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 al[3] 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 transcripts[5] 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 *IG* heavy chain gene rearrangement using PCR-based assays[7,8] showed that these 6 cases were monoclonal lymphomas, and, therefore, the simultaneous presence of 2 or more *BCL6* translocations in the same individual GL cases represented the evolution of subclones of the original tumor population. Two *BCL6* translocations detected in the same individual cases also could be due to biallelic *BCL6* translocations within the same lymphoma clones.

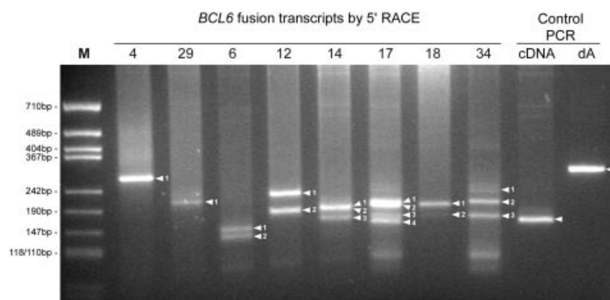

Figure 1. Detection of *BCL6* fusion transcripts by 5′ RACE. Nested PCR and the control PCR products of 5′ RACE were separated by gel electrophoresis. Ethidium-bromide–stained gel shows 2 representative cases of the 13 cases with one *BCL6* translocation partner gene (cases 4 [DLBCL] and 29 [DLBCL]), and all 6 cases with 2 or more independent *BCL6* translocation partner genes (case 6 [DLBCL], 12 [DLBCL], 14 [DLCLML], 17 [DLCLML], 18 [MALT], and 34 [DLBCL]). The case number is shown above each lane. cDNA: purified control cDNA was amplified with control forward and reverse primers. dA: poly dA-tailed control cDNA was amplified with oligo-dT anchor primer and the control reverse primer. Arrows point to bands representing *BCL6* chimeric transcripts (indicating 1 to 4 fusion partners found in individual cases): case 4 (IGH γ3), case 29 (transferrin receptor), case 6 (chromosome 3 clone RP11-49A15 and chromosome 3q clone RP11-45H13), case 12 (IGH γ3 and chromosome 7 clone RP11-611L7), case 14 (chromosome 2 clone RP11-551D18, uncharacterized sequence, and chromosome X clone RP13-12804), case 17 (chromosome 13q clone RP11-114G1, chromosome 7 clone RP11-63005, chromosome 2 clone RP11-211K17, and chromosome 3q clone RP11-489M17), case 18 (chromosome 9 clone RP11-381G8 and chromosome 5 clone CTD-2074D8), case 34 (chromosome 7q11-q22 clone RP5-1164F5, IGLL, and chromosome 19 clone CTD-2331H12). The complete sequences of the chimeric transcripts are available upon request.

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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Supported by a grant from the Research Grants Council of Hong Kong Special Administrative Region, P. R. China (HKU 7072/99M [G.S.]).

References

1. Bastard C, Tilly H, Lenormand B, et al. Translocations involving band 3q27 and *Ig* gene regions in non-Hodgkin's lymphoma. Blood. 1992;79:2527-2531.
2. Dalla-Favera R, Ye BH, Lo Coco F, et al. *BCL6* and the molecular pathogenesis of B-cell lymphoma. Cold Spring Harb Symp Quant Biol. 1994;59:117-123.
3. Akasaka T, Lossos IS, Levy R. *BCL6* gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. Blood. 2003;102:1443-1448.
4. Ye BH, Chaganti S, Chang C-C, et al. Chromosomal translocations cause deregulated *BCL6* expression by promoter substitution in B cell lymphoma. EMBO J. 1995;14:6209-6217.
5. Chen W, Iida S, Louie DC, Dalla-Favera R, Chaganti RS. Heterologous promoters fused to *BCL6* by chromosomal translocations affecting band 3q27 cause its deregulated expression during B-cell differentiation. Blood. 1998;91:603-607.

6. Xu WS, Liang RHS, Srivastava G. Identification and characterization of *BCL6* translocation partner genes in primary gastric high-grade B-cell lymphoma—heat shock protein 89α is a novel fusion partner gene of *BCL6* translocation. Genes Chromosomes Cancer. 2000;27:69-75.
7. McCarthy KP, Sloane JP, Wiedemann LM. Rapid method for distinguishing clonal from polyclonal B cell populations in surgical biopsy specimens. J Clin Pathol. 1990;43:429-432.
8. Ramasamy I, Brisco M, Morley A. Improved PCR method for detecting monoclonal immunoglobulin heavy chain rearrangement in B cell neoplasms. J Clin Pathol. 1992;45:770-775.

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.[1] 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),[1] 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,[2,3] 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.[4] Whether the reverse is also true will determine which method will be the preferred one.

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References

1. Akasaka T, Lossos IS, Levy R. *BCL6* gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. Blood. 2003;102:1443-1448.
2. Lossos IS, Jacobs Y, Cleary ML, Levy R. Re: Akasaka, H., et al, Molecular anatomy of BCL6 translocations revealed by long-distance polymerase chain reaction-based assays. Cancer Res. 60:2335-2341,2000. Cancer Res. 2001; 61:7363-7364.
3. Pasqualucci L, Neumeister P, Goossens T, et al. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature. 2001;412:341-346.
4. Lossos IS, Akasaka T, Martinez-Climent JA, Siebert R, Levy R. The BCL6 gene in B-cell lymphomas with 3q27 translocations is expressed mainly from the rearranged allele irrespective of the partner gene. Leukemia. 2003;7:1390-1397.

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[5] (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