Point Mutations and Deletions of the Bcl10 Gene in Solid Tumors and Malignant Lymphomas

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Abstract

The Bcl10 gene, which encodes a protein with proapoptotic activity, recently has been identified on chromosome 1p22. In this study, we analyzed somatic mutations and deletions of the Bcl10 gene in a series of 439 tumor tissues from various histological origins that are known to have frequent loss of heterozygosity at chromosome 1p22. According to the LOH study at intragenic polymorphic sites, deletion of Bcl10 in informative cases was detected in 50% of malignant mesotheliomas, 33% of gastric carcinomas, 23% of breast carcinomas, 20% of hepatocellular carcinomas, 17% of lymphomas, 15% of colorectal carcinomas, 13% of laryngeal carcinomas, and 10% of male germ cell tumors (GCTs). In contrast, we detected Bcl10 mutations in 4 of 120 lymphomas (3.3%) and 2 of 78 GCTs (2.6%), respectively, but no mutation was found in the remaining solid tumors analyzed. Taken together, these data imply that Bcl10 may occasionally be involved in the pathogenesis of lymphoma and GCTs. However, the absence or low frequency of the mutation suggests that either Bcl10 is inactivated by other mechanisms or it is not the only target of chromosome 1p22 deletion in human tumors.

Introduction

The development of human tumors results from clonal expansion of genetically modified cells that acquired selective growth advantage through accumulated alterations of proto-oncogenes and tumor suppressor genes (1). Inactivation of a tumor suppressor gene frequently is accompanied by loss of portions of the chromosome on which the tumor suppressor gene resides (2). Deletions and rearrangements of chromosome 1p22 have been reported in many types of human tumors, raising the possibility that tumor suppressor genes are present in this region (3–7).

Cytogenetic studies of MALT4 lymphoma have identified abnormalities of chromosome 1p22, in particular translocation t(1;14)(p22;q32), as uncommon but recurrent events (8). Recently, Willis et al. (9) cloned a t(1;14)(p22;q32) translocation breakpoint from a low-grade MALT lymphoma and identified a recurrent breakpoint upstream of the promoter of a novel gene, Bcl10. The Bcl10 gene is a cellular homologue of the herpesvirus-2 E10 gene; both contain amino-terminal CARD homologues to that found in several apoptotic regulatory and effector molecules, including Apaf-1, caspase-1, caspase-2, caspase-9, RAIDD, RICK/RIP/CARDIAK, and ARC (9–15).

Bcl10 expressed in a MALT lymphoma exhibited a frameshift mutation resulting in truncation beyond the CARD (9, 10). Unlike the wild-type Bcl10 protein, the truncated mutants failed to induce apoptosis but retained nuclear factor-kB activation (9–12). Some proteins that induce apoptosis function as suppressors of transformation, and such transformation is suppressed efficiently by some human tumor suppressor proteins, such as wild-type p53 (16). Cotransfection of wild-type Bcl10 reduced the numbers of transformed colonies generated by oncogenes such as ras, HPV17, E1a, and mutant p53, whereas cotransfection with mutant Bcl10 markedly enhanced the number of colonies and caused the transformed colonies to appear earlier (9). These data indicate that failure of Bcl10-mediated apoptosis caused by gene mutation may contribute to the development of human tumors.

To date, there have been contradictory reports on Bcl10 gene mutations in human tumors. Two research groups reported frequent Bcl10 mutations in primary lymphomas and/or cell lines from MM, GCTs, and colon carcinoma (9, 10), whereas the other two groups reported the absence of Bcl10 mutation in tumor tissues of lymphoma and GCTs, and cell lines from MM and GCTs (17, 18). To characterize the Bcl10 mutation in human tumors, the following questions were investigated in this study: (a) whether human tumor tissues from various histological origins that are known to have frequent LOH at chromosome 1p22 have somatic mutations of Bcl10 gene; and (b) if so, whether the Bcl10 somatic mutation is relevant to the frequent LOH at chromosome 1p22 in human tumors.

