



Complex chromosome rearrangements may locate the bcr/abl fusion gene sites other than 22q11

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ABSTRACT

Background and Objectives. From 5-8% of Philadelphia (Ph) positive patients with chronic myeloid leukemia (CML) show variant translocations in which at least a third chromosome in addition to 9q34 and 22q11 is involved. The formation mechanisms and clinical significance of variant Ph translocations are still unclear. The BCR/ABL chimeric gene encoding for chimeric proteins is always present and maps on the 22q regardless of the type of translocation. We studied two apparently Ph negative CML patients with unusual karyotypes both showing a typical b3a2 rearrangement.

Design and Methods. Dual-color fluorescence *in situ* hybridization (FISH) can visualize BCR and ABL genes and localize the BCR/ABL fusion gene. We used FISH to study the formation mechanisms of variant Ph translocations in two patients.

Results. The chimeric BCR/ABL gene was located on a locus other than the expected 22q11 in both patients. In the first case the fusion signal was present on the 9q34 band whereas in the second patient it was detected on chromosome 8, involved in masked Ph formation.

Interpretation and Conclusions. The location of the hybrid BCR/ABL gene on chromosomes other than 22q is a rare event which can only be observed using the FISH technique. When these unusual translocations occur the hypothesis most often put forward is that several consecutive cytogenetic events have taken place. The factors which regulate the formation of these breakpoints have yet to be clarified. The FISH technique allows the identification of chromosome rearrangements that could not otherwise be detected by conventional banding procedures. The location of the hybrid BCR/ABL gene on sites other than 22q11 represents a rare type of variant Ph translocation. The real frequency and clinical significance of such rearrangements need to be investigated.

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Key words: variant Ph translocations, Ph chromosome; BCR/ABL, FISH, chronic myeloid leukemia

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The t(9;22)(q34;q11) is observed in more than 90% of patients with chronic myeloid leukemia (CML).¹ It is termed the *standard* translocation and gives rise to the Philadelphia (Ph) chromosome which molecularly results in the formation of a chimeric gene, the BCR/ABL, encoding for proteins with abnormal tyrosine kinase activity.² Cytogenetic variants of the Ph chromosome can be observed in 5-8% of CML patients³ and in these cases, at least, a third chromosome other than 9 and 22 is involved. The segment from the third chromosome is usually translocated to the 9q34 band, whereas the 22q11→22qter region moves to the breakpoint of the third chromosome. When this latter breakpoint is very close to the telomere, such that the segment which moves to the end of the *q* of chromosome 9 is very small, both chromosomes 9 may appear cytogenetically normal.⁴ Fluorescence *in situ* hybridization (FISH) can, however, demonstrate the translocation of the ABL gene from the 9q34 band to Ph chromosome as occurs in the standard t(9;22).⁵ Indeed, in variant Ph translocations the same molecular BCR/ABL recombinations as in the t(9;22) are usually found.⁶ In some cases of CML with a normal karyotype, FISH revealed the translocation of 3' ABL sequences from 9q34 to 22q11 adjacent to the 5' portion of the BCR gene with the formation of the BCR/ABL hybrid gene.⁷ These findings indicate that the formation of the BCR/ABL hybrid gene always takes place in CML and that this is located on 22q11 regardless of the observed chromosomal rearrangement. In rare cases the BCR/ABL hybrid gene is located on chromosomal sites other than the Ph chromosome.

We report two new CML cases with complex karyotypes and unusual locations of the BCR/ABL hybrid gene detected by FISH. In the first patient QFQ banding showed normal chromosomes 9, a t(21;22)(q22;q11) and loss of the der(22)t(21;22), whereas FISH demonstrated that BCR sequences were translocated on the 9q34 band giving rise to a BCR/ABL fusion signal. The second patient showed a masked Ph translocation involving chromosomes 8 and 10 in addition to 9 and 22 and the BCR/ABL located on the der(8). A typical b3a2 rearrangement was present in both cases.

Case reports

Patient #1

A 46-year old woman was admitted to another institution for the first time in November 1995

because of fatigue, shortness of breath on exertion and weight loss. Physical examination was unremarkable. Hemoglobin (Hb) concentration was 86 g/L; white blood cell (WBC) count $4.85 \times 10^9/L$; platelet count $494 \times 10^9/L$. Peripheral blood contained immature myeloid cells (promyelocytes 6%, myelocytes 15%), some blasts (2%), and platelet anisocytosis. Marrow was normocellular with a prevalence of immature myeloid cells, increased megakaryocytes, and reduced erythroid lineage with dysplastic features. Marrow biopsy suggested a diagnosis of chronic myeloproliferative syndrome with increased megakaryocytes and fibrotic evolution. The patient was not treated. In November 1996 the patient was admitted to our Department. The hematologic features were Hb: 80 g/L; WBC: $5.7 \times 10^9/L$; platelets $345 \times 10^9/L$. Marrow showed myeloid hyperplasia with a prevalence of immature cells. Cytogenetic analysis yielded all abnormal metaphases: 45,XX, der(21)t(21;22)(q22;q11), -22. Leukocyte alkaline phosphatase (LAP) score was 4 (normal values 20-100). FISH analysis confirmed the presence of the BCR/ABL fusion gene and molecular analysis showed a b3a2 rearrangement. A diagnosis of CML with atypical marrow fibrosis was made. Treatment with α -interferon was immediately discontinued because of intolerance and the patient was treated with supportive therapy and hydroxyurea 1 g/day for 10 days /month for five months. As of January 1998, the patient has been transfused monthly with red blood cells (1-2 units every month). At time of writing (May 1999), her Hb was 80 g/L, WBC $3.2 \times 10^9/L$ and platelets $211 \times 10^9/L$. Marrow showed 10% of blast cells. Cytogenetic analysis confirmed the karyotype observed at diagnosis, with no evolution.

