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A chronic myeloid leukemia case with a unique variant Philadelphia translocation: t(9;22;21)(q34;q11;p12)

WALID AL-ACHKAR[1], ABDULSAMAD WAFA[1], FATEN MOASSASS[1] and THOMAS LIEHR[2]

[1]Molecular Biology and Biotechnology Department, Human Genetics Division, Atomic Energy Commission, Damascus, Syria; [2]Jena University Hospital, Institute of Human Genetics, Jena, Germany

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Abstract. The so-called Philadelphia (Ph) chromosome is present in more than 90% of chronic myeloid leukemia (CML) patients. Approximately, 5-10% of these patients show complex translocations involving a third chromosome in addition to chromosomes 9 and 22. Since at present the majority of CML cases are treated with imatinib, variant rearrangements do not exhibit specific prognostic significance. However, events of therapy resistance remain to be studied. In this study, we report a unique case of CML exhibiting an uncommon t(21;22)(p12;q11). This translocation has been characterized by fluorescence *in situ* hybridization (FISH) and array-proven multicolor banding (aMCB). Using specific probes for the BCR and ABL genes, results of FISH showed a three-way variant Philadelphia translocation (9;22;21)(q34;q11;p12) with a BCR/ABL fusion residing on the der(22) and the 3'BCR region translocated on the short arm of the derivative chromosome 21. In addition, the aMCB technique is significant in the detection of the breakpoints of genetic changes. The underlying mechanisms and prognostic significance of these changes are discussed.

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disease that originates in an abnormal pluripotent bone marrow stem cell and is consistently associated with the Philadelphia (Ph) chromosome, usually leading to a BCR/ABL gene fusion. The Ph chromosome produced as a result of t(9;22)(q34;q11) is observed in over 90% of cases, whereas variant Ph translocations are observed in 5-10% of cases (1). By standard cytogenetics, variant translocations have been classified as simple when they involve the distal section of chromosome 22 and another chromosome distinct from chromosome 9, and as complex when chromosomes 9, 22 and at least one or more other chromosomes are involved (1). The BCR-ABL fusion gene is formed by the transposing of 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 constitutively active tyrosine kinase (2). Imatinib mesylate (Glivec, formerly STI571) was designed specifically to inhibit the tyrosine kinase activity of the BCR/ABL protein and other tyrosine kinases, such as cABL, c-KIT and platelet-derived growth factor receptor (PDGF). By binding to an active site of the tyrosine kinase, Glivec switches off downstream signaling, cells are prevented from proliferating and apoptosis ensues (3). Various studies showed that a high efficacy of imatinib therapy achieves a complete or major cytogenetic response, i.e., a reduction to 0-34% Ph-positive cells. This positive effect is achieved in cases with a simple t(9;22) combined with complex translocations, resulting in BCR/ABL gene fusion, as well as in cases with clonal evolution (4,5).

In this case report, we present a unique translocation, t(21;22), which was further characterized by fluorescence *in situ* hybridization (FISH) and array-proven high-resolution multicolor banding (aMCB) as t(9;22;21)(q34;q11;p12) with a BCR/ABL fusion residing on the der(22) and the 3'BCR region translocated on the short arm of derivative chromosome 21, nonetheless successfully treatable with imatinib.

Materials and methods

Case report. A 36-year-old male was diagnosed as suffering from CML in the chronic phase (CP). In August 2007, the white blood cell count (WBC) of the patient was $11.8x10^9$/l, constituting 53% neutrophils, 21% lymphocytes, 4% monocytes, 4% eosinophiles, 16% basophiles and 2% blasts. The platelet count was $118x10^9$/l and the hemoglobin level was 12.9 g/dl. A previous physical examination revealed splenomegaly. The patient was treated with imatinib mesylate at 400 mg/day for eight months in total, and the previous relevant symptoms appeared to have improved. The serum lactate dehydrogenase (LDH) level was 301 U/l (normal level up to 414 U/l) and serum alkaline phosphatase level was 94 U/l (normal level up to 90 U/l). In February 2008, the patient presented for the second time with a WBC of $54.5x10^9$/l consisting

