A unique cytogenetic abnormality, t(2;7)(p13.1;p21.3), in a Philadelphia-positive chronic myeloid leukemia

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Abstract. The Philadelphia (Ph) chromosome is present in more than 90% of patients suffering from chronic myeloid leukemia (CML). It is the product of a reciprocal translocation between the long arms of chromosomes 9 and 22, resulting in the transfer of the 3' portion of the proto-oncogene ABL from 9q34 to the 5' portion of the breakpoint cluster region (BCR) on 22q11. Currently, most CML cases are treated with Imatinib and variant rearrangements are thought to have no specific prognostic significance, although the events of therapy resistance have not yet been studied. In this study we report a novel case of CML exhibiting an uncommon t(2;7)(p13.1;p21.3) besides t(9;22)(q34;q11). This unusual translocation has been characterized by fluorescence *in situ* hybridization (FISH) and array-proven multicolor banding (aMCB), the latter being extremely significant in characterizing breakpoint regions in detail. The underlying mechanisms and prognostic implications of this cytogenetic abnormality are discussed in this study.

Introduction

Chronic myeloid leukemia (CML) is a clonal malignant disorder of pluripotent hematopoietic stem cells, characterized by the presence of the Philadelphia (Ph) chromosome in more than 90% of patients. The Ph chromosome results from a reciprocal translocation of chromosomes 9 and 22, which leads to the transfer of the 3' portion of the proto-oncogene ABL from 9q34 to the 5' portion of the breakpoint cluster region (BCR) on 22q11. This results in a fused BCR/ABL gene and the production of an abnormal tyrosine kinase protein that causes the altered and disease-causing myelopoiesis found in CML. Since tyrosine kinase activity is required for the transforming function of the BCR/ABL fusion protein, the specific inhibitor of the kinase Imatinib is an effective treatment for CML patients. In a previous study, the 5-year estimated overall survival rate of 89% for patients who received Imatinib as an initial therapy was higher than that reported in any previously published prospective study of the treatment of CML and only 7% of all patients progressed to the accelerated phase or blast crisis (1).

In this study, we present a previously unreported translocation of chromosomes 2 and 7 being present with the Ph chromosome in a CML patient successfully treated with Imatinib. The additional rearrangement was characterized in detail by fluorescence *in situ* hybridization (FISH) and high resolution array-proven multicolor banding (aMCB) as t(2;7)(p13.1;p21.3).

Materials and methods

Case report. A 45-year-old female was diagnosed as suffering from CML in chronic phase (CP). In December 2008, the white blood cell count (WBC) was 15.3x10^9/l with 58.3% neutrophils, 34.8% lymphocytes, 5.2% monocytes, 0.8% eosinophiles and 0.9% basophiles. The platelet count was 978x10^9/l and the hemoglobin level was 11.5g/dl. The previous physical examination revealed hepatosplenomegaly. The patient was treated with imatinib mesylate at 400 mg/day for eight months and the relevant symptoms disappeared. In July 2009, she passed away under the treatment due to unknown reasons. The study was approved by the ethics committee of the Atomic Energy Commission of Syria and patient consent was obtained.

Cytogenetic analysis. Chromosome analysis using GTG-banding was performed according to standard procedures (2). A total of 20 metaphases derived from the unstimulated bone marrow of the patient were analyzed. Karyotypes were described according to the international system for human cytogenetic nomenclature (3).

Molecular cytogenetics. FISH using a whole chromosome painting (WCP) probe for chromosomes 2 and 7 (MetaSystems, Altlussheim, Germany) and subtelomeric probes for 7pter and 7qter (Abbott Molecular/Vysis, Des Plaines, IL, USA) were applied according to the manufacturer's instructions

