalin-fixed paraffin-embedded tissue, heat retrieval of antigen was performed on 4- μ m sections before antibody staining. Serial dilutions of the hybridoma culture supernatant and various antigen retrieval methods were tried. A 1/60 dilution of the hybridoma culture supernatant and treatment of sections in target retrieval solution, pH 6.0 (DAKO, UK), in a microwave oven for 30 to 35 minutes gave the best results of immunostaining and was used for study of the protein expression in both normal and malignant lymphoid tissues. The protein expression was evaluated by three different observers (HY, AD, MQD). The proliferation antigen Ki67 was detected with monoclonal antibody MIB1 (1/70 dilution; DAKO) using the avidin-biotin method preceded by antigen heat retrieval. The Ki67 proliferation indices were determined by counting 10 randomly chosen fields. Only homogenous tumor areas were included for the quantification to ensure that the Ki67-positive cells counted maximally represented tumor cells.

Statistical Analysis

Statistical evaluation of quantitative data from two groups was performed using nonparametric Mann-Whitney *U*—Wilcoxon Rank Sum *W* test with SSPS software.

Results

Characterization of BCL10 Antibodies

The eight positive single clones were examined for their specificity by Western blotting analysis with the full-length recombinant BCL10 protein. Seven clones showed specific recognition of the BCL10 protein. To map the amino acid residues recognized by these monoclonal antibodies, Western blotting analysis of the HEK 293 cells transfected with various BCL10 deletion constructs as well as the recombinant amino-terminal BCL10 product was performed. Three clones including clone 151 recognized the full-length (amino acids 1 to 233), the truncated (amino acids 1 to 168), and the carboxyl-terminal (amino acids 101 to 233) BCL10 products expressed in HEK 293 cells, but did not react with the recombinant amino-terminal BCL10 product (amino acids 1 to 122) (Figure 1), indicating that these clones recognized amino acids between 122 to 168. The remaining four clones recognized the full-length and the carboxyl-terminal BCL10 product but only weakly reacted with or did not recognize the truncated BCL10 product, suggesting that these antibodies recognized amino acid residues further toward the carboxyl terminus than those recognized by clone 151. No clones recognized epitopes within the amino-terminal CARD. All seven monoclonal antibodies were further tested by immunohistochemistry of formalin-fixed paraffin-embedded tissue sections from MALT lymphomas with t(1;14)(p22;q32). All seven clones showed characteristic staining of MALT lymphoma with t(1;14)(p22;q32)

Figure 1. Western blotting analysis. Mouse BCL10 monoclonal antibody clone 151 recognizes the expressed BCL10 products in 293 cells by constructs containing the full-length wild-type (293 BCL10 wt, amino acids 1 to 233), the truncated (293 m106, amino acids 1 to 168), and the carboxyl-terminal BCL10 sequence (293 C-terminus, amino acids 101 to 233), and does not react with the recombinant BCL10 amino terminal product (BCL10 N-terminus, amino acids 1 to 122). Tonsil, MALT lymphoma, and the 293 cells transfected with control vector show two forms of BCL10: a predominant 32-kd band and a weaker 37-kd band, indicated by **arrowheads**.

(see below). The staining was specific because no staining was seen if hybridoma culture supernatant was omitted or immunoabsorbed with the recombinant BCL10 protein before immunohistochemistry. Of the seven monoclonal antibodies, clone 151 gave the best immunostaining on paraffin-embedded tissue sections and was used for all subsequent experiments. Western blotting analysis of frozen tissues from tonsil, lymph node, and spleen showed that BCL10 was present as a predominant 32-kd band with a weaker 37-kd band (Figure 1).

BCL10 Expression in Normal Lymphoid Tissues

BCL10 protein was expressed in normal spleen, reactive tonsil, lymph node, and MALT of the appendix (Table 1). In B-cell follicles, the protein was expressed abundantly in the germinal center B cells, moderately in the marginal zone, but only weakly in 40 to 60% of the mantle zone B cells (Figure 2, A–D). Within the germinal center, dark-zone centroblasts expressed more BCL10 than light-zone centrocytes (Figure 2A). Both the germinal center and marginal zone B cells expressed BCL10 protein in the cytoplasm (Figure 2, B and D). The subcellular localization of BCL10 in the mantle zone B cells could not be confidently determined because of their low expression level and scanty cytoplasm.

BCL10 protein was also expressed in fetal thymus. At early stages of gestation (16 to 25 weeks), BCL10 was expressed in the medulla and occasionally in the cortex (Figure 2E), whereas at late stages of gestation (>28 weeks), the protein was found exclusively in the medulla. Double-immunostaining for BCL10 and CD20 or CD3 (DAKO, UK) revealed that BCL10-positive cells are both B- and T-cell lineages, with the majority of positive cells being T cells (Figure 2, G and H). In both cases, BCL10 was expressed only in the cytoplasm (Figure 2F).

