Infrequent *BCL10* Mutations in B-Cell Non-Hodgkin's Lymphomas

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The *BCL10* gene was recently isolated from the breakpoint region of t(1;14)(p22;q32) in mucosa-associated lymphoid tissue (MALT) lymphomas. Somatic mutations of *BCL10* were found in not only t(1;14)-bearing MALT lymphomas, but also a wide range of other tumors. To clarify the actual frequency and spectrum of *BCL10* mutations in primary B-cell non-Hodgkin's lymphomas (NHL), we examined a total of 139 NHL cases comprising 25 with MALT lymphomas, 54 with follicular B-cell lymphomas (FCL), and 60 with diffuse large B-cell lymphomas (DLBL). Polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) and sequencing analyses led to the identification of four nucleotide changes in FCL and one in DLBL. In contrast, no *BCL10* mutations were found in our series of MALT lymphomas. While screening for mutations, we also found three polymorphic sequence variants at codons 5 and 213 and in intron 1 of the *BCL10* gene. Our results strongly suggest that somatic mutations of *BCL10*, if they occur at all, are rare in B-cell NHLs and do not commonly contribute to their molecular pathogenesis.

Key words: Chromosome translocation — Apoptosis — SSCP — Malignant lymphoma

Recurrent chromosomal translocations are well recognized and have been extensively characterized in nodal B-cell lymphomas.[1, 2] These translocations were found to involve and deregulate an impressive list of proto-oncogenes including *BCL1*, *BCL2*, *BCL6*, and *c-myc*. In contrast, data on the molecular genetic mechanisms underlying the pathogenesis of marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) type are only now beginning to emerge. Recurrent abnormalities found in MALT lymphomas include trisomies of chromosomes 3, 7, 12, and 18, the t(1;14)(p22;q32), and the t(11;18)(q21;q21).[3–9] The t(11;18) translocation is reported to be one of the most frequent and specific chromosomal translocations in MALT lymphomas[7–9] and a novel gene, named *MALT1* or *MLT*, has recently been cloned by ourselves and others from the breakpoint of the t(11;18).[10–12] On the other hand, the frequency of the t(1;14) translocation is relatively low and this translocation appears to be characteristic of aggressive MALT lymphomas.[13]

The *BCL10* gene has been isolated from the breakpoint region of the t(1;14) in MALT lymphomas.[14, 15] BCL10, also called CIPER or CARMEN, was found to be a cellular homolog of the equine herpesvirus-2 *E10* gene: both contain an amino-terminal caspase recruitment domain (CARD), which is homologous to that found in several apoptotic molecules.[14–17] By analogy to BCL2 involvement in follicular B-cell lymphomas (FCL), BCL10 was

expected to be anti-apoptotic. In functional assays, however, wild-type BCL10 turned out to be pro-apoptotic. It is noteworthy that BCL10 in t(1;14)-bearing MALT lymphomas exhibited a variety of truncating mutations.[14, 15] As expected, the truncated BCL10 was shown to lose its pro-apoptotic action and display a gain-of-function transforming activity, so that it is conceivable that such mutations may confer a survival benefit upon MALT lymphoma cells. Similar truncating mutations were also identified in a subset of FCL and other tumor cell lines with a loss of heterozygosity (LOH) in chromosome 1p22.[14] However, several recent studies have reported that *BCL10* mutations are very rare in various human tumors, thus raising questions regarding the pathological role of the *BCL10* gene in these tumors.[18–23] Furthermore, the actual frequency and spectrum of the involvement of the *BCL10* mutations in B-cell non-Hodgkin's lymphomas (NHL) remain to be established.

In the present study, we examined 25 cases with MALT lymphomas, 54 with FCL, and 60 cases with diffuse large B-cell lymphomas (DLBL) for somatic mutations of the *BCL10* gene by means of polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis. Our analysis suggests that *BCL10* may not be a major target for mutations in B-cell NHLs.

MATERIALS AND METHODS

Patients' samples   A total of 139 tumor DNA samples obtained from patients with B-cell NHL (25 with MALT

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lymphomas, 54 with FCL, and 60 with DLBL) were analyzed in this study. The pathologic diagnosis was established according to the REAL classification.[24] Involved lymph nodes and extra-nodal lesions were used for tumor samples. Four of 25 MALT lymphoma cases were known to carry the t(11;18), but the cytogenetic abnormalities of the remaining 21 were unspecified.