Materials and Methods

Tissue Samples. A series of 439 paraffin-embedded tissues of 120 malignant lymphomas (88 B-cell-lineage tumors and 32 T-cell-lineage tumors), 78 GCTs, 15 MMs, 41 colorectal adenocarcinomas, 60 invasive ductal carcinomas of breast, 43 advanced gastric adenocarcinomas, 50 HCCs, and 32 laryngeal squamous cell carcinomas were selected for SSCP and LOH analysis of the Bcl10 gene. The malignant lymphomas tested consisted of 68 diffuse large B-cell lymphomas, 13 MALT lymphomas, 10 angiocentric lymphomas, 2 mantle cell lymphomas, 5 follicular lymphomas, 8 anaplastic large cell lymphomas, 12 peripheral T-cell lymphomas, unclassified, and 2 angioimmunoblastic T-cell lymphomas. The GCTs comprised 35 mature teratomas, 16 yolk sac tumors, 1 embryonal carcinoma, 16 seminomas, and 6 tumors with mixed histology. The tumor samples of laryngeal carcinoma and breast carcinoma included 20 and 28 cases of lymph node metastases, respectively.

Microdissection. Malignant cells were selectively procured from H&E-stained sections using a 30G1/2 hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) affixed to a micromanipulator, as described previously (19). We also microdissected infiltrating lymphocytes for corresponding normal DNA from the same slide in all cases. This microdissection technique used in this study has been proven to be precise and effective for procurement of tumor cells without contamination by normal cells (19). DNA extraction was
performed by a modified single-step DNA extraction method, as described previously (19).

SSCP Analysis. Genomic DNA from normal lymphocytes or tumor cells was amplified by PCR using seven primer pairs covering the entire coding region of the \textit{Bcl10} gene. The primer pairs were as follows: exon 1, 5'-GGACCCGAGAA- GAGGCCCATCTCC-3' and 5'-GATCTCTTCTGCCTGACCTC-3' (product size, 187 bp); exon 2-1, 5'-AAGACTGCAACTAACTGCACTG-3' and 5'-AATGGATCTACAAATTTCTCCAGCC-3' (200 bp); exon 2-2, 5'-CAGCAGAAATTTCTTGTCGAACA-3' and 5'-AGACCTTTGGTITGTCTCTG- CAACTGA-3' (184 bp); exon 3-1, 5'-AGGGCTGGAAAATTGTTAGA-3' and 5'-AAACACCATTACATTAAATCTGACCC-3' (231 bp); exon 3-2, 5'-TAAAAACGGGACAGCTTG-3' and 5'-CGTGGTAATGTCCCCCAGT-3' (200 bp); exon 3-3, 5'-AATGACCAGCATACTTGTGCAAC-3' and 5'-TAAAATAAATTTGAATGCAAATAAAGTG-3' (191 bp). The oligonucleotide primers for exons 2-2, 2-3, 3-1, 3-2, and 3-3 were designed with the program Oligo (National Biosciences, Plymouth, MN) using sequences obtained from GenBank (accession no. AF097732), and primers were not informative (homozygosity); Het, retention of heterozygosity.

Complete or nearly complete absence of one allele in the tumor DNA of informative cases, as defined by direct visualization, was considered as LOH.

Results

\textbf{Bcl10 Mutations.} Genomic DNA was isolated and analyzed for potential mutations in all three exons of the \textit{Bcl10} gene by PCR-SSCP analysis. Enrichment and direct sequence analysis of aberrantly migrating bands led to the identification of mutations in 6 of the 439 samples (1.4%): 4 of 120 malignant lymphomas (3.3%), and 2 of 78 GCTs (2.6%; Table 2; Fig. 1, A and B). Of the six mutations identified, five were observed in exon 3, and one was observed in exon 2. Fifteen MNs, 41 colorectal adenocarcinomas, 60 invasive ductal carcinomas of breast, 43 advanced gastric adenocarcinomas, 50 HCCs, and 32 laryngeal squamous cell carcinomas did not show any \textit{Bcl10} gene mutations in the SSCP analysis. The four lymphomas with the mutations consisted of one angiocentric T-cell lymphoma of the nasal cavity (case ML 21), one B-cell-origin diffuse large cell lymphoma of the spleen (case ML 15), one B-cell-origin diffuse large cell lymphoma of the mesenteric lymph node (case ML 23), and one high-grade MALT lymphoma (case GL 13). Both of the two GCTs with \textit{Bcl10} mutations were mature teratomas (cases GT 37 and GT 53). None of the corresponding normal samples showed evidence of mutations by SSCP (Fig. 1, A and B), indicating that the mutations detected in these specimens had risen somatically.

