ANALYSIS OF VARIANT TRANSLOCATION t(4;9;22) BY A FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IN A CASE OF CHRONIC MYELOCYTIC LEUKEMIA

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ABSTRACT

Chronic myelocytic leukemia is characterized by formation of the abelson murine leukemia viral oncogene homolog/breakpoint cluster region fusion gene, usually as a consequence of the Philadelphia translocation between chromosomes 9 and 22. Approximately 5%–10% of chronic myelocytic leukemia patients show variant translocations involving other chromosomes in addition to chromosomes 9 and 22. We report a chronic myelocytic leukemia case carrying variant Philadelphia translocation involving both chromosomes 9 and 22 as well as chromosomes 4. A detailed molecular cytogenetic characterization was performed by fluorescence in situ hybridization, which disclosed the presence of the 5'breakpoint cluster region/3'abelson murine leukemia viral oncogene homolog 1 fusion gene was localized on the Philadelphia chromosome, whereas the 5'abelson murine leukemia viral oncogene homolog 1/3'breakpoint cluster region gene was not detected on the derivative (9) chromosome because 3'breakpoint cluster transferred onto partner chromosomes 4. Serial translocations or a single simultaneous event are alternative hypotheses proposed to justify the occurrence of these complex rearrangements.

Key words: Acute myelocytic leukemia, Philadelphia chromosome, translocation, fluorescence in situ hybridization

INTRODUCTION

Approximately 90%–95% of chronic myelocytic leukemia (CML) cases are characterized by the Philadelphia chromosome (Ph), which arises from the reciprocal t(9;22)(q34;q11). About 5%–10% of CML patients show a variant translocation, involving one or more chromosomes in addition to 9 and 22. Three-, four-, and five-way variant translocations have been reported. Some variant t(9;22) cases, the Ph chromosome can be recognized cytogenetically, whereas the der(9) is rearranged with a different partner chromosome. Other CML patients show a “masked Ph” in which additional material is translocated on the Ph chromosome as a result of a rearrangement between the der(22)t(9;22) and another...
At the molecular level, this exchange of DNA results in the transfer of the c-ab2 proto-oncogene from its normal residence, at chromosome 9 band q34, to a restricted chromosome 22 band q1 1 segment of about 5.8 kb, termed the breakpoint cluster region (bcr) 3 - 6. The mechanisms of these rearrangements are difficult to determine; serial translocations or a single simultaneous event could be alternatively hypothesized 7. In a few variant t(9;22) cases with a masked Ph chromosome, the fusion gene is located on chromosomal sites other than 22q11 8 - 13. All chromosomes have been reported to act as the third chromosome, but breakpoint clustering at 1p36, 3p21, 5q13, 6p21, 9q22, 11q13, 12p13, 17p13, 17q21, 17q25, 19q13, 21q22, 22q12, and 22q13 has been reported 14 - 15. Herein, we describe a CML-chronic phase male with a complex translocation involving chromosome with variant t(9;22).

CASE REPORT

1. Study

Cytogenetic analysis of a 56-year-old male with leukocytosis and anemia revealed a variant t(9;22) translocations: 46,XY,t(4;9;22)(p16;q34;q11)(Figure 1). Reverse-transcriptase polymerase chain reaction (PCR) demonstrated the P210 rearrangement (b3a2). The diagnosis of chronic-phase CML was made. The patient started Imatinib therapy.

Cytogenetic analysis

Karyotype analysis was carried out on bone marrow cells according to standard methods. Conventional cytogenetic analysis of a 24- to 48-hour culture was performed on bone marrow cells at diagnosis with standard techniques and evaluated with Giemsa–trypsin–Giemsa banding at about the 400-band level according to International System for Human Cytogenetic Nomenclature (ISCN 2009). Twenty metaphases were scored. No mosaicism was observed in the samples analyzed 16.

FISH analysis

Fluorescence in situ hybridization (FISH) studies were carried out on bone marrow samples at the time of diagnosis. Briefly, 500 ng of labeled probe was used for FISH experiments; hybridization was performed at 37°C in 2× standard saline citrate (SSC), 50% (vol/vol) formamide, 10% (wt/vol) dextran sulphate, 5 µg COT1 DNA (Bethesda Research Laboratories, Gaithersburg, MD), and 3 µg sonicated salmon sperm DNA in a volume of 10 µL. Post-hybridization washing was at 42°C in 2× SSC/50% formamide (three times) followed by three washes in 0.1× SSC at 60°C. Biotin-labeled DNA was detected with DEAC-conjugated streptavidin. In cohybridization experiments, other probes were directly labeled with FluorX and Cy5. Chromosomes were identified by 4′,6-diamidino-2-phenylindole (DAPI) staining. Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton
Instruments, Boston, MA). Cy3 (red; New England Nuclear, NJ), FluorX (green; Amersham, Arlington Heights, IL), Cy5 (IR; New England Nuclear), and DAPI (blue) fluorescence signals, which were detected by using specific filters, were recorded separately as gray-scale images. Pseudocoloring and merging of images were performed with Adobe Photoshop software.

