To the editor:

Multiple BCL6 translocation partners in individual cases of gastric lymphoma

Cytogenetic and molecular analyses have demonstrated that alteration of 3q27 and/or BCL6 is one of the most common genetic abnormalities in B-cell lymphomas.1 BCL6 translocations involve not only immunoglobulin (IG) genes but also a number of non-IG loci as partners.2 Using the relatively insensitive method of Southern blot hybridization, BCL6 has been found to be rearranged in 30% to 40% of diffuse large B-cell lymphoma (DLBCL) and 6% to 14% of follicular lymphomas (FLs).2

In a recent article, Akasaka et al3 examined the prevalence of BCL6 gene rearrangement by the highly sensitive methodology of long-distance inverse polymerase chain reaction (LDI-PCR) on genomic DNA from 41 cases of FL that underwent transformation to DLBCL. Significantly, among the 14 cases (34%) detected to have BCL6 translocations, 3 of these cases (21%) were found to harbor 2 independent BCL6 translocations.

Since translocations involving BCL6 usually juxtapose heterologous promoters and associated 5' untranslated sequences derived from other chromosomes to the BCL6 coding domain,4 we employed an alternative, and even more sensitive, strategy of 5' RACE (rapid amplification of cDNA ends) using a kit from Roche Diagnostic (Indianapolis, IN) to detect BCL6 fusion transcripts5 in 39 archival cases of primary gastric lymphoma (GL), including 4 MALT lymphoma (mucosa-associated lymphoid tissue), 12 DLCLML (diffuse large cell lymphoma with residual MALT lymphoma), and 23 DLBCL cases. The sensitivity of the detection of BCL6 translocations by 5' RACE is higher than that of LDI-PCR because for each copy of a chromosomal fusion gene, there are multiple copies of chimeric transcripts. 5'RACE followed by cloning and DNA sequencing of the PCR fragments was performed as described in Xu et al,6 and the presence of all translocations detected by 5'RACE were further confirmed by direct reverse transcriptase–polymerase chain reaction (RT-PCR).

Significantly, among the 19 cases (49%) found to have BCL6 translocations, 6 (32%) were found to harbor 2 or more independent BCL6 translocations (Figure 1), including 1 MALT lymphoma, 2 DLCLML, and 3 DLBCL cases. Analysis of clonality by LDI-PCR on chromosomal DNA and 5' RACE on mRNA—can detect not only the main lymphoma clone but also the emerging minor subclones with new BCL6 translocations and possibly altered pathogenicity.

Our unexpected findings by 5' RACE of 2 or more independent BCL6 translocation partners in a significant number (32%) of GL cases with BCL6 translocations, together with the similar findings of Akasaka et al in FL cases that underwent transformation to DLBCL by LDI-PCR,1 suggest that multiple BCL6 translocations in individual cases of B-cell lymphomas are more common than appreciated and raise important issues regarding the molecular description and pathogenesis of B-cell lymphomas. The higher sensitivity of these 2 PCR-based techniques—LDI-PCR on chromosomal DNA and 5' RACE on mRNA—can detect not only the main lymphoma clone but also the emerging minor subclones with new BCL6 translocations and possibly altered pathogenicity.


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References

Response:

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In an interesting and timely report, Chen et al detected the presence of more than one BCL6 translocation in 6 of the 39 specimens of gastric lymphoma. This report confirms and extends our recent observation of 2 independent BCL6 translocations in 21% of follicular lymphoma (FL) specimens that harbored at least one BCL6 translocation. As Chen et al point out, the current methods do not distinguish between 2 different translocations within the same cell or 2 different cell populations within the same tumor, each with a different translocation. In either case, detection of multiple BCL6 translocations in the same malignant clone suggests that these translocations represent late events in lymphomagenesis. These observations, together with our finding that BCL6 translocations may be lost when FL transforms to diffuse large B-cell lymphoma (DLBCL), suggest that BCL6 is not playing a pivotal role in lymphomagenesis in these cases. Since the same mechanism may be responsible both for BCL6 mutation and for BCL6 translocation,2 it is possible that these alterations in the BCL6 gene are markers of genomic instability within the tumor.

In their report, Chen et al state that 5’ RACE is more sensitive than long-distance inverse polymerase chain reaction (LDI-PCR) for the detection of BCL6 translocation. However, a side-by-side comparison of the sensitivity of these 2 methods has not been performed. In our experience, BCL6 translocations detected by LDI-PCR were detectable also by the 5’ RACE method. Whether the reverse is also true will determine which method will be the preferred one.

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References


To the editor:

ADAMTS13 autoantibody detection by quantitative immunoblotting

The activity of ADAMTS13 (a von Willebrand factor–cleaving metalloprotease) is decreased in congenital and acquired thrombotic thrombocytopenic purpura (TTP) either because of a gene defect or because of transient inhibition by an autoantibody.1,2 Current diagnosis in patients is based on various activity assays, most of which are time-consuming. Not only would a quicker diagnostic method be advantageous, but such a method that differentiates between congenital and antibody-induced TTP would be additionally useful because different treatment strategies might be more appropriate for congenital than antibody-induced TTP.

ADAMTS13 recently has been characterized and cloned.3,4 Here we describe a highly sensitive assay using a recombinant ADAMTS13 (rADAMTS13) to screen plasma samples for antibodies against ADAMTS13. This antibody test also may allow treatment to be monitored, especially for potential anamnestic responses caused by replacement of the protease.

ADAMTS13 activity and the inhibitor titer of the plasma samples were determined as described earlier.6 To visualize the inhibitors, 3 µL (100 mU) of rADAMTS13 containing concentrated cell supernatant was electrophoresed on gradient polyacrylamide gels (4%-12%) in the presence of sodium dodecyl sulfate under nonreducing conditions and subjected to a standard Western blotting procedure.7 The blots were incubated for 2 hours at room temperature with diluted plasma samples from TTP patients or from healthy donors, or with a monoclonal mouse anti–human ADAMTS13 antibody (242Q2).

The blots were developed by further incubation with an alkaline phosphatase (ALP)–labeled donkey F(ab)2 fragment anti–human IgG for the plasma samples, and with an ALP-labeled rabbit F(ab’)2 fragment anti–mouse IgG for the monoclonal antibody (both from Accurate, Westbury, NY). The blots were stained with an ALP-substrate kit (BioRad, Hercules, CA). Quantitative densitometry analysis and molecular mass determination of the stained protein bands were performed from scanned images of the immunoblots using a calibrated scanner and image processing software (Image Master; Amersham Pharmacia, Uppsala, Sweden).

In the plasma samples in which an inhibitor was detectable with the activity assay, a band appeared with a molecular mass of 190 kDa (Figure 1A, lanes 2, 3, and 5), but no band was found in plasma pooled from healthy donors (lane 7), a TTP patient after treatment (lane 4), or a patient with hereditary TTP (lane 6). An equivalent 190-kDa band was obtained when the immunoblot was...