All six mutations identified were caused by single-nucleotide substitution. Among these, four were missense mutations that affect codons 80, 161, 175, and 200 of \textit{Bcl10}, and would result in amino acid substitutions (Table 2). The remaining two mutations included one nonsense mutation (GL 13) and one silent mutation (GT 37). The nonsense mutation showed a T-to-A transversion at bp 521, causing a premature stop at codon 174. The detection of \textit{Bcl10} gene mutations was reproducible through triplicate experiments that included tissue microdissection, PCR, SSCP, and sequencing analysis, ensuring the specificity of the results.

\textbf{Allelic Status.} In the course of the SSCP analysis of the \textit{Bcl10} gene, we identified four patterns of aberrant bandshift (two in exon 1 and two in exon 3), which were detected in both the tumor tissues and the corresponding normal tissues (Fig. 1E). These patterns were also observed in DNAs from the lymphocytes of healthy individuals (Ta-
ble 1). Thus, we considered these alteration patterns as polymorphisms. On the DNA sequencing, these polymorphisms exhibited T instead of G at bp 13, C instead of G at bp 24, T instead of A at bp 485, and A instead of G at bp 638. Because the heterozygosity rates of the polymorphisms at bp 13 and bp 485 were too low for LOH study (Table 1), only the polymorphisms at bp 24 and bp 638 were used for the LOH study of the Bcl10 gene. Overall, 196 of 392 cases analyzed (50%) were informative for one or two polymorphic markers, and 40 of 196 (20%) informative cases showed LOH. The LOH data of each type of tumors are summarized in Table 1.

In the six cases with Bcl10 mutations, two (ML 15 and ML 21) showed LOH for one or two polymorphic marker(s). Another two mutation cases (GL 13 and GT 37) were not informative for the polymorphic markers. The remaining two cases (ML 23 and GT 53) were heterozygous for at least one of these markers but did not show LOH (Table 2).

Discussion

To extend the previous findings on the Bcl10 gene mutations in some human tumors and cell lines (9, 10) to a wider range of primary human tumors, we investigated somatic mutations and deletions of the Bcl10 gene in a series of 439 tumor tissues from various histological origins that are known to have frequent LOH at chromosome 1p22. We detected four and two Bcl10 mutations in lymphomas and GCTs, respectively, but no mutation was found in the remaining solid tumors analyzed, which suggests that Bcl10 mutations are involved in the pathogenesis of human tumors.

Although functional studies have not yet been performed, some of the mutations identified in the present study are likely to disrupt or alter the normal function of the Bcl10 protein. One of the mutations (case GL 13) identified is a nonsense variant (174 Leu to stop; Table 2), which is predicted to cause premature termination of the protein synthesis, and hence resemble typical loss-of-function mutations. Another of the mutations (case ML 21) would result in an amino acid substitution from Pro to Leu. Proline differs from the other common amino acids in having a secondary amino group, and the resulting cyclic structure would markedly influence protein architecture (20). Therefore, it is possible that Bcl10 mutant protein with an amino acid substitution from 175 Pro to Leu might have a change in its structure and might lose its function, which remains to be confirmed by functional and structural analysis.

To date, the somatic mutations of the Bcl10 gene in tumor patients have been identified in exons 2 and 3 (9, 10). Exon 2 encodes the evolutionarily conserved CARD domain at the amino terminus, and exon 3 encodes most of the non-CARD carboxyl terminus of the Bcl10 protein (9–14). The CARD motif within Bcl10 induces the self-oligomerization of this protein (11–13), and the non-CARD carboxyl terminus complexes with caspase-9 and promotes its processing to the active protease (11). Mutational analysis of these CARD and non-CARD regions demonstrated that both regions are required for inducing apoptosis (11). In the current study, one missense mutation (80 Asp to Gly) was identified in the CARD domain. Therefore, in this case, the mutated CARD might not mediate oligomerization of Bcl10 protein. The other four mutations identified in the non-CARD carboxyl terminus (161 Thr to Met, 174 Leu to stop, 175 Pro to Leu, and 200 Gly to Asp), might impair death signaling through poor interaction of the mutant proteins with caspase-9.