Correspondence to: Dr Walid Al-Achkar, Molecular Biology and Biotechnology Department, Human Genetics Division, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria
E-mail: ascientific@aec.org.sy

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of 44% neutrophils, 11% lymphocytes, 1% monocytes, 29% basophiles and 15% blasts. The platelet count was 303x10^9/l and the hemoglobin level was 13.5 g/dl. The serum LDH level was 403 U/l and the serum alkaline phosphatase level was 104 U/l. The patient was treated again with imatinib mesylate at 400 mg/day for 14 months in total. The patient was then lost during follow-up.

Cytogenetic analysis. Chromosome analysis using GTG-banding was performed according to standard procedures (6). A total of 20 metaphase cells derived from the unstimulated bone marrow of the patient were analyzed. Karyotypes were described according to the international system for human cytogenetic nomenclature (7).

Molecular cytogenetics. 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 (6). aMCB sets based on micro-dissection-derived region-specific libraries for chromosome 9, 21 and 22 were applied as previously described (8,9). A total of 20 metaphase spreads were analyzed, using a fluorescence microscope (Axio Imager Z1 mot, Zeiss, Hertfordshire, UK) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI. Image capturing and processing were carried out using an ISIS imaging system (MetaSystems, Altlussheim, Germany) for the MCB evaluation.

Results

Karyotyping was performed following the initiation of chemotherapy treatment, showing the following karyotypic changes.

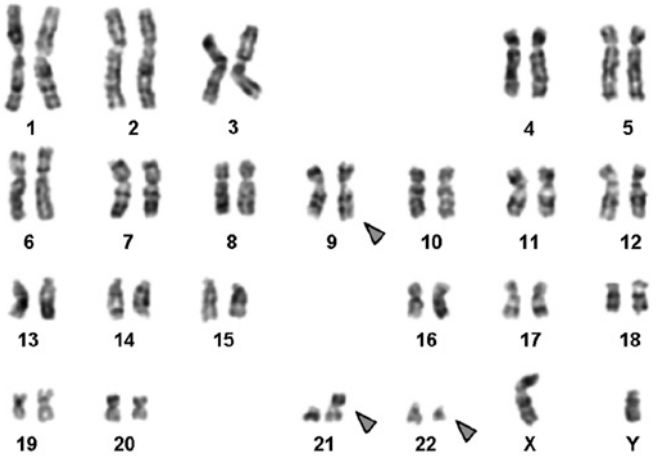

Figure 1. GTG-banding revealed a complex karyotype involving one further chromosome besides chromosomes 9 and 22. Derivative chromosomes are shown by the arrowheads.

A complex karyotype 47,XY,t(9;22),der(21;22),+der(22)[3]\46,XY,t(9;22),der(21;22)[10]\46,XY,t(9;22)[7] was determined by 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 revealed that a typical Ph chromosome with a BCR/ABL fusion gene was present. However, sections of chromosome 22 were present on a der(21) (Fig. 2A). Thus, aMCB using probes for the corresponding chromosomes was performed as previously reported (9). A complex translocation among the three chromosomes was detected (Fig. 2 B-D) and the final karyotypes obtained were: 47,XY,t(9;22)(q34;q11),der(21;22)

