Short communication

Insertion (22;9)(q11;q34q21) in a patient with chronic myeloid leukemia characterized by fluorescence in situ hybridization

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Abstract

An unusual cytogenetic rearrangement, described as $\text{ins}(22;9)(q11;q34q21)$, was detected in a 49-year-old male patient diagnosed with chronic myeloid leukemia (CML). Reverse transcriptase polymerase chain reaction (RT-PCR) revealed a b3a2 fusion transcript. In order to confirm the cytogenetic findings and fully characterize the inverted insertion, we performed fluorescence in situ hybridization (FISH) assays using locus-specific and whole chromosome painting probes. Our FISH analysis showed the presence of the *BCR/ABL* fusion gene, verified the insertion and determined that the breakpoint on chromosome 22 where the insertion took place was located proximal to the *BCR* gene and distal to the *TUPLE1* gene on 22q11. © 2001 Elsevier Science Inc. All rights reserved.

1. Introduction

Chronic myeloid leukemia (CML) is cytogenetically characterized by the presence of the Philadelphia chromosome (Ph), which results from a reciprocal $t(9;22)(q34;q11)$ translocation and is found in about 90–95% of CML patients. The molecular consequence of the $t(9;22)$ is the fusion of the 3' segment of the *ABL* gene from 9q34 to the 5' part of the *BCR* gene located at 22q11, giving rise to a chimeric *BCR/ABL* gene, which encodes a fusion protein with prominent tyrosine kinase activity and transforming ability [1]. Ph variants are found in 5% of CML patients. Such variants consist of complex translocations among three or more chromosomes including 9 and 22 and sometimes result in spuriously Ph negative but *BCR/ABL* positive CML [2]. In these cases, fluorescence in situ hybridization (FISH) is a useful technique to determine the location of the *BCR/ABL* fusion gene [3]. Here, we present the characterization by FISH of a complex inverted insertion of chromosome 9 into chromosome 22 in a case of CML.

2. Materials and methods

2.1. Case report

In January 1999, a 49-year-old man was referred to the Department of Hematology of the Hospital Miguel Servet (Zaragoza, Spain) for evaluation of fatigue, weight loss, and abdominal discomfort. Physical examination revealed a palpable abdominal mass in the left side, 12 cm below costal limits. Neither the personal nor the familiar history showed relevant data. Hematological parameters were as follows: hemoglobin 104 g/L; hematocrit: 32%, platelets: 432×10^9 /L; white blood cell (WBC) count: 630×10^9 /L. Peripheral blood (MGG stain) with 25% neutrophils, 17% band, 13% metamyelocytes, 32% myelocytes, 4% promyelocytes, 2% blasts, 1% eosinophils, 4% basophils, and 2% lymphocytes. Leukocyte alkaline phosphatase score was reduced to 4. All laboratory values were normal with the exception of high levels of lactate dehydrogenase (1151 UI/L, normal below 460 UI/L) and B₁₂ vitamin levels (7329 pg/mL, normal 180–900 pg/mL). Bone marrow aspirate showed hypercellularity with myeloid hyperplasia (94.2%), including 2.8% myeloblasts, and 2.8% basophils. Decreased erythropoiesis (2%) and increased number of hypobulbated and small size megakaryocytes. Bone marrow biopsy showed marked hy-

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percellularity with loss of fat spaces and mild reticulin fibrosis of irregular distribution. Abdominal ultrasonography confirmed splenomegaly. Cytogenetic analysis at diagnosis revealed a t(9;22) variant, ins(22;9)(q11;q34q21), and the presence of a b3a2 fusion transcript was detected by reverse transcriptase polymerase chain reaction (RT-PCR). The patient was diagnosed as Philadelphia positive CML at stage 3 of Kantarjian. To additionally characterize this rare cytogenetic finding, we initiated FISH studies.

Cytoreduction was started with hydroxyurea 2 g qid po until decreased WBC counts and spleen size were evident. One month later the administration of alfa-Interferon [4], 3 mU qid sc was initiated with escalating doses until 9 mU qid and cytosine arabinoside 10 mg/m² 10 days every 4 weeks. Six months later a partial hematological remission was achieved with normal WBC counts, normal leukocyte differential distribution, and spleen size reduced to 50% of initial value. To date, 16 months after the diagnosis, the patient has remained stable in a chronic phase with partial hematological remission; a cytogenetic response has not appeared yet.

2.2. Cytogenetic analysis, FISH and RT-PCR

Chromosomal analysis of tumor cells was performed on bone marrow. Samples were processed using standard short-term unstimulated cultures. Metaphases were G-banded according to a conventional trypsin-Giemsa procedure.

