Response:

Multiple BCL6 translocation partners in individual cases of gastric lymphoma

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, 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. 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. 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. 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. 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
developed with the monoclonal antibody against the rADAMTS13 preparation (lane 8).

The results from this immunoblot assay correlated well with the quantitative determination of inhibitors by the collagen-binding assay.

The sensitivity of inhibitor detection was determined by using serial dilutions from 1/100 to 1/25 600 of a plasma sample from a patient with an inhibitor titer of 13 inhibitor units (InhU)/mL (Figure 1B). When the blots were quantified by densitometry, there was a linear correlation between the inhibitor concentrations of the samples and the band intensities in the range of 0.0005 to 0.010 InhU/mL. Taking into account that the plasma samples were diluted at least 100-fold, we estimated that the method detected noninhibitory antibodies, which will further explain the role of ADAMTS13 proteases and the pathogeneses of TTP.

Corbin and colleagues analyze in their report the biochemical and biologic effects of several mutations in the kinase domain of the Bcr-Abl protein identified in patients who developed resistance to imatinib. Some mutations (6 of 9 studied here) confer only a modest resistance index (less than 2-fold increase in $IC_{50}$), with $IC_{50}$ values lower than plasma values commonly observed in patients treated with standard dosages of imatinib. Therefore, the authors suggest that these mutations could not represent the cause of resistance.

In our opinion, some quality criteria (ie, analysis of clones instead of polymerase chain reaction products, detection of a...