Case Report

New Complex Chromosomal Translocation in Chronic Myeloid Leukaemia: t(9;18;22)(q34;p11;q11)

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A Chronic myeloid leukaemia (CML) case with a new complex t(9;18;22)(q34;p11;q11) of a 29-year-old man is being reported. For the first time, this translocation has been characterized by karyotype complemented with fluorescence in situ hybridization (FISH). In CML, the complex and standard translocations have the same prognosis. The patient was treated with standard initial therapy based on hydroxyurea before he died due to heart failure four months later. Our finding indicates the importance of combined cytogenetic analysis for diagnosis and guidance of treatment in clinical diagnosis of CML.

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1. INTRODUCTION

Chronic myeloid leukaemia (CML) is a myeloproliferative disorder characterized in about 95% of cases by the presence of the Philadelphia (Ph) chromosome (t(9;22)(q34;q11))[1]. It is a result from a reciprocal translocation between the long arms of chromosomes 9 and 22 [2]. About 3-4% of Ph-positive patients with CML have variant translocations [3, 4], involving another chromosome [5]. These variant translocation have been classified as “simple” and as “complex” when chromosomes 9, 22, and at least one other chromosome are involved [6]. Both translocations have the same prognosis. Here, we present the first report of a complex variant Ph translocation involving chromosome 18: t(9;18;22)(q34;p11;q11).

2. MATERIALS AND METHODS

2.1. Case report

In July 2000, a 29-year-old man was admitted for fatigue with anaemic syndrome to the Averroes Hospital (Casablanca, Morocco). For this patient, the diagnosis of CML has been retained in front of the existence of a splenomegaly and in view of the following blood formula: Hb 8.7 g/dl; platelets $566 \times 10^9 /L$; and white blood cells $189 \times 10^9 /L$ with 34% neutrophils and 31% myelocytes. Morphologic examination of the bone marrow biopsy was consistent with CML in chronic phase. Treatment with hydroxyurea initially (5 cp/day) resulted in a clinical response, with normalization of the peripheral blood values. In May 2001, his WBC was 21800 /µl, with Hb of 12.5 g/dl; the differential leukocytes count was 13% myelocytes and 5% metamyelocytes. At diagnosis, the cytogenetic analysis on bone marrow metaphases revealed a complex karyotype with der(18). Four months later the patients died due to a heart failure.

2.2. Cytogenetic analysis

Unstimulated bone marrow cells obtained at the time of primary diagnosis were cultured for 24 hours in RPMI 1640 medium supplemented with 20% fetal calf serum, 1% L-glutamine, 1% antibiotics, and without mitogens. After incubation, the cells were exposed to Colcemid (0.01 µg/ml) for 30 minutes, followed by hypotonic treatment (0.075 M KCl), and were fixed with methanol-glacial acetic acid solution.
(3 : 1). Chromosomes were spread on cold, wet slides, dried, and banded with the RHG technique. Karyotype was interpreted according to International System for Human Cytogenetic Nomenclature (ISCN 2005) recommendation [7]. A minimum of 20 metaphases were analyzed and no signs of clone were found in the samples.

2.3. FISH analysis

Fluorescence in situ hybridization (FISH) was performed on methanol-acetic acid fixed bone marrow cells [8]. Commercially available, differentially labelled probes (LSI bcr/abl extrasil (ES) dual color; Vysis) were used to detect the rearranged BCR/ABL gene. In addition, biotin-labelled whole chromosome painting probes (WCPs) (Cambio, UK) were used to detect abnormal chromosome 18 and 22. The hybridisation protocol followed the manufacturer’s recommendations. fluorescent signals were captured with a cooled charge-coupled device (CCD) camera attached to a microscope with triple-band filters (Olympus Optical Co., Tokyo, Japan) and processed using an image analysis system.

3. RESULTS

Twenty metaphases were analyzed at diagnosis and R-banding showed the following karyotype: 46,XY,t(18;22)(p11;q21). Both chromosomes 9 looked unsuspicious (see Figure 1). The RT-PCR analysis revealed the BCR/ABL fusion with b3a2 junction, which indicated the breakpoint within the major breakpoint cluster region of the BCR gene (M-BCR)(data not shown). Conventional cytogenetic results were refined by FISH analysis. Dual Color FISH (DC-FISH) analysis of interphase nuclei and metaphase using LSI BCR/ABL extrasil (ES) translocation probe showing the yellow (red-green) fusion on the der(22), a BCR signal (green) was observed on the normal 22, an ABL signal (red) on the normal 9, a smaller ABL signal retained on the other chromosome 9 (see Figure 2(a)). Further investigation, using a combination of the library probe for chromosome 9 and the library probe for chromosome 22 showed one chromosome 9 painted red, and the other homologue with part of the long arm painted green indicating a rearrangement (see Figure 2(b)). The karyotype was thus redefined.
The complex abnormalities in CML remain intriguing because of the wide range of chromosomal involvement with no apparent clinical effect [9,10]. Variant type Ph translocations have been described using cytogenetic analysis [4] and molecular techniques. Whether these translocations result from sequential events or from a “concerted genomic rearrangement” with a simultaneous break of the chromosomal regions involved followed by a mismatched joining of the broken ends in a one-step mechanism remain to be determined [11]. The genesis of variant translocations via two-step mechanisms is also possible [12]. The application of FISH and RT-PCR allowed us to refine the cytogenetic data [13]. Recently, it was shown significant positive correlation between breakpoint location and CG composition [14]. In this study, we have demonstrated the existence of a BCR/ABL chimeric transcript, giving rise to a b3a2 mRNA transcript, and we showed that material from chromosome 9 was translocated to chromosome 22, as well as from chromosome 22 to chromosome 18. The variant Ph translocations involving chromosome 18 seem very uncommon because any case of t(18;22)(p11;q11) has been previously described. These results suggest that combinatorial probe hybridization is very useful for defining both the standard translocation, t(9;22), and the variant form of Ph translocations. The comparison of conventional cytogenetic and FISH analysis results, suggests that FISH is a more sensitive method to detect and to explain the variant translocations in CML.

4. DISCUSSION

The complex abnormalities in CML remain intriguing because of the wide range of chromosomal involvement with no apparent clinical effect [9,10]. Variant type Ph translocations have been described using cytogenetic analysis [4] and molecular techniques. Whether these translocations result from sequential events or from a “concerted genomic rearrangement” with a simultaneous break of the chromosomal regions involved followed by a mismatched joining of the broken ends in a one-step mechanism remain to be determined [11]. The genesis of variant translocations via two-step mechanisms is also possible [12]. The application of FISH and RT-PCR allowed us to refine the cytogenetic data [13]. Recently, it was shown significant positive correlation between breakpoint location and CG composition [14]. In this study, we have demonstrated the existence of a BCR/ABL chimeric transcript, giving rise to a b3a2 mRNA transcript, and we showed that material from chromosome 9 was translocated to chromosome 22, as well as from chromosome 22 to chromosome 18. The variant Ph translocations involving chromosome 18 seem very uncommon because any case of t(18;22)(p11;q11) has been previously described. These results suggest that combinatorial probe hybridization is very useful for defining both the standard translocation, t(9;22), and the variant form of Ph translocations. The comparison of conventional cytogenetic and FISH analysis results, suggests that FISH is a more sensitive method to detect and to explain the variant translocations in CML.

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REFERENCES
