

BCR translocation to derivative chromosome 2: A new case of chronic myeloid leukemia with a complex variant translocation and Philadelphia chromosome

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Abstract. The well-known typical fusion gene BCR/ABL is observed in connection with a complex translocation event in 5-8% of cases of chronic myeloid leukemia (CML). The present study described an exceptional CML case with complex chromosomal aberrations not previously observed. Aberrations included a translocated BCR to the derivative chromosome 2 [der(2)] that also involved a four-chromosome translocation, implying chromosomal regions 1p32 and 2q21, besides 9q34 and 22q11.2, which were characterized by molecular cytogenetics.

Introduction

Chronic myeloid leukemia (CML) is a clonal malignant disorder of pluripotent hematopoietic stem cells progressing from a chronic to an accelerated to a blast phase (1). The cytogenetic hallmark of CML is the Philadelphia (Ph) chromosome, resulting from t(9;22)(q34;q11), which reflects the rearrangement of the ABL and BCR genes (2). The Ph chromosome is present in more than 90% of CML cases (3). In Ph-positive CML, expression of the BCR/ABL chimeric protein p210 with an increased tyrosine kinase activity is essential for multiple signaling pathways to confer the leukemia phenotype (4).

Complex chromosomal rearrangements involving one or more additional chromosomes have been described in more than 600 CML cases (5). Using conventional cytogenetic analysis, two variant subgroups have traditionally been identified: complex t(9;22;V) where V represents a third translocation partner chromosome, and simple t(9;V) or t(22;V) (6). Only a few cases exhibit a chromosomal fragment from the third chromosome translocated to the der(22)t(9;22) producing

a 'masked Ph' (7). In the majority of Ph-variant cases, the segment 22q11-qter shifts to a third chromosome, while a part of the third chromosome is located on 9q34. Deletions on the derivative chromosome 9 occur with a much higher frequency in patients with variant Ph translocations (45%) compared to those with classic Ph (17%) (8).

We present a CML case with a translocated BCR to der(2), involving four different chromosomal breakpoints characterized by molecular cytogenetics.

Materials and methods

Case report. A 47-year-old female patient was admitted to our Human Genetics Division initially presented with a WBC of $9.66 \times 10^9/l$ and splenomegaly. Chromosome analysis using banding cytogenetics revealed a karyotype in accordance with the clinical diagnosis of CML in the chronic phase. She was treated with hydroxyurea (1000 mg/day) for four years and three months. At her initial admission, her hematological parameters were: 85.4% neutrophils, 7.7% lymphocytes and 6.9% immature cells. The platelet count was $372 \times 10^9/l$ and the hemoglobin level was 11.8 g/dl. She was initially treated with hydroxyurea for 18 months. Then, 33 months later, following hydroxyurea treatment, her WBC was $130.91 \times 10^9/l$ (79.8% neutrophils, 8.5% lymphocytes and 11.7% immature cells). The platelet count was $340 \times 10^9/l$, and the hemoglobin level was 11.9 g/dl.

Banding cytogenetics. Chromosome analyses were performed by the GTG-banding technique according to standard procedures (9). Twenty metaphases, obtained from the unstimulated bone marrow of the patient, were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (10).

Fluorescence in situ hybridization (FISH). FISH was conducted using commercially available probes. LSI BCR/ABL dual-color dual-fusion translocation probe (Abbott Molecular/Vysis, USA), whole chromosome painting (WCP) probe for chromosomes 1, 2 and 22 (MetaSystems, Germany) and α satellite probe (CEP) for chromosome 9 (Abbott Molecular/Vysis) were applied according to the standard method (11). Twenty metaphase spreads were analyzed, using a fluorescence micro-

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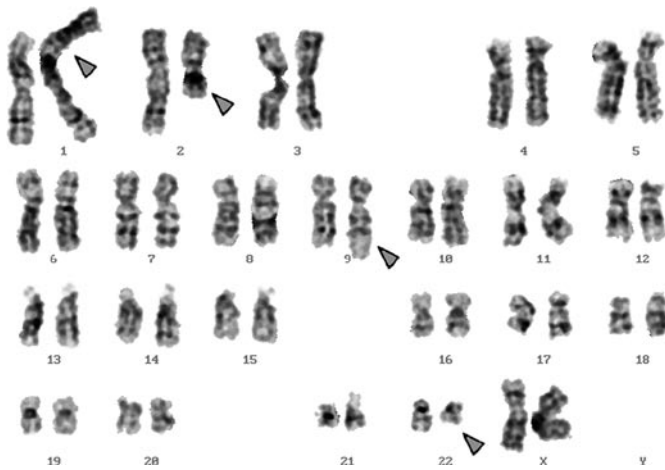


Figure 1. GTG-banding shows a complex karyotype involving two further chromosomes besides chromosomes 9 and 22. Derivative chromosomes are indicated by arrowheads.