Table 1. BCL 10 Protein Expression in Normal and Neoplastic Tissues

Tissue type	Number of cases	BCL 10 expression	
		Cytoplasmic	Nuclear (>10%)
Normal Solid tissue			
Tongue	3	—	—
Esophageal	3	—	—
Duodenal	3	—	—
Rectal	3	—	—
Liver	3	—	—
Gall bladder	2	—	—
Pancreas	2	—	—
Bronchus	3	—	—
Lung	4	—	—
Thyroid	2	—	—
Adrenal gland	2	—	—
Kidney	2	—	—
Bladder	4	—	—
Cervix	3	—	—
Uterus	3	—	—
Ovary and tube	3	—	—
Placenta and cord	3	—	—
Testis	6	—	—
Heart	2	—	—
Skin	3	—	—
Breast	15	+ luminal epithelial cells	—
Normal lymphoid tissue			
Appendix	4	4	—
Tonsil	8	8	—
Lymph node	6	6	—
Spleen	5	5	—
Thymus	8	8	—
Neoplastic lymphoid tissue			
MALT			
t(1;14) +ve	4		4 (100%)
t(1;14) -ve	36		20 (55%)
Mucosal DLBCL	20	20	—
FL	21	19	2 (10%)
MCL	17	17	—
Nodal DLBCL	18	14	4 (22%)

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; —, denotes lack of BCL10 expression.

Of 21 types of normal solid tissues examined, only breast showed BCL10 protein expression. BCL10 expression appeared to be restricted to the cytoplasm of the luminal epithelial cells of breast (Table 1).

BCL10 Expression in Malignant Lymphoma

The results were summarized in Table 1. Unlike marginal zone B cells, each of the four MALT lymphomas with t(1;14)(p22;q32) showed strong BCL10 expression in both the nucleus and cytoplasm in all tumor cells (Figure 3, A–D). Strong BCL10 nuclear expression in these tumor cells distinguished them from nonlymphomatous-reactive lymphocytes, which showed either weak or no cytoplasmic BCL10 expression. The BCL10 expressing tumor cells invaded gastric glands forming lymphoepithelial lesions (Figure 3C) and disseminated to other parts of the gastric mucosa intermingling with reactive lymphocytic infiltrates (Figure 3E). Discrete tumor cells as identified by strong BCL10 nuclear expression were also found in the marginal zone of the spleen in one gastric MALT lymphoma where splenic tissue was available. Strong nuclear BCL10 expression in tumor cells was in sharp con-

trast to the weak cytoplasmic BCL10 expression in normal marginal zone B cells (Figure 3F).

Although such high levels of nuclear BCL10 expression were not seen in 36 cases of MALT lymphoma without t(1;14)(p22;q32), 20 (55%) of these cases showed moderate nuclear and cytoplasmic BCL10 expression in 10 to 90% of tumor cells with the remaining tumor cell population expressing the protein only in the cytoplasm (Figure 3G). The remaining 16 cases displayed only cytoplasmic BCL10 expression (Figure 3H). To correlate BCL10 expression pattern with disease progression, the histology of all gastrectomy specimens of MALT lymphoma was reviewed. Cases showing BCL10 nuclear expression invaded the gastric serosa in 16 of 21 (76%), whereas this occurred in 4 of 8 (50%) of those displaying cytoplasmic BCL10 alone. We compared Ki67 proliferative indices between MALT lymphomas showing nuclear BCL10 expression (20 cases) and those displaying only cytoplasmic expression (10 cases). Tumors with nuclear BCL10 expression generally showed slightly higher Ki67 proliferative indices than those with cytoplasmic BCL10, however, no statistical significance was found between the two groups.

Figure 2. BCL10 protein expression in normal lymphoid tissues. **A:** A low magnification ($\times 150$) of tonsil shows the level of BCL10 expression is much higher in centroblasts of the dark zone (DZ) than in centrocytes of the light zone (LZ). **B:** Germinal center B cells show BCL10 expression in the cytoplasm (original magnification, $\times 480$). **C:** A low magnification ($\times 250$) of spleen shows differential BCL10 expression in marginal zone (MGZ), mantle zone (MZ), and follicle center (FC) B cells. **D:** Splenic marginal zone B cells show BCL10 expression in the cytoplasm (original magnification, $\times 480$). **E:** Thymus (16 weeks of gestation) shows BCL10 expression mainly in the medulla (M) and occasionally in the cortex (C) (original magnification, $\times 80$). **F:** Cytoplasmic BCL10 expression in medullary lymphocytes (original magnification, $\times 480$). **G:** Double-immunostaining with BCL10 and CD3 antibodies (original magnification, $\times 480$); BCL10 is in brown, showing paranuclear staining, whereas CD3 is in blue, displaying a membrane-staining pattern. **H:** Double-immunostaining with BCL10 and CD20 antibodies (original magnification, $\times 480$). BCL10 is in brown, showing paranuclear staining, whereas CD20 is in blue, displaying a membrane-staining pattern.