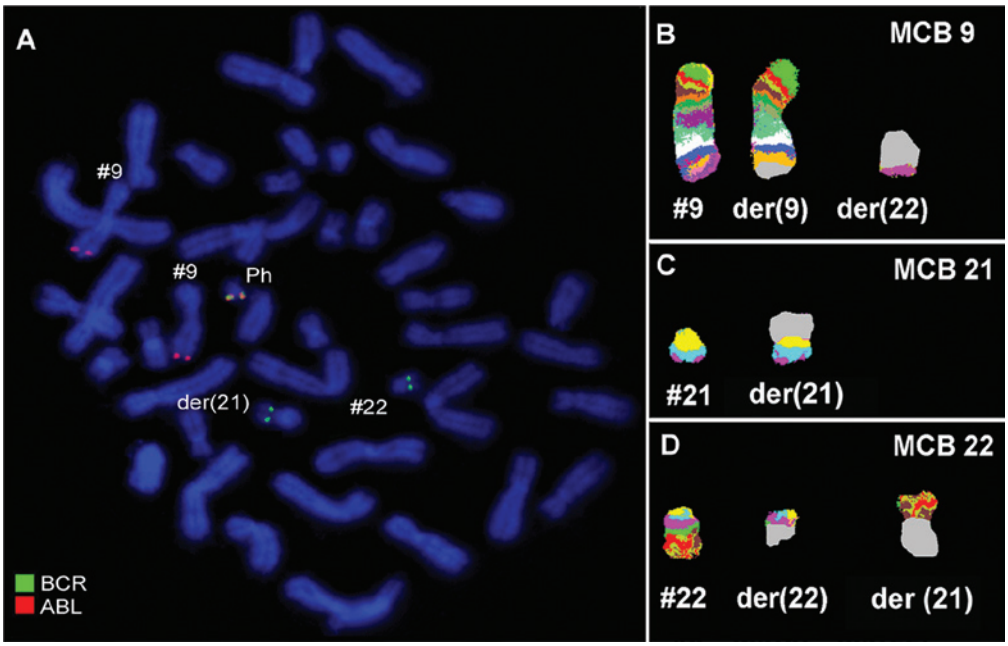

Figure 2. (A) Fluorescence *in situ* hybridization (FISH) using probes for BCR (green) and ABL (red) confirmed an involvement of chromosome 21 in the rearrangement, presence of the BCR/ABL translocation and Philadelphia (Ph) chromosome in this case. (B-D) Array-proven multicolor banding (aMCB) was applied to determine the involved breakpoints in this complex rearrangement. Each lane shows the results of aMCB analysis using probe sets for chromosomes 9, 21 and 22. The normal chromosomes are shown in the first column and the derivatives of the three chromosomes in the subsequent ones. The aMCB probe's unstained regions on the derivative chromosomes are shown in gray. #, chromosome; der, derivative chromosome; Ph, Philadelphia chromosome.

(p12;q11),+der(22)[3]\46,XY,t(9;22)(q34;q11),der(21;22)(p12;q11)[10]\46,XY,t(9;22)(q34;q11)[7].

Discussion

According to the literature, a number of other CML cases with t(9;22;21)(q34;q11;q22) (10-15), one case with t(9;22;21)(q34;q11;q21) (16) and one with t(9;22;21)(q34;q11;q11.2) (17) have been reported, respectively. To the best of our knowledge, only one case of Ph chromosome-positive CML with a unique translocation of three chromosomes t(9;22;21)(q34;q11;p12) was detected, and this translocation has yet to be observed at 21p12 in CML (18).

Chromosomes are known to be involved in variant rearrangements in CML (19). However, it has been suggested that the distribution of the break-points 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 (19). However, the fusion gene remained on chromosome 22.

The review by Johansson *et al* (19) observed that a major breakpoint in chromosome 21 is q22, and q11 is very rare. The translocation with 21q22 is also common in other hematologic malignancies, whereas 21q11 has been reported in only a few cases of myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL) and acute myelogenous leukemia (AML) (20). However, CML with variant chromosomal abnormalities generally has a similar prognosis to that of cases with the typical t(9;22)(q34;q11) translocation (1). Further patient studies with involvement of 21p12 are required in order to establish prognosis in such cases.

In conclusion, we reported a unique case of a Ph chromosome-positive CML in the CP with a new variant Ph translocation involving three chromosomal aberrations 9q34, 21p12 and 22q11, and the 3'BCR region translocated on the short arm of derivative chromosome 21, which has not previously been described. Of note is that the patient had a favorable response to imatinib.

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