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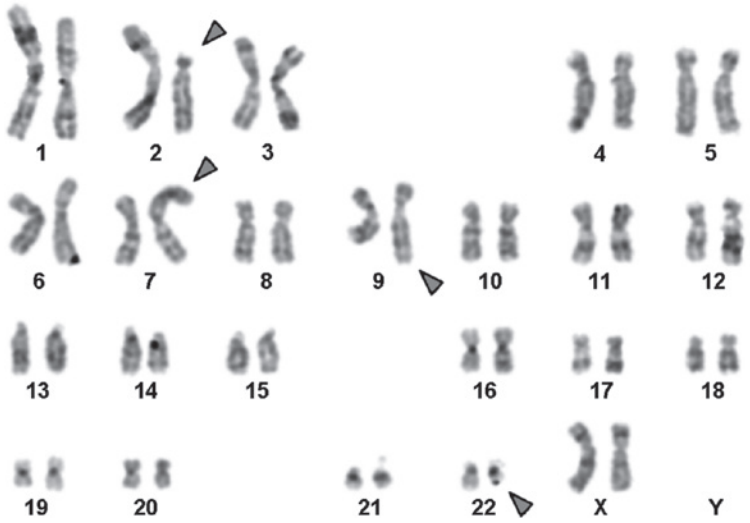

Figure 1. GTG-banding revealed an abnormality involving one further chromosome besides chromosomes 9 and 22. Derivative chromosomes are indicated by the arrowheads.

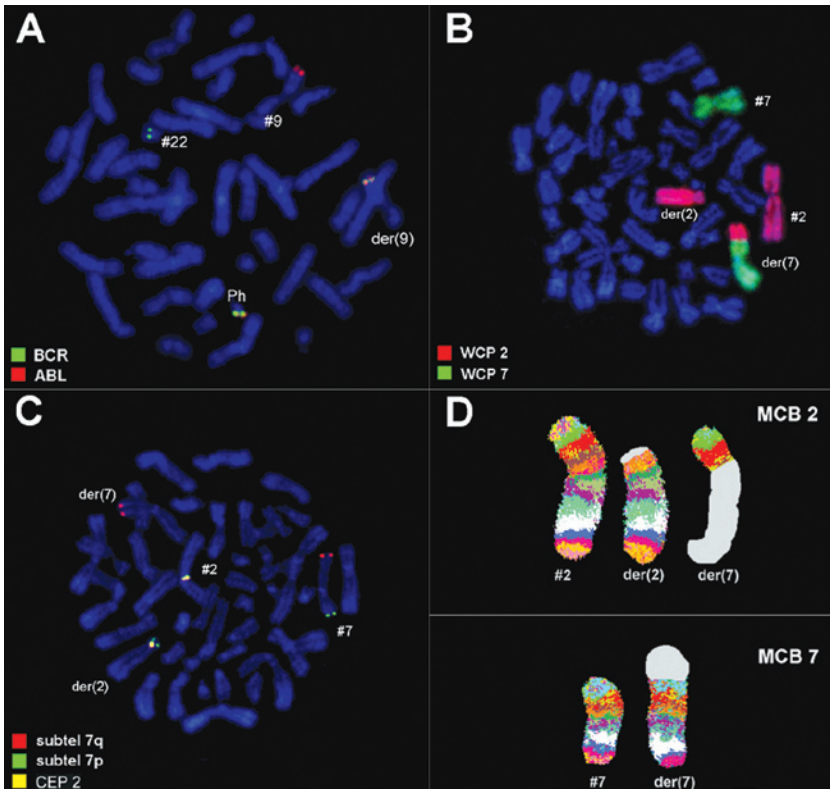

Figure 2. Karyotype and chromosomal aberrations were confirmed using molecular cytogenetic approaches. (A) Fluorescence *in situ* hybridization (FISH) using probes for BCR (green) and ABL (red) confirmed the presence of the BCR/ABL translocation and the Philadelphia (Ph) chromosome. (B) The application of FISH analysis using WCP probes for chromosomes 2 and 7 is shown. (C) The application of FISH analysis using subtelomeric and CEP probes for chromosomes 2 and 7 is shown. (D) Array-proven multicolor banding (aMCB) was applied to determine which chromosomes were involved in the complex rearrangement. Each lane shows the results of aMCB analysis using probe sets for chromosomes 2 and 7. The normal chromosomes are shown in the first column and the derivative of two chromosomes in the subsequent ones. The aMCB-probes unstained regions on the derivative chromosomes are shown in gray. #, chromosome; der, derivative chromosome; WCP, whole chromosome painting; CEP, chromosome enumeration probe.