Similar to the BCL10 expression pattern in normal germinal center B cells, 19 of 21 follicular lymphomas expressed the protein in the cytoplasm and the remaining two expressed the protein in both the nucleus and cytoplasm in 30 and 80% of tumor cells, respectively. Mantle cell lymphomas showed either weak (14 of 17, 82%) or no (3 of 17, 18%) BCL10 expression. As the expression level is low, the subcellular localization of BCL10 protein in this lymphoma subtype could not be confidently determined. All diffuse large B-cell lymphomas of mucosal sites showed weak cytoplasmic BCL10 expression. Of nodal diffuse large B-cell lymphomas, 14 of the 18 cases expressed BCL10 protein in the cytoplasm and the remaining four cases expressed the protein in both the nucleus and cytoplasm in 10 to 20% of tumor cells.

Discussion

BCL10 mRNA, as previously shown by *in situ* hybridization, was highly expressed by germinal center B cells, moderately by marginal zone B cells, but weakly by mantle zone B cells.³ BCL10 protein, as shown in the present study, was also differentially expressed among various B-cell subsets of the B-cell follicle, from abundant expression in antigen activated highly proliferating germinal center centroblasts to low or no expression in nondividing naïve mantle zone B cells. These results suggest that BCL10, like BCL2¹⁵ and BCL6,^{16,17} is developmentally regulated during B-cell maturation and may play an important role in the germinal center reaction, during which naïve B cells undergo a series of complex biochemical

Figure 3. BCL10 protein expression in malignant B-cell lymphoma. **A** and **B**: A low-grade pulmonary MALT lymphoma with t(1;14)(p22;q32). Original magnifications: ×150 (**A**), ×480 (**B**). **C** and **D**: A low-grade gastric MALT lymphoma with t(1;14)(p22;q32). **Arrowhead** indicates lymphoepithelial lesion. Original magnifications: ×150 (**C**), ×480 (**D**). **E**: BCL10-expressing tumor cells disseminate to other part of the gastric mucosa intermingling with reactive lymphocytes (original magnification, ×250). **F**: Discrete tumor cells are also found in the marginal zone of the spleen, where the strong nuclear BCL10 expression in tumor cells is in sharp contrast to the weak cytoplasmic BCL10 expression in normal marginal zone B cells (original magnification, ×150). **G**: A low-grade gastric MALT lymphoma without t(1;14)(p22;q32) shows BCL10 expression predominantly in the nucleus (original magnification, ×480). **H**: A low-grade gastric MALT lymphoma without t(1;14)(p22;q32) shows BCL10 expression in the cytoplasm (original magnification, ×480).

and genetic processes and become memory B cells expressing immunoglobulin of high affinity.¹⁸

The BCL10 protein was also differentially expressed at various stages of T-cell maturation in thymus. At an early stage, the T cells, which reside in the cortex and undergo T-cell receptor rearrangements and positive selection, lacked BCL10 expression; while at a late stage, T cells move to the medulla and undergo negative selection to delete those highly reactive to MHC and self peptides,¹⁹ expressed BCL10 in the cytoplasm. These data suggest that BCL10 may also play a role during T-cell maturation.

The level of BCL10 expression and its subcellular localization required for its pro-apoptosis and NF- κ B activation *in vivo* are unknown and are likely to be different. The differential BCL10 expressions among various B-cell

populations of the B-cell follicle may reflect their individual requirements for different BCL10 functional properties. In view of its overall pro-apoptotic activity as shown in *in vitro* assays and high expression in germinal center centroblasts, BCL10 may sensitize these cells to apoptotic signalings and help to eliminate those producing low-affinity immunoglobulins. A similar role of BCL10 may be expected in the medulla of the thymus, deleting autoreactive T cells. These speculations are strongly supported by findings in BCL10 transgenic mice. In these transgenic mice, both the spleen and thymus were severely atrophic at postnatal stages because of high levels of apoptosis of both B and T cells, respectively.²⁰

Among other normal tissues, BCL10 protein was found by immunohistochemistry only in breast but not in 22

other types of tissues examined. The lack of BCL10 protein expression in these normal tissues is intriguing. It is possible that BCL10 protein is expressed in these normal tissues but at a low level below the detection limit of the current immunohistochemical system. Alternatively, BCL10 protein expression may be tissue and cell type-specific.

