A rare chronic myeloid leukemia case with Philadelphia chromosome, BCR-ABL e13a3 transcript and complex translocation involving four different chromosomes

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Abstract. The so-called Philadelphia (Ph) chromosome is present in more than 90% of chronic myeloid leukemia (CML) patients. Around 5-10% of these patients show complex translocations involving other chromosomes in addition to and/or besides chromosomes 9 and 22. CML cases with fusion transcripts, such as e13a3, in which ABL exon 3 rather than exon 2 has fused to BCR, are extremely rare. Such reported cases with the e13a3 transcript showed the Ph chromosome on karyotype analysis. This study reported a rare Ph chromosome-positive CML case with new complex chromosomal aberrations and an e13a3 BCR-ABL transcript that has yet to be established. A four-chromosome translocation involving chromosomal regions 12p11.2, 19q13.3, 9q34.1 and 22q11.2, besides a trisomy 8 and a derivative chromosome 12, were identified using high resolution multicolor banding. Reverse transcription polymerase chain reaction products showed the presence of BCR-ABL fusion transcript e13a3, and this signifies the major BCR breakpoint. The significance of the observed rearrangements and their possible role in the progression of CML was investigated.

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expansion of a clone of pluripotent hematopoietic stem cells carrying the chimeric BCR-ABL fusion gene. The Philadelphia (Ph) chromosome created as a result of t(9;22) (q34;q11) is observed in more than 90% of CML patients. The BCR-ABL fusion gene is formed by transposing the 3' portion of the ABL oncogene from 9q34 to the 5' portion of the BCR gene on chromosome 22, and this fusion gene encodes a constitutive active tyrosine kinase (1). BCR breakpoints localize on 1 of 3 breakpoint cluster regions (bcr): major bcr spanning BCR exons 12-16, minor bcr spanning BCR alternative exons 2' and 2 and μbcr spanning downstream of exon 19. The majority of the BCR-ABL fusion transcripts are e13a2, e14a2, with e1a2 and e19a2 being less common (2). Extremely rare CML cases with fusion transcripts, such as e13a3, in which ABL exon 3 rather than exon 2 is fused to BCR, were previously reported (3-6). Cases reported to have the e13a3 transcript showed the Ph chromosome on karyotype analysis. A cryptic or variant rearrangement in which the typical Ph chromosome is not visible at the karyotype level is noted in 5-10% of CML patients (7). This rearrangement can be found in cases of normal or complex karyotypes. The mechanisms for these rearrangements are difficult to determine. This study reported a rare Ph chromosome-positive CML case involving the e13a3 BCR-ABL transcript and new complex aberrations, including four chromosomal breakpoints.

Materials and methods

Case report. A 25-year-old female was diagnosed as suffering from CML in the chronic phase (CP) after a blood cell count was initiated in November 2009 due to hepatosplenomegaly and loss of weight. The hematologic parameters were: white blood cells (WBC) 122x10^9/l (96.6% neutrophils, 1.55% lymphocytes, 0.170% monocytes, 1.68% eosinophiles and 0.04% basophiles). The platelet count was 156x10^9/l and the hemoglobin level was 10.3 g/dl. No treatment had been administered prior to the test. The patient was referred to our laboratory one month later, after treatment with hydroxyurea (2,000 mg/day). Thus, the previous relevant symptoms disappeared. The more recent hematological parameters were: WBC 4.5x10^9/l (42.9% neutrophils, 44.1% lymphocytes and 13% monocytes). The platelet count was 375x10^9/l and the hemoglobin level was 9.9 g/dl.