FISH identification of the *ABL1* gene was performed using a pool of PAC, RP5-835J22, and RP5-1132H12 (red), which encompass a region from approximately 40 kb upstream of exon 1b to 20 kb downstream of exon 11. BAC RP11-164N13 (green) was used to identify the *BCR* gene. FISH cohybridization with these clones on a metaphase bearing a classic t(9;22)(q34;q11) results in a red signal on normal chromosome 9, a green signal on normal chromosome 22, and fusion signals on both the der.(9) and Ph chromosomes(Figure 1A).

2. Results

Figure 1 displays cohybridization with *ABL1* and *BCR* clones that revealed a fusion signal on the Ph [der. (22)] chromosome. No fusion signal was observed on the der. (9) chromosome. The use of BAC clones proximal to *ABL1* showed a small deletion of 115 Kb of chromosome 9 sequences on der.(9). The breakpoint was mapped between the clones RP11-379C10 [9 and der. (9)] and RP11-339B21 [9 and der(4)]. RP11-395P17clone showed only a fluorescent signal on normal chromosome 9 because it was inside the deleted region (Figure 1B). RP11-339B21, RP11-167N5, RP11-5J2 and RP11-17L7 (AL353695.7) BACs were translocated on der. (4) chromosome (Figure 1C). Chromosome 4 breakpoint was characterized inside the BAC probe RP11-919N24, showing a splitting signal on der.(4) and der.(9) chromosomes (Figure 1E). Indeed, RP11-61B7, RP11-572O17 and RP11-478C1 clones were translocated on der.(9) chromosome (Figure 1D), while RP11-422O10, RP11-520M5 Figure 1E) and RP11-466A9 (Figure 1C) were retained on der.(4) chromosome. RP11-248J22 (chromosome 22-specific clone) was mapped on 22 and der.(4) chromosomes(Figure 1C).
Figure 1. (A–E) FISH analysis for characterization of the t(4;9;22) translocation detected in this study. (A) PAC, RP5-835J22, and RP5-1132H12 (red) display identification of the ABL1 gene and BAC RP11-164N13 (green) identifies the BCR gene. (B) Breakpoint locations on chromosomes 9 and 4 were revealed by RP11-379C10 [9 and der(9)] and RP11-339B21 [9 and der(4)]. (C) RP11-248J22 (chromosome 22-specific clone) was mapped on 22 and der(4) chromosomes. (D) Translocation on der(9) chromosome by BACs RP11-61B7, RP11-572O17 and RP11-. (E) BAC probe 478C1RP11-919N24, showing a splitting signal on der(4) and der(9) chromosomes.
DISCUSSION

According to the literature, in 2-10% CML cases the fusion gene BCR/ABL is a result of a complex translocation. At present it appears that variant translocations can affect any chromosome. In our case, the 5'BCR/3'ABL fusion gene was localized on the Ph chromosome, whereas the 5'ABL/3'BCR gene was not detected on the der.(9) chromosome because 3'BCR transferred onto partner chromosomes (chromosome 4). It was difficult to establish whether a single event or multistep events had occurred. The possible mechanism for variant translocation formation is a single-event rearrangement via the simultaneous breakage of several chromosomes followed by mismatched joining 17. Although the few reported cases are undoubtedly insufficient to be able to draw any conclusion on the involvement of specific genes in CML pathogenesis, nevertheless these findings indicate that a detailed cytogenetic characterization of CML variant cases is crucial to identify the loss of genomic sequences and to evaluate the occurrence of deletions on the third chromosome involved in the rearrangement 18.

CONCLUSION

Serial translocations or a single simultaneous event are alternative hypotheses proposed to justify the occurrence of these complex rearrangements. FISH analysis with appropriate BAC/PAC clones allowed us to precisely characterize the complex chromosomal rearrangements that were not detected by conventional cytogenetic analysis.

LIST OF ABBREVIATION

ABL: Abelson murine leukemia viral oncogene homolog  
BAC: Bacterial artificial chromosome  
BCR: Breakpoint cluster region  
CML: Chronic myelocytic leukemia  
Der:Derivative chromosome  
FISH: Fluorescence in situ hybridization  
PAC: P1-derived artificial chromosome  
Ph: Philadelphia  
P210: Bcr-abl fusion protein  
t:Translocation  
RP: Roswell Park

CONSENT

I declare that this work was done with agreement of the patient.

COMPETING INTERESTS

I declare that there is no Conflict of interest
REFERENCES