One consequence of chromosomal translocations involving the immunoglobulin loci in lymphomagenesis is overexpression of the gene. High-level BCL10 expression was indeed observed in each of the four MALT lymphomas with t(1;14)(p22;q32). However, this observation is paradoxical, because under all conditions BCL10 has exhibited pro-apoptotic functions.^{3,4,6-10} The high-level expression of an apparently pro-apoptotic molecule in the context of an expected oncogenic translocation has been seen previously in the case of both BCL6 and *c-myc*, as both molecules can induce apoptosis under certain circumstances.^{21,22} In the case of *c-myc*, deregulated expression occurs in the presence of concurrent genetic abnormalities including either concurrent deregulation of BCL2 in follicular lymphoma or p53 mutation in the case of Burkitt's lymphoma, which prevents *c-myc*-induced apoptosis and results in unchecked proliferation.²³

The paradox of high-level BCL10 expression in MALT lymphoma may have a number of explanations. First, the finding of pro-apoptotic and tumor suppressor activities of BCL10 in *in vitro* experiments may not truly reflect its biological behavior *in vivo*. As BCL10 only weakly promotes apoptosis in *in vitro* assays and also activates NF- κ B,^{3,4,6-10} BCL10 may well behave as an antagonist to apoptosis *in vivo* in certain cellular contexts. Secondly, mutation may convert the tumor suppresser gene into an oncogene. In view of the contrasting biological activities between the wild-type BCL10 and its truncated mutants, this model is attractive. However, examination of the genomic DNA samples from three MALT lymphomas with t(1;14)(p22;q32) showed only one case with potentially pathogenic mutations.¹² The consequences of truncated BCL10 mutants from alternative RNA splicing and RNA editing events in lymphomagenesis remain to be investigated.²⁴ It should be noted that the truncated forms of BCL10 predicted to arise from alternative splicing would not be detected by any of the monoclonal antibodies we have generated to date. Finally, the altered subcellular localization of the BCL10 protein may explain the paradox. Unlike their normal cell counterpart, the marginal zone B cell, which expressed BCL10 only in the cytoplasm, each of the four MALT lymphomas with t(1;14)(p22;q32) showed BCL10 expression in both the nucleus and cytoplasm. It is possible that the nuclear BCL10 rather than the cytoplasmic form confers the tumorigenic activity.

BCL10 nuclear expression was also seen in MALT lymphomas without t(1;14)(p22;q32) although at a much lower level. As these tumors were more often those with aggressive clinicopathological features, this prompted us to correlate nuclear BCL10 expression with proliferation markers. Tumors with nuclear BCL10 expression generally showed slightly higher Ki67 proliferative indices than

those with only cytoplasmic BCL10, however, no statistical significance was found between the two groups. This may reflect that the number of cases examined was too small and that the effect of BCL10 deregulation on tumor cell proliferation is not strong enough to dramatically increase the tumor cell proliferative activity.

Unlike MALT lymphoma, both mantle cell and follicular lymphomas generally showed BCL10 expression patterns comparable to those seen in their normal cell counterparts, suggesting that deregulation of BCL10 expression is unlikely to be involved in the development of these tumors.

The mechanisms underlying the nuclear localization of BCL10 protein in malignant B cells are unclear. BCL10 nuclear expression was independent of both t(1;14)(p22;q32) and the level of protein expression. The findings that the frequency of nuclear BCL10 expression was 10 times as high as that of BCL10 gene mutation in MALT lymphoma suggest that events other than genomic mutations are responsible for the nuclear localization of the protein.^{12,13} BCL10 does not contain any known nuclear localization signals.²⁵ The presence of nuclear BCL10 suggests that the protein may have functions other than apoptosis regulation. An isoform of ARC a CARD-containing protein has been shown to be involved in nucleolar RNA processing and the CARD of pro-caspase 2 has been shown to mediate nuclear transport indicating that at least some CARD-containing proteins may have unexpected functions.^{26,27}

In summary, our results show differential expression of BCL10 among different B-cell populations of the B-cell follicle, indicating its importance in B-cell maturation. The subcellular localization of BCL10 was frequently altered in MALT lymphoma in comparison with its normal cell counterparts, suggesting that this may be important in lymphomagenesis. The strong BCL10 nuclear expression in a B-cell lymphoma is highly indicative of t(1;14)(p22;q32).

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