together with a chromosome enumeration probe (CEP) for chromosome 2 (Abbott Molecular/Vysis) (2). aMCB sets based on microdissection-derived region-specific libraries for chromosomes 2 and 7 were applied as previously described (4). Twenty metaphase spreads were analyzed using a fluorescence microscope (AxioImager.Z1 mot, Carl Zeiss Ltd., Hertfordshire, UK) 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

mFISH imaging system (MetaSystems) for the evaluation of the MCB.

Results

Karyotyping was performed prior to and following the initiation of chemotherapy treatment. The result prior to chemotherapy was 46,XX,t(9;22)[18]/46,XX[2] and following therapy it was 46,XX,t(2;7),t(9;22)[20] in GTG-banding (Fig. 1). It was further specified by molecular cytogenetic studies (Fig. 2). A dual-color-FISH applying probes specific to BCR and ABL revealed a typical Ph chromosome with a BCR/ABL fusion gene (Fig. 2A). The corresponding WCP probes confirmed a Ph-independent translocation between chromosomes 2 and 7 (Fig. 2B), also substantiated by subtelomeric probes for 7pter and 7qter (Fig. 2C). aMCB narrowed down the breakpoints to 2p13.1 and 7p21.3.

Discussion

The present study identified one additional chromosomal alteration in a Ph-positive CML-CP case as t(2;7)(p13.1;p21.3). To the best of our knowledge, this translocation has not been previously observed in CML (5). Moreover, neither breakpoint has been reported to be involved in variant Ph-rearrangement in CML (6).

The breakpoint 2p13 has been reported in patients with acute bilineal leukemia (7,8) and B-cell chronic lymphocytic leukemia (9,10). The gene BCL11A may be involved in these diseases (10,11). However, in Hodgkin's lymphoma, breaks at 2p13 (12) have been reported to be associated with the oncogene REL (13). Finally, 2p13 has previously been reported in a translocation t(2;14) in a Ph-positive acute lymphoblastic lymphoma (ALL) case (14).

The 7p21.3 region also merits further study. This region includes the ETS variant gene 1 (ETV1), which encodes an ETS translocation variant 1 protein in humans (15,16). The ETS genes (including FLI, ERG, ETV4 and ETV1) encode a family of eukaryotic transcription factors that includes more than 30 members that are found in organisms from sponges to humans (17). The ETS genes are involved in multiple processes, including cell proliferation and cancer cell invasion (18). Some of these genes become oncogenic by retroviral insertional mutagenesis (17) [as in gastric cancer (18), prostate cancer (19) and breast cancer metastasis (18)] or by chromosomal translocations [as in myeloid leukemia and Ewing's sarcomas (17)]. For example, ETV1 has been mapped at position 7p21.3 and has been found to be subject to a translocation between chromosomes 7 and 22 in a human Ewing's sarcoma (17).

The 2p13.1 region includes the Anthrax toxin receptor 1 gene (ATR1) (20). ATR1 is a transmembrane protein and a tumor-specific endothelial marker (TEM) that is involved in colorectal cancer (21).

TEM-8 belongs to a family of TEMs that were identified as being predominantly expressed in tumor endothelium (22). Moreover, a high TEM-8 expression appears to be correlated with advanced tumor stage in breast and colorectal cancer (23). An overexpression of ATR1 has also been found in several neuroblastoma (NB) cell lines (24).

TEM-8 has been shown to function as a cell surface TEM that is highly conserved in mice and human tumor endothelium, thus making TEM-8 an attractive marker for the establishment of an *in vivo* model system in an attempt to assess anti-angiogenic strategies in combating tumor growth (22).

In conclusion, in this study we reported a unique case of a Ph chromosome-positive CML in CP with a new translocation, possibly therapy-initiated, involving the two chromosomal aberrations 2p13.1 and 7p21.3, which has not previously been described. Notably, the reported patient had a good response to Imatinib.

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