

Complex variant philadelphia translocation involving the short arm of chromosome 9 in a case of chronic myeloid leukemia

Here we describe how we detected the BCR/ABL fusion gene on the short arm of der(9) combining classical GTG banding and Fluorescence In Situ Hybridization (FISH) in a case of chronic myeloid leukemia (CML). To our knowledge, variant translocations involving the short arm of chromosome 9, in literature, are almost rare in chronic myeloid leukemia. It is not clear if this complex genetic translocation represents clonal evolution or a unique, initial presentation variant of the Philadelphia chromosome (Ph).

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At diagnosis, approximately 95 % of cases of CML have the characteristic t(9;22)(q34;q11) that results in the Ph chromosome [der(22q)].¹ This translocation fuses sequences of the BCR gene on chromosome 22 with regions of the ABL gene on chromosome 9.² Variant translocations involving additional chromosome have been observed in 5-10% of CML patients.³ In some variant t(9;22) the Ph chromosome can be detected cytogenetically, while other CML cases show a *masked Ph* with additional material of another chromosome.

We present our cytogenetic findings in a CML patient with masked Ph as a result of a rearrangement between the der(22)t(9;22) and the short arm of the der(9)t(9;22).

Case Report

A 39-year-old woman first presented at our laboratory in January 2004 for a routine blood examination. Haematological findings were as follows: haemoglobin, 11.8 g/dl; platelets, 410.0×10³/μL; white blood cells, 78.69×10³/μL and 77% polymorphonuclear cells; serum lactate dehydrogenase (LDH) level was 885U/L. Bone marrow aspirate was hypercellular with granulocytic and megakaryocytic hyperplasia. On physical examination the patient presented splenomegaly (2.0 cm below the left costal margin) without hepatomegaly. She was diagnosed as having CML in the chronic phase (CP), then received treatment with orally administered imatinib mesylate at daily of 400 mg and after 3 months (May 2004) of therapy the patient showed haematologic and cytogenetic response.

Materials and Methods

Cytogenetics

Cytogenetic examinations were performed on unstimulated 24-hour *in vitro* culture (RPMI-1640 medium supplemented with 20% fetal calf serum, glutamine and Colcemid) of a bone marrow specimen. Chromosomes were banded with a trypsin-Giemsa technique (GTG banding). Karyotype was described according to the ISCN nomenclature.⁴

Fluorescence In Situ Hybridization (FISH)

FISH to metaphases was carried out with chromosome-9-specific and chromosome-22-specific whole chromosome painting (WCP) probes and Locus Specific Identifier BCR/ABL ES Dual Color Translocation Probe (Vysis, Inc.).

Figure 1. G-banded metaphase showing a karyotype of 46, XX, t(9;9;22)(p13;q34;q11) (A). FISH with BCR (green) and ABL (red) probes in metaphase spread and interphase nuclei. The BCR/ABL fusion signal (yellow) was located on 9p (B). FISH with chromosome 9 (green) and chromosome 22 (red) whole chromosome painting probes confirmed the Ph translocation to the short arm of chromosome 9(C).

Results

Chromosome analysis was performed on 20 metaphases. The modal chromosome number was 46. The cytogenetic diagnosis from the GTG banding study was 46, XX, t(9;9;22)(p13;q34;q11) (Figure 1A). No mosaicism was observed.

BCR and ABL cosmid probes demonstrated the BCR/ABL fusion gene on the short arm of der(9) in all metaphases. In addition FISH analysis revealed 5'ABL deletion on the long arm of der(9) as shown by the absence of the expected faint red signal (Fig. 1B).

Dual-color FISH with chromosome 22 and 9 painting probes revealed four signals of chromosome 22 painting probe: on the normal chromosome 22, on 9p and on 9q of the same chromosome and on der(22); the chromosome 9 painting probe gave signals on the normal chromosome 9, on der(9) and on der(22) (Figure 1C).

In May 2004 we repeated FISH analysis with BCR/ABL cosmidic probes, revealing the fusion gene in only 24% of cells. It was not possible to perform the cytogenetic analysis since no division cell was detected.

Discussion

The Ph chromosome is the characteristic chromosomal abnormality found in CML. Among the Ph-negative patients, the BCR/ABL fusion gene was generally located on either 22q or 9q.^{5,6}

In 1984 Bernstein et coll. reported a CML case where the usual translocation of 22q11 to 9q34 was followed, at first, by translocation of the Ph to 9p and, in a second step, by translocation of 9p to the distal long arm of a dicentric marker 9. Their case then evolved into a more aggressive phase of the disease associated to an increase in karyotype complexity.⁷

Our case could represent the first step of such a genetic evolution, still confined to the less aggressive chronic phase of CML. The follow up of the patient with the collection from additional cases is necessary to comment on

the clinical significance of this rare rearrangement.

We conclude that FISH is useful to easily detect Ph chromosome variant translocations and accurately quantify disease at diagnosis, during and after treatment including cytogenetic remission.

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