PCR-SSCP Tumor DNA samples were screened by means of PCR-SSCP for mutations in the coding region of exons 1–3 of BCL10. Genomic DNA was isolated from the samples with the standard method. For the SSCP analysis, the coding region of BCL10 was divided into five fragments (exon 1, exon 2.1, exon 2.2, exon 3.1, and exon 3.2) as described elsewhere.[14] The primers used were designed as described elsewhere.[14] Each 20 μl of PCR reaction mixture contained 100 ng of genomic DNAs, 1× PCR buffer, 100 μM of each dNTP, 0.2 μl of [α-^{32}P]dCTP, 1 μM of each primer and 0.5 unit of *Taq* polymerase (Takara, Kyoto). For exon 1, the following touchdown protocol was used: 2 cycles at 94°C for 30 s, at 65°C for 45 s and at 72°C for 45 s, 2 cycles at 94°C for 30 s, at 63°C for 45 s and at 72°C for 45 s, 2 cycles at 94°C for 30 s, at 61°C for 45 s and at 72°C for 45 s, followed by 30 cycles each at 94°C for 30 s, at 58°C for 45 s and at 72°C for 45 s. For the other exons, the PCR parameters were: at 94°C for 30 s, at 60°C for 45 s, and at 72°C for 45 s for 35 cycles. The PCR products were electrophoretically separated at 40 W on 6% non-denaturing polyacrylamide gels both at 4°C without glycerol and at room temperature in the presence of 5% glycerol. Gels were dried at 80°C and exposed to autoradiographic film at −80°C for 6 to 18 h.

Sequencing analysis The PCR products that revealed mobility shifts in SSCP analysis were confirmed by sequencing analysis. The bands were excised from the gels following autoradiography and suspended in 50 μl of TE buffer overnight at room temperature. One microliter of the eluted DNA was used for PCR under conditions identical to those described above for SSCP analysis, except that radiolabeled dCTP was omitted. Each PCR product was purified from the 2% low melting-point agarose gel and subcloned into the pBluescript SK(−) vector. The inserts were then sequenced in both directions using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, CA) on an ABI 373 DNA automated sequencer (Applied Biosystems, Foster City, CA).

Southern blot analysis Ten micrograms of genomic DNAs from 25 MALT lymphoma samples and normal placenta were digested with *Eco*RI or *Hin*dIII and loaded on each lane. A human *BCL10* cDNA fragment (nucleotide number 601–1212, GenBank accession number AJ-006288) was RT-PCR amplified, radio-labeled by using a random primer DNA labeling kit (Nippon Gene, Tokyo), and used as a probe for Southern blot analysis. Hybridization and washing were performed as described elsewhere.[25]

RESULTS

We first searched for somatic mutations in the coding region of BCL10 in 139 primary B-cell NHLs by using genomic PCR-SSCP and sequencing analyses. The primers used were designed as described by Willis *et al.*[14] For the genomic PCR-SSCP analysis, the coding region of BCL10 was divided into five fragments (exon 1, exon 2.1, exon 2.2, exon 3.1, and exon 3.2). PCR products were electrophoresed under two different conditions to facilitate the detection of SSCP variants. PCR-SSCP variants in exons 1 and 3.2 were noted with high frequency and the vast majority of them could be subgrouped into the same electrophoretic patterns (data not shown), suggesting that these variants were most likely derived from genetic polymorphisms. The variants were then excised from the gel and subjected to sequencing analysis. This analysis revealed the nucleotide changes, which in turn were found to correspond to the genetic polymorphisms (G/T at codon 5, G/A at codon 213, and G/C at nucleotide 58 in intron 1) recently reported by two groups.[18, 19]

In addition to these polymorphic SSCP variants, we were also able to identify SSCP variants whose electrophoretic patterns could not be ascribed to these genetic polymorphisms. Representative examples of the SSCP variants identified in the analysis of DLBL and FCL are
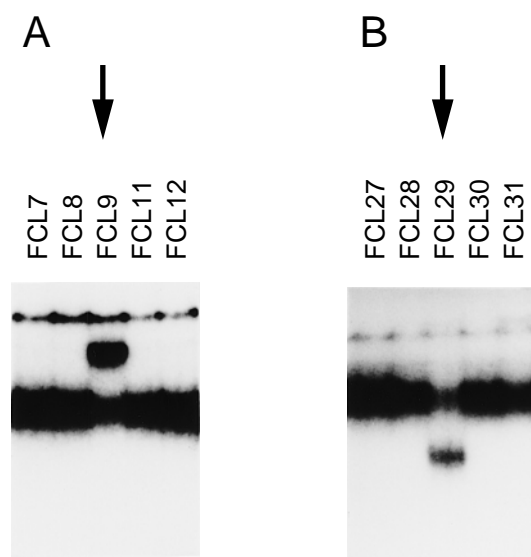

Fig. 1. Representative results of PCR-SSCP analysis of the exon 3.1 of BCL10. Arrows indicate aberrant SSCP patterns in patient FCL9 (A) and patient FCL29 (B). PCR-SSCP was performed as described in "Materials and Methods."