Banding cytogenetics. Chromosome analysis was performed using the GTG-banding technique according to standard

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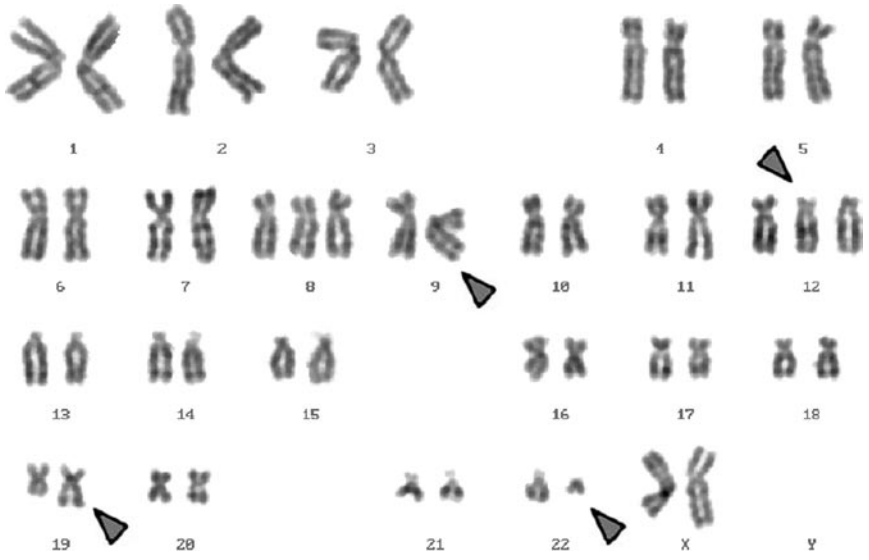

Figure 1. GTG-banding revealed a complex karyotype involving two further chromosomes besides chromosomes 9 and 22. Derivative chromosomes are indicated by the arrowheads.

procedures (8). A total of 20 metaphases, obtained from the unstimulated bone marrow of the patient, were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (9).

Molecular cytogenetics. Fluorescence *in situ* hybridization (FISH) using a LSI BCR/ABL dual color dual fusion translocation probe (Abbott molecular/Vysis, Des Plaines, IL, USA) was applied according to the manufacturer's instructions. Array-proven multicolor banding (aMCB) probe sets based on microdissection-derived region-specific libraries for chromosomes 9, 12, 19 and 22 were applied as previously described (10,11). A total of 20 metaphase spreads were analyzed, using a fluorescence microscope (AxioImager.Z1 mot, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI (4',6-diamino-2-phenylindole). Image capturing and processing were carried out using an Isis imaging system (MetaSystems, Altlussheim, Germany) for the MCB evaluation.

RNA isolation and reverse transcriptase-polymerase chain reaction. Total RNA was extracted from 1.5 ml of fresh peripheral blood immediately after collection using the InviTrap RNA kit (Invitek, Germany). A negative control was used to monitor RNA isolation. RNA concentration was estimated by absorbance at 260 nm and its purification was determined by a 1.8:2.0 ratio of absorbance values at 260:280 nm. The RNA solutions were stored at -80˚C. Reverse transcription (RT) for the complementary DNA (cDNA) synthesis, polymerase chain reaction (PCR) and nested PCR amplification of the BCR-ABL gene were performed using a ready-to-use Genequality BCR-ABL kit (AB Analitica, Italy) and a GeneAmp® PCR System 9700 thermocycler. Two positive controls were used to monitor amplification, one with β2-microglobuline and the second b3a2 with the patient sample. The negative controls used serial water. PCR products were electrophoresed on an ethidium bromide-stained 2.5% agarose-TAE-gel and observed under UV light. The type of amplified BCR/ABL cDNA was established on the basis of the size compared with the molecular markers and the primers used (b3a2, 353 bp; b2a2, 278 bp; b3a3, 179 bp and b2a3 (e13a3), 104 bp).