FISH was performed according to manufacturer's instructions using: whole painting probes for chromosomes 9 and 22 labeled with fluorescein (FITC) and cyanine 3 (Cy3), respectively (Cambio, Cambridge, UK), and two locus-specific probes to detect *ABL* (SpectrumOrange)/*BCR* (SpectrumGreen), and *TUPLE1* (SO)/*ARSA* (SG) genes (Vysis, Downers Grove, IL, USA). Cytogenetic and FISH data were described according to the International System for Human Cytogenetic Nomenclature [5].

RT-PCR was performed according to routine protocols [6].

3. Results

A total of 50 metaphases were evaluated and in all of them, the karyotype was described as: 46,XY,der(9)t(9;22)(q13;q11),der(22)ins(22;9)(q11;q34q21). As it can be observed in Fig. 1, the derivative chromosome 22 contains a segment of chromosome 9 (4D), the fusion signal *BCR5'*-*ABL3'* appears near the telomere (4B) and both the *ABL5'* and *TUPLE1* signals are located close to the centromere (4B and 4C). The derivative chromosome 9 contains part of chromosome 22 (3D), one signal for the *ARSA* gene located at 22q13 (3C) and no signals for *ABL* and *BCR* genes (not shown). According to these findings, the derivative chromosomes were described as: ish der(9)t(9;22)(q13;q11)(wcp9+,wcp22+,ABL-,BCR-,ARSA+),der(22)(22pter→22q11.2::9q34→9q21::22q11.2::9q34→9qter)(wcp22+,wcp9+,TUPLE1+,ABL++,BCR+). Fig. 1 shows

the ideograms of both normal and derivative chromosomes 9 and 22 and the chromosome pictures after G-banding, painting FISH and locus-specific FISH with *BCR/ABL* and DiGeorge probes. A b3a2 mRNA transcript was found by RT-PCR indicating that the breakpoint was located within the major breakpoint cluster region (M-bcr) of the *BCR* gene.

4. Discussion

Philadelphia chromosome cytogenetic variants in CML are becoming easily characterized using FISH techniques [2,7]. These t(9;22) variants usually involve a third chromosome and complex rearrangements involving only chromosomes 9 and 22 have been rarely described [8–10]. Like classical t(9;22), these variants usually lead to a *BCR/ABL* fusion, which can be confirmed by RT-PCR [11]. The clinical course of these patients does not differ from that of patients carrying the classical t(9;22) [2]. Cytogenetic responses generally begin to appear from 3 to 12 months of treatment with IFN- α [12]. In our patient, although a partial hematological remission was achieved, the cytogenetic response had not appeared after 15 months of treatment with IFN- α . We performed a complete genetic analysis of leukemic cells from this CML patient and found a complex balanced rearrangement between chromosomes 9 and 22 without the Philadelphia chromosome, but with molecular confirmation of the *BCR/ABL* fusion gene. G-banding karyotyping revealed a large derivative chromosome 22 and a short derivative chromosome 9, which were described as: 46,XY,der(9)t(9;22)(q13;q11),der(22)ins(22;9)(q11;q34q21). Fig. 1 shows both normal 9 and 22 chromosomes (I), the different fragments after chromosome breakage (II), and the complex alteration (III) as they were seen by the different techniques. The *ABL5'* (4B) signal is located near the centromere on the der(22) thus confirming the inverted insertion we had proposed initially. To locate the second breakpoint on chromosome 22 we used the *TUPLE1* gene, which is proximal to *BCR*, and found that this gene was retained on 22q11 (4C), hence the region where the 9q segment was inserted must be between those two genes. According to the genomic sequence of chromosome 22 (<http://www.sanger.ac.uk/HGP/Chr22/>), our findings suggest that the breakpoint must be approximately in a region between 3.2 Mb (*TUPLE1*) and 7.1 Mb (*BCR*) from the centromere. Although we had cytogenetically described the der(22) as a simple ins(22;9)(q11;q34q21), after FISH, the description of such chromosome became der(22)(22pter→22q11.2::9q34→9q21::22q11.2::9q34→9qter).

In order to explain the genesis of this variant t(9;22), we propose two hypotheses: a two-step mechanism, in which a classical t(9;22) took place followed by an inverted insertion of an interstitial segment of the der(9) into the proximal region of the Philadelphia chromosome; or a single-step mechanism, in which all the fragments were split and rearranged in a complex fashion at the same time. The first hy-

pothesis could not be confirmed because at diagnosis the patient did not have a Ph chromosome; it is possible that the clone with the insertion had a proliferative advantage over the clone with the t(9;22), due perhaps to a second fusion between genes involved in the insertion. Another possible explanation that we can not rule out is that both the translocation and the insertion took place as a two-step mechanism in the same cell, leading to a single malignant clone.

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