In MALT lymphoma, Zhang et al. (10) identified a Bcl10 point mutation that generates an amino acid substitution at position 174. In addition, the mutation of codon 175 of Bcl10 was identified in follicular lymphoma (9). Of the six Bcl10 gene mutations identified in the present study, two mutations generated amino acid changes at residues 174 and 175 of exon 3. In exon 2, the mutations involving amino acid substitutions at residue 80 were observed in our study and the previous study (9). These observations suggest that these areas in exon 2 (codon 80) and in exon 3 (codons 174 and 175) might be some of the common mutation sites of the Bcl10 gene in human tumors.

The previous study by Zhang et al. (10) found one nucleotide change at bp 638 (G to A) of Bcl10 gene and described it as a missense mutation. However, in this study, we observed this nucleotide change in both tumors and their corresponding normal tissues (Table 1; Fig. 1E). Moreover, the change was found in lymphocytes of healthy individuals, which indicates that it is a polymorphism of the Bcl10 gene. Using this and another intragenic polymorphism, we have found LOHs with a range from 10 to 50% according to the tumor types, which is comparable to the LOH data using microsatellite markers at chromosome 1p22 (3–7). However, unexpectedly, we found only six Bcl10 mutations in lymphomas and GCTs. These findings could be explained in several ways. First, it is possible that other tumor suppressor genes in addition to Bcl10 reside at chromosome 1p22 or that some of the LOHs represent evidence of genomic instability affecting this area without targeting specific genes. Second, the second allele could be inactivated by Bcl10 gene silencing through promoter methylation, but this possibility remains unknown at this stage. Third, the discrepancy might be partially explained by the ongoing mutation of the Bcl10 gene in MALT lymphomas. In the course of searching for Bcl10 mutations, Willis et al. (9) observed that different sections from different histological blocks from the same tumor showed different SSCP bands; they considered this phenomenon as ongoing mutation. Because we did not sequence individual genomic clones, the frequency of Bcl10 mutations might be significantly underestimated in the present study. Finally, it is also possible that the primers we used were not suitable to detect all of

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Fig. 1. Representative results showing SSCP analysis for mutations (A and B), sequencing analysis (C and D), and SSCP analysis for LOH (E) of the Bcl10 gene. A and B, SSCP of DNA from tumors (T) of case GL 13 (A) and GT 53 (B) show wild-type bands and additional aberrant bands when compared with SSCP from corresponding normal lymphocytes (N). C and D, cyclic sequencing analysis using DNA template from aberrant bands from case GL 13 (C) and case GT 53 (D). The arrows indicate the nucleotide substitutions in tumor tissues. E, detection of allelic loss by amplification of a region encompassing the biallelic polymorphism at bp 638 in exon 3. Left panel, SSCP showing a noninformative case. Middle panel, SSCP showing retention of heterozygosity. Right panel, a pattern of LOH. Loss of two bands was observed in DNA from tumor cells (T) compared with the SSCP from normal cells (N).
the Bcl10 mutations present on the tumors analyzed in this study. However, this possibility is unlikely because we have also tried SSCP analysis with the same primer sets described in the previous studies (9) and found that the primer sets had the same mutation detection efficiency (data not shown).

In summary, we searched for Bcl10 gene mutations in a wide range of human tumors with frequent LOH at chromosome 1p22, where Bcl10 resides, and found that this gene is somatically mutated in some lymphomas and GCTs. Because wild-type Bcl10 is proapoptotic and behaves as a tumor suppressor gene, the Bcl10 mutations identified in this study might lead to a longer survival of affected tumor cells and might contribute to tumorigenesis of some lymphomas and GCTs. And, because deletion of chromosome 1p22 has been considered as an important event in the pathogenesis of many human tumors (3–7), studies are now needed to locate any other possible tumor suppressor genes in this region.

References

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