Results

Karyotyping was performed before and after the initiation of chemotherapy treatment, showing the following karyotypic changes: a complex karyotype 48,XX,+8,+der(12)t(12;19),t(9;12;19;22)/47,XX,+8,t(9;12;19;22)/47,XX,+der(12)t(12;19),t(9;12;19;22)/46,XX,t(9;12;19;22) was determined using GTG-banding (Fig. 1) and was further specified by molecular cytogenetic studies (Fig. 2). A dual-color-FISH using a probe specific for BCR and ABL showed a typical Ph chromosome with a BCR/ABL fusion gene. However, sections of chromosome 22 were present on a der(19) (Fig. 2F). A multi-color (M)-FISH, was applied to exclude further cryptic rearrangements (Fig. 2A). Thus, the four chromosomes 9, 12, 19 and 22 were found to be involved. aMCB using probes for the corresponding chromosomes were performed as previously reported (11). A complex translocation among the four chromosomes was detected (Figs. 2B-E), and the following final karyotypes were obtained: 48,XX,+8,+der(12)t(12;19)(p11.2;q13.3),t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[8]/47,XX,+8,t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[2]/47,XX,+der(12)t(12;19)(p11.2;q13.3),t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[2]/46,XX,t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[8].

It is likely that due to low chromosomal resolution, the complexity of the karyotype was initially missed. RT-PCR analysis of the fusion transcript showed a band corresponding to the e13a3 (b2-a3) transcript. However, the band was ~104 bp (Fig. 3).

Discussion

We described a rare Ph chromosome-positive CML case with the e13a3 BCR-ABL transcript and a new complex variant translocation t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2) that was

