“Masked” Philadelphia chromosome in acute lymphoblastic leukemia

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Philadelphia chromosome (Ph), which is primarily associated with chronic myeloid leukemia (CML) and a significant fraction of acute lymphoblastic leukemia (ALL), is generated by the reciprocal translocation t(9;22)(q34;q11.2). Although the Ph chromosome is readily recognizable by G-banding in most cases, around 1% of CML cases lack cytogenetic evidence of the Ph chromosome.¹,² Nevertheless, in such Ph-negative CML cases, molecular evidence of the BCR-ABL1 fusion gene can be obtained by reverse transcriptase-mediated poly-

Figure 1. G-banding karyotype. Chromosome 22 indicated by an arrow was labeled by the BCR-ABL1 fusion signal on FISH (Figure 2).
Figure 2. FISH of the metaphase spread karyotyped in Figure 1. The probe consists of a red-labeled LSI-ASS-ABL probe that covers the ~650-kb region encompassing the ASS and ABL1 genes and a green-labeled LSI BCR Dual Fusion probe that covers the ~1,500-kb region with a ~300-kb gap encompassing the immunoglobulin λ light chain variable gene and the BCR gene. Hybridization signals are indicated by arrows of each color. As no green signal resides on chromosome 9, which chromosome 9 is involved in the generation of the BCR-ABL1 fusion cannot be determined. Fluorescence filters are as follows: FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine B isothiocyanate; and DAPI/FITC/TRITC triple band-pass filter for the merged picture.

A 26-year-old woman was admitted to the Department of Hematology due to marked leukocytosis. Her hemoglobin level was 10.8 g/dL, white cell count was 350.38 × 10³/µL, and platelet count was 40 × 10³/µL. The white cell differential was 2.2% lymphocytes, 0.2% eosinophils, 0.4% segmented neutrophils, 0.4% banded neutrophils, and 96.8% blasts. The level of lactate dehydrogenase was 4,065 IU/L and uric acid was 8.8 mg/dL. The bone marrow was hypercellular and contained 97.0% blasts negative for peroxidase staining. Flow cytometry revealed that blasts were CD10+, CD19+, CD20+, CD21+, CD22−/weak, CD24+, CD25−/weak, CD34+, CD66c+, HLA-DR+, cCD79a+, and TdT+, and lacked cytoplasmic expression of immunoglobulin µ heavy chain, indicating the pre-pre-B-cell immunophenotype.

RT-PCR of mRNA extracted from the bone marrow amplified the minor breakpoint cluster region (bcr)-type transcripts encoding the p190 BCR-ABL1 chimeric protein (Supplementary Figure S1). Interphase FISH analysis using Vysis LSI BCR/ABL1 Dual Color, Dual Fusion Translocation Probe (Abbott Laboratories, Abbott Park, IL, U.S.A.), applied to the bone marrow smear slide detected two red, one green, and one yellow fusion signals (Supplementary Figure S2), instead of the standard one red, one green, and two yellow signal pattern. G-banding of metaphase spreads obtained from bone marrow aspirates detected no visible cytogenetic abnormality in both pairs of chromosomes 9 and 22 (Figure 1). However, when the FISH probe was applied to the metaphase spread, a small red signal representing ABL1 was found to reside on a cytogenetically normal chromosome 22, leading to the generation of the yellow fusion signal (Figures 1 and 2). As these observations suggest that the body of ABL1 included in the probe was inserted within the BCR, the karyotype according to the ISCN 2013 can be described as follows: 46,XX[10].ish ins(9;22)(q34;q11.2q11.2)(ABL1+;BCR+,ABL1+)[10].

Two mechanisms for the formation of the BCR-ABL1 fusion gene in masked Ph-positive cells have been proposed: either by cryptic insertion of ABL1 into the BCR region (or vice versa) or by a multiple step model where...
a standard t(9;22)(q34;q11.2) is followed by a second translocation between the der(9)t(9;22) and der(22) t(9;22) and/or another chromosome, thereby restoring the normal chromosome morphology. In both instances, more than the two DNA breaks associated with standard t(9;22)(q34;q11.2) are theoretically required. Although these mechanisms have been recognized in the major-bcr breakpoint in CML, it is likely that a similar molecular mechanism was responsible for the development of masked Ph in this particular ALL case involving the minor-bcr breakpoint.

The patient was treated with HyperCVAD alternating with a high-dose methotrexate and cytarabine regimen, in combination with tyrosine kinase inhibitors and rituximab because of CD20 positivity. She achieved complete response in spite of the development of severe infectious complications during the treatment. She is currently undergoing allogeneic hematopoietic stem cell transplantation. The clinical and prognostic significance of masked Ph in ALL compared with standard t(9;22) (q34;q11.2) remains to be determined.

REFERENCES


“Masked”フィラデルフィア染色体を認めた急性リンパ芽球性白血病

川原勇成 1, 飯岡 大 1, 福塚勝弘 2, 大野仁嗣 1,2

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**Supplementary Figures**

**Figure S1.** RT-PCR for the minor-bcr-type (A) and major-bcr-type (B) *BCR-ABL1* fusion transcripts. The sizes of PCR products representing the e1a2 minor-bcr-type breakpoint are 633 bp (1<sup>st</sup> round) and 444 bp (2<sup>nd</sup> round). The b2a2 major-bcr-type breakpoint generates 383-bp products by the nested PCR. PCR primers are as described previously.  

**Figure S2.** FISH for the *BCR-ABL1* fusion gene applied to the bone marrow smear slide. Red (*ABL1*) and green (*BCR*) signals involved in the generation of yellow (*BCR-ABL1* fusion) signals are indicated by arrows of each color; red signals are so small that a green color predominates in fusion signals. One hundred and seventy (98%) of 174 nuclei counted had two red, one green, and one yellow signals under the triple band-pass filter.