Table I. *BCL10* Mutations in B-Cell Non-Hodgkin's Lymphomas

Patient No.	Codon	Base change	Coding change
DLBL26	162	ACG→ATG	Thr→Met
FCL3	162	ACG→ATG	Thr→Met
FCL9	162	ACG→ACA	Silent
FCL29	162	ACG→ATG	Thr→Met
FCL50	162	ACG→ATG	Thr→Met

DLBL, diffuse large B-cell lymphoma; FCL, follicular lymphoma.

shown in Fig. 1. The results of sequencing analyses of the distinct variants are summarized in Table I. A total of four nucleotide changes of *BCL10*, one of them silent, was identifed in our series of FCL. One nucleotide change was also found in a DLBL patient. No truncating mutations were identified in our series. In contrast to FCL and DLBL, no mutations of *BCL10* were identified in 25 of our MALT lymphomas. This observation is in agreement with the results of a recent report showing that no mutations were detected in 15 MALT lymphomas.[19]

We also performed Southern blot analysis of the 25 MALT lymphoma DNA samples with a *BCL10* cDNA fragment, but did not find any gross *BCL10* gene rearrangements (data not shown). Because the breakpoints of the t(1;14)(p12;q32) translocation were typically found in the upstream flanking region of the *BCL10* gene and were readily detectable by conventional Southern blot analysis,[14, 15] it is likely that our series of MALT lymphomas did not carry the t(1;14) translocation.

DISCUSSION

MALT lymphoma is now regarded as a distinct clinicopathological entity.[26] Cytogenetic studies have shown that recurrent abnormalities in MALT lymphomas are trisomies of chromosomes 3, 7, 12, and 18, the t(1;14)(p22;q32), and the t(11;18)(q21;q21).[3–9] The *BCL10* gene, a novel apoptotic signaling gene that encodes an amino-terminal CARD, has been isolated by two groups from the breakpoint region of the t(1;14).[14, 15] In contrast to other lymphomagenic genes, wild-type BCL10 does not appear to confer a growth advantage. It is noteworthy that BCL10 from t(1;14)-bearing MALT lymphomas was found to exhibit a variety of mutations, most of which resulted in truncations. Such mutations were also observed in a subset of FCL and non-hematopoietic tumors.[14] However, these findings have been challenged by a series of recent studies raising questions regarding the pathological role of *BCL10* as a tumor suppressor gene in various human tumors.[18–23]

The present study aimed to investigate the actual frequency and spectrum of mutations of the *BCL10* gene in various primary B-cell NHLs. Our results suggest that nucleotide changes at codon 162 of *BCL10* may occur in a small subset of DLBL and FCL. This is somewhat at variance with the results of Fakruddin *et al.*, who found no *BCL10* mutations in 15 FCLs.[18] This slight discrepancy between their results and ours can be explained by the difference in the number of FCL samples (15 versus 56). Another possible explanation is that the discrepancy reflects epidemiological differences of FCL. Since normal genomic DNAs were not available for this study and the same change at codon 162 was found in four out of five FCL cases, it is equally possible that this change may represent a rare Japanese-specific genetic polymorphism. If this is a genetic polymorphism, then the frequency of *BCL10* mutations is very rare in our series of Japanese NHLs. In any cases, it will be interesting to examine whether this change is associated with the pathogenesis of FCL. On the other hand, our analysis did not identify any nucleotide changes in the 25 MALT lymphomas. This observation is in agreement with the results of Fakruddin *et al.*, who did not find any mutations in 15 MALT lymphomas.[18]

Recently, Dyer *et al.* suggested that some *BCL10* mutations may be found only in RNA and not in genomic DNA, which raises the possibility that BCL10 may undergo posttranscriptional sequence modification.[27, 28] They reported that nucleotide insertions or deletions were common within the two homopolymeric runs of eight A's and seven T's in the *BCL10* gene. In some cases, these abnormalities were observed as a result of direct cDNA sequencing. In contrast, only four out of 500 genomic DNA sequences were found to exhibit such insertions or deletions within the homopolymeric runs, suggesting that such changes are largely nontemplated and may constitute an unusual form of RNA editing.[27, 28] Careful re-evaluations are required to clarify this important issue. If Dyer *et al.*'s findings are confirmed, then the frequency of *BCL10* mutations identified in our study by means of genomic PCR-SSCP analysis would presumably be an underestimate.

In summary, the present study describes the actual frequency and spectrum of the involvement of *BCL10* mutations in primary B-cell NHLs. Our results suggest that somatic mutations of *BCL10*, if they occur at all, are rare in B-cell NHLs and do not commonly contribute to their molecular pathogenesis. Further studies are warranted to screen a wider range of human malignant disorders for mutations at both DNA and RNA levels.

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Note added in proof: After acceptance of this manuscript, normal PBLs from a patient FCL50 have been analyzed by PCR-SSCP. The base change (ACG/ATG) at codon 162 has been found to represent a rare genetic polymorphism.