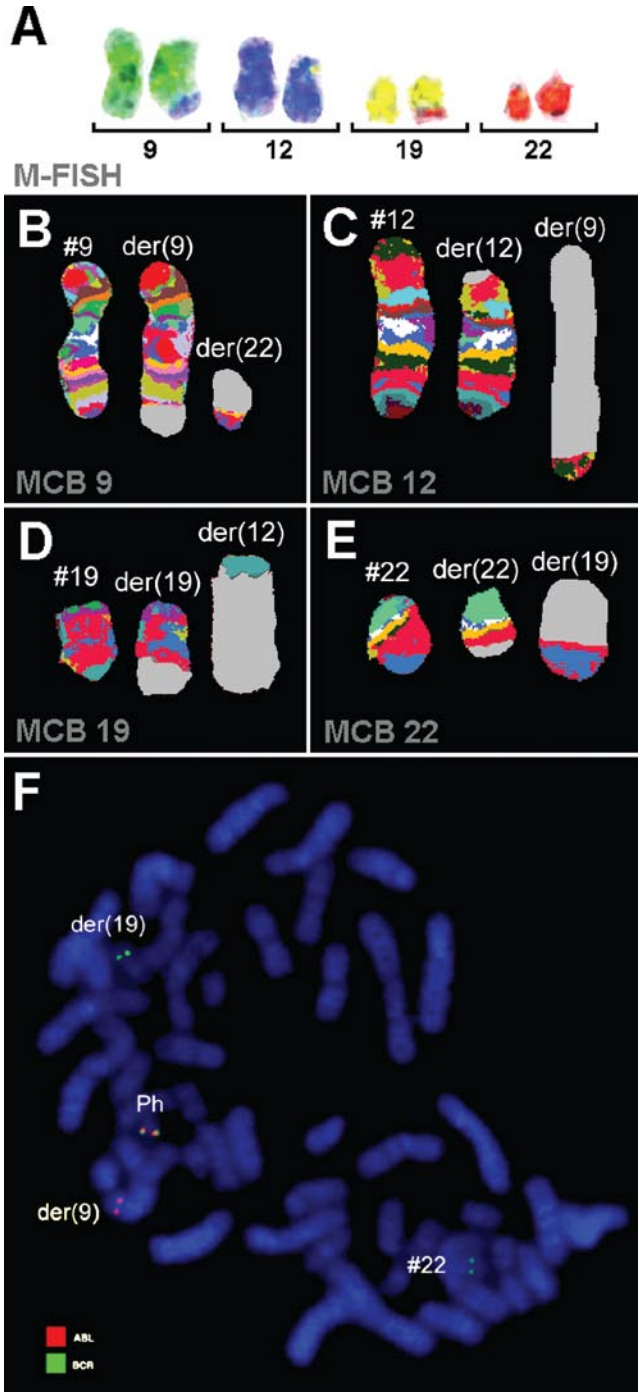

Figure 2. Karyotype and chromosomal aberrations were confirmed using molecular cytogenetic approaches. (A) M-FISH confirmed the complexity of the karyotype: 48,XX,+8,+der(12),t(9;12),t(12;19),t(19;22). (B-E) The application of aMCB analysis using probe sets for chromosomes 9, 12, 19 and 22 is shown. The normal chromosomes are shown to the left of the image, and the derivative of the four chromosomes to the middle and right. Using aMCB probes, the light gray areas show unstained regions on the derivative chromosomes. (F) Fluorescence *in situ* hybridization (FISH) using probes for BCR (green) and ABL (red) confirmed the involvement of chromosome 19 in the rearrangement present in this case. #, chromosome; der, derivative chromosome; Ph, Philadelphia chromosome.

detected. Apart from a trisomy 8 and an additional derivative chromosome 12, two additional chromosomal alterations were present. To the best of our knowledge, this translocation has yet to be elucidated in CML (12).

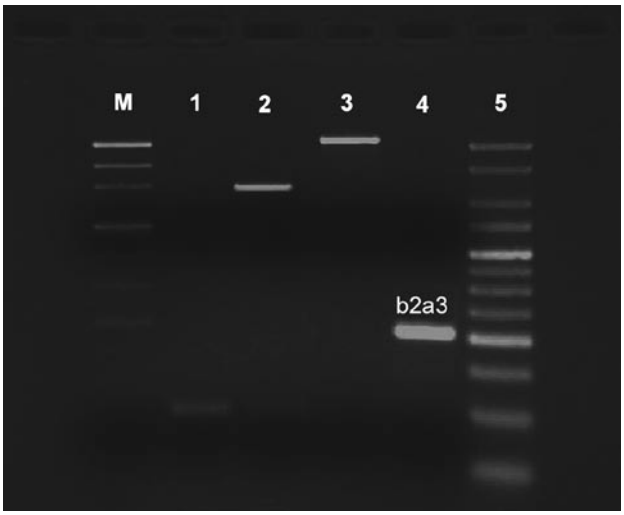

Figure 3. Gel electrophoresis of the nested RT-PCR products. Lane M, 501-110 bp marker; lane 1, negative control; lane 2, positive control (b3a2, 353 bp); lane 3, β2-microglobuline (535 bp); lane 4, BCR-ABL (b2a3, 104 bp) from the patient and lane 5, M¼100 bp molecular weight marker.

In 5-10% of Ph chromosome CML cases complex translocations in addition to those and/or besides chromosomes 9 and 22 (13) are noted. At present it appears that in such rearrangements any other chromosome may be involved. However, it has been suggested that the distribution of chromosomes and breakpoints is non-random with the chromosomal bands most susceptible to breakage being 1p36, 3p21, 5q31, 6p21, 9q22, 10q22, 11q13, 12p13, 17p13, 17q21, 17q25, 19q13, 21q22, 22q12 and 22q13 (7), showing one match with the present case, i.e., 19q13.

The progression of CML from CP to blast crisis is frequently associated with non-random secondary chromosomal aberrations such as +8, i(17q), +19 and an extra Ph chromosome (14).

Trisomy 12 is the most frequently reported chromosome abnormality in B-cell chronic lymphocytic leukemia (B-CLL). It is found in one third of cytogenetically abnormal CLL by conventional karyotype (15) and in approximately 11-46% of cases when interphase FISH is used (16).

The majority of CML patients express e13a2 (b2-a2) or e14a2 (b3-a2) of BCR-ABL mRNA encoding for p210 Bcr-Abl tyrosine kinase. These two types are detected by RT-PCR (17-19). CML with the e13a3 transcript is extremely rare, and RT-PCR for e13a2 and e14a2 is usually negative because ABL exon 2 is deleted (20). Our case was diagnosed using conventional G-band, FISH, M-FISH, MCB and RT-PCR.

In conclusion, we reported a rare Ph chromosome-positive CML case in CP with the rare e13a3 BCR-ABL transcript and new complex variant translocation involving the chromosomes 9, 12, 19 and 22.

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