Detection of ABL Gene Translocation and 5’- Deletion by Chromogenic In Situ Hybridization (CISH™)

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INTRODUCTION

Constitutive activation of the ABL tyrosine kinase (e.g. BCR-ABL and ETV6-ABL) is a key event in the pathogenesis of CML and certain other myeloproliferative diseases. In addition to the oncogenic BCR-ABL fusion, deletion of the ABL 5’ region is found in 10% of CML patients, and is associated with a poor prognosis. Detection of ABL translocation and deletion is important in the diagnosis and surveillance of CML, particularly in the context of targeted therapies such as imatinib (Novartis). The aim of these studies was to develop and validate a method for demonstrating ABL translocation and 5’ deletion in routinely prepared bone marrow (BM) or peripheral blood smears using bright-field microscopy.

MATERIALS AND METHODS

Specimens: 12 fresh BM/blood smears, 4 formalin-fixed and paraffin-embedded (FFPE) bone marrow biopsies, and 5 archival Wright’s stained BM smears from normal persons (N=3) or from patients with CML (N = 7) or other hematological disorders (N=11) were studied for ABL gene translocation by CISH.

SPT™ BCR/ABL Translocation Probe (Zymed): Repetitive sequences (e.g. Alu and LINE), which normally contribute to nonspecific staining in ISH, were removed from BACs flanking the ABL locus using subtraction probe technology (SPT). The resultant split-apart ABL ISH probes were 250 kb (centromeric) and 260 kb (telomeric) in size, with a 200 kb gap between the probes which contains the actual ABL locus (Figure 1). The ABL.c (centromeric part) is digoxigenin (DIG) labeled. The ABL.t (telomeric part) is biotin labeled.

CISH: The BM/blood smears were dried and then fixed with paraformaldehyde (provided in the BM/Blood Smear Translocation Detection Kit, Zymed) for 15 min at room temperature. Pretreatment was not required for paraformaldehyde fixed smear specimens. The 4 µm FFPE bone marrow biopsy sections were deparaffinized, followed by heat pretreatment and enzyme digestion (CISH Tissue Pretreatment Kit, Zymed). Paraformaldehyde fixation was not necessary for the archival Wright’s stained BM smears. Ready-to-use Spot-Light® BCR/ABL probe pair (Zymed) was applied on each section before the denaturation and hybridization. After the stringent wash, the DIG and biotin labeled BCR/ABL probe pair was detected by chromogenic methods, using AP-anti-DIG and HRP-Streptavidin, followed by incubation with DAB and FastRed substrates (Figure 2).

FISH: The probe pair was validated by FISH using FITC-anti-DIG and Alexa 594-Streptavidin in cytogenetic prepared CML specimens with known BCR/ABL translocation and normal lymphocyte (Figures 3a, b, c).

RESULTS

The chromogenic ISH (CISH) signals were readily visualized with a 100x oil objective under a bright-field microscope in hematoxylin counterstained BM/blood smears.

Cells without ABL translocation showed two pairs of juxtaposed brown and red dots (Figures 4a, 5). Cells with ABL translocation showed separation of one red-brown signal pair (Figures 4b, 6). Cells with ABL translocation and concomitant ABL centromeric region deletion showed deletion of the red component of one red-brown signal pair (Figures 4c, 7). All 7 CML cases showed ABL translocation, and among them one showed ABL translocation and 5’-ABL deletion.

No BCR/ABL translocation detected in a normal bone marrow smear, using Zymed SpotLight® BCR/ ABL probe pair and CISH™ Bone Marrow/Blood Smear Detection Kit.

No BCR/ABL translocation detected in normal lymphocyte, using Zymed SpotLight® BCR/ ABL probe pair.

No BCR/ABL translocation detected in CML cell smear, using Zymed SpotLight® BCR/ ABL probe pair and CISH™ Bone Marrow/Blood Smear Detection Kit.

BCR/ABL translocation detected in CML cell smear.
DISCUSSION

The repetitive sequences were removed from the probe pair before they were labeled. The resultant probe pair generates strong and clear CISH signals. The Zymed ABL translocation probe pair uses a split-apart strategy to detect the translocation, hybridizing to the centromeric and telomeric regions that immediately flank the ABL gene. The ABL translocation breakpoints are distributed over a region of 200 kb. All the breakpoints reported so far in the ABL gene are located between the gap of centromeric and telomeric probes. Therefore, CISH using Zymed ABL translocation probe pair provides a rapid and easy-to-handle technique for routine screening of ABL rearrangement in any of the leukemias listed in Figure 1. This screening approach also enables localization of previously uncharacterized translocation partners. Most importantly, the split-apart probe pair can overcome the pitfall of the join-together probe pair due to random signals overlapping.

Approximately 10% of patients with CML have deletion of the 5' region of ABL and the 3' region of the BCR gene on 9q+ chromosome (Sinclair et al., 2000). The deletions at 5' region of ABL gene, in many cases, can span several megabases. The evidence suggests that these large deletions are associated with a poor prognosis of CML (Sinclair et al., 2000; Kolomietz et al., 2001; Cohen et al., 2001). ABL-c is deleted in up to 10% of CML. The Zymed probe has an advantage over the traditional bring-together probes to detect fusion genes since the ABL split apart assay reveals both translocation and associated deletion.

ETV6-ABL translocation, t(9;12)(q34;q13), thus far has been reported in only 6 cases of ALL, ANLL and CML (Andrasson et al., 1997; Hannemann et al., 1998).

CONCLUSION

This study indicates that ABL rearrangement can be evaluated by light microscopy, using CISH methods with Spot-Light BCR/ABL translocation probe pair in BM/Blood smear and FFPE BM biopsy. Such methods can substantially facilitate diagnosis and surveillance of CML, with particular relevance in hematology and pathology laboratories that are not equipped for FISH.

REFERENCES