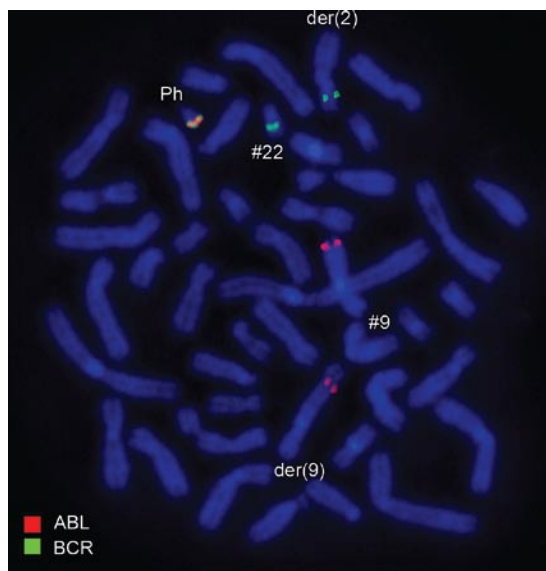


Figure 2. Using probes for BCR (green) and ABL (red), fluorescence *in situ* hybridization (FISH) confirmed the involvement of chromosome 2 in the rearrangement present in this case. #Chromosome; der, derivative chromosome; Ph, Philadelphia chromosome.

scope (Axio Imager Z1 mot; Zeiss, Germany) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain 4'6-diamino-2-phenylindole. Image capturing and processing were carried out using an Isis image analysis system (MetaSystems).

Results

Karyotyping was performed at 3 and 10 months after the initiation of hydroxyurea treatment. The same karyotypic changes were noted. A complex karyotype 46,XX,t(1;2;9;22) was determined in GTG-banding (Fig. 1), and was further studied by molecular cytogenetics (Figs. 2-4). Using a commercially available probe specific for BCR/ABL, dual-color FISH showed that the typical Ph chromosome with the BCR/ABL translocation was present. However, BCR was translocated to

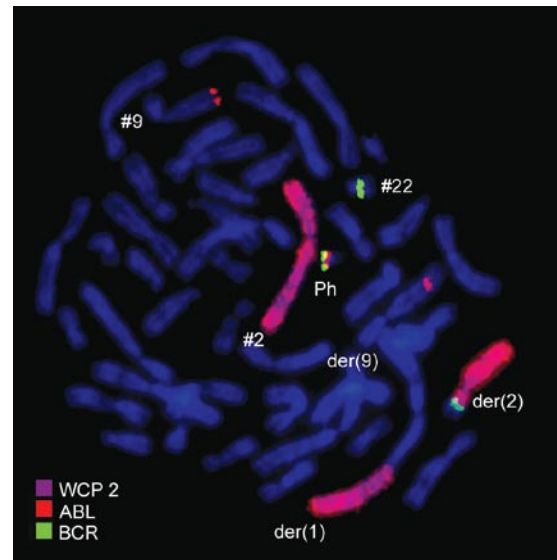


Figure 3. Using probes for BCR (green), ABL (red) and WCP2 (red), fluorescence *in situ* hybridization (FISH) confirmed the involvement of chromosome 2 in the rearrangement present in this case. #Chromosome; der, derivative chromosome; Ph, Philadelphia chromosome.



Figure 4. Using probes for WCP1 (green) WCP2 (red) and CEP9 (red), fluorescence *in situ* hybridization (FISH) confirmed the involvement of chromosome 1 with chromosomes 2 and 9 in this case. #Chromosome; der, derivative chromosome.

der(2) (Figs. 2 and 3). Another commercially available probe specific for WCP1 + WCP2 + CEP9 confirmed the involvement of chromosome 1 with chromosomes 2 and 9 (Fig. 4). Thus, FISH was performed using probes for the involved chromosomes according to GTG-banding (Figs. 2-4). The result obtained was: 46,XX,t(1;2;9;22)(p32;q21;q34;q11.2).

Discussion

The present study identified one additional translocation, 46,XX,t(1;2;9;22)(p32;q21;q34;q11.2), in CML-CP. To the best

of our knowledge, this translocation has never been described in the literature (12).

In 5-8% of CML cases, the fusion gene BCR/ABL is the result of a complex translocation (13). At present, it appears that variant translocations can affect any chromosome. However, it has been suggested that the distribution of the breakpoints is non-random, with the chromosomal bands 1p36, 3p21, 5q31, 6p21, 9q22, 10q22, 11q13, 12p13, 17p13, 17q21, 17q25, 19q13, 21q22, 22q12 and 22q13 being the most susceptible to breakage (5). None of the above-mentioned breakpoints were noted in our study. However, the fusion gene is located on chromosome 22.

Two possible mechanisms for variant translocation formation have been suggested. The first is a single-event rearrangement via the simultaneous breakage of several chromosomes followed by mismatched joining (14). Nacheva *et al* proposed a classic Ph translocation followed by a further translocation event between chromosomes 9 and 22 plus a third chromosome (15). The mechanism of the formation of a variant Ph translocation may have prognostic importance in that a two-event mechanism represents clonal evolution, whereas a variant translocation occurring via a single genomic rearrangement may confer a similar prognosis to the classic Ph translocation (16).

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