Patient #2

A 66-year old woman presented with a 2-month history of an abscess on the hand. Past history was significant for hypertension, peptic ulcer and colon diverticula. Laboratory studies showed: Hb 82 g/L; WBC $43.4 \times 10^9/L$ (neutrophils 50%, eosinophils 1%, basophils 8%, lymphocytes 11%, monocytes 3%, myelocytes 25%, blasts 2%); platelets $515 \times 10^9/L$. Physical examination was non-contributory. The marrow was hypercellular and a diagnosis of CML in the chronic phase was made. The patient's LAP score was 2 (n.v. 20-100). Cytogenetic analysis showed a masked Ph due to the complex t(8;22;10;9) (q22; q11;q22;q34). Molecular analysis confirmed the presence of a b3a2 rearrangement. The patient was treated with antibiotics and hydroxyurea for two months. She then started therapy with α -interferon 6×10^6 U for two months and then 9×10^6 U for 8 months. The patient is currently stable (21 months from diagnosis) but three successive cytogenetic studies have revealed the presence of the masked Ph chromosome in 100% of the examined metaphases without karyotype evolution.

Design and Methods

Cytogenetics

Cytogenetic analysis was performed on 24-hour unstimulated bone marrow (BM) cultures using standard procedures. Acetic acid/methanol fixed chro-

mosomes were identified by Q-bands and utilized for FISH analysis.

Fluorescence in situ hybridization

Fresh slides prepared for cytogenetic analysis were used for the FISH studies. Before hybridization slides were pre-treated with RNase and proteinase K followed by post-fixation and denaturation.⁸ The probes used were: chromosome 8 biotinylated α -satellite probe (D8Z1, Oncor, Gaithersburg, MD, USA); chromosome 9 α -satellite and chromosome 22 painting probe digoxigenin labeled (Boehringer Mannheim). Dual color FISH was carried out with BCR and ABL probes (Vysis) directly labeled with Spectrum Green and Spectrum Orange fluorochromes respectively. Denaturation, hybridization and post-hybridization washes used for each probe were as specified by the manufacturer. In one-color experiments, biotinylated probes were detected by using avidin-fluorescein isothiocyanate (FITC) and biotinylated goat anti-avidin (Vector); anti-digoxigenin-FITC (Boehringer) was used for digoxigenin probes.⁹ Chromosome 8 α -satellite added to BCR/ABL probe was detected using CY3-avidin (Amersham). Chromosomes were counterstained with propidium iodide (PI) or diamidino-2-phenylindole (DAPI). Results were analyzed by using a Nikon microscope equipped with a color CCD camera (Hamamatsu Photonics K.K, Japan).

RNA PCR analysis

Bone marrow samples were diluted in an NH_4Cl Tris solution to lyse the red cells and the white cell fraction was pelleted. After one wash in PBS, total RNA was extracted using a previously described method.¹⁰ Two micrograms of total RNA were reverse transcribed according to standard protocols. Nested PCR reactions were performed as described by Hughes *et al.*¹¹ and run on 1.8% agarose gel.

Results

Figure 1 shows partial Q-banded karyotypes of the two reported patients.

In patient #1 all the examined metaphases exhibited only one apparently normal chromosome 21 and 22 and a der(21) due to a t(21;22)(q22;q11) while chromosomes 9 were cytogenetically not involved. Thus a formal diagnosis of CML could not be made on the basis of cytogenetic analysis because Ph chromosome and 9q34 involvement were not present. By using a whole chromosome painting (wcp) probe for chromosome 22, FISH revealed a normal chromosome 22 and 22 sequences at the end of a 21q, as expected (data not shown). Dual color FISH, using BCR-ABL probes, showed a fusion signal at the end of the q of a C-size chromosome that turned out to be a chromosome 9 after re-hybridization of the same metaphase with a chromosome 9 centromeric probe (Figure 2A and B). The other two BCR and ABL alleles were located on normal chromosomes 22 and 9 respectively.

The karyotype of patient 2 was complex and showed a *masked* Ph with involvement of chromosomes 8 and 10 besides 9 and 22.

We suggest: 46,XX,t(8;22;10;9)(q22;q11;q22;q34). The wcp FISH with the 8,22 and 10 specific probes



Figure 1. Partial Q-banded karyotypes of the two reported patients. *Top*: patient #1. The chromosomes 9 are apparently uninvolved. The chromosome 21 on the right shows additional material at the end of the q which turned out to be chromosome 22 derived after FISH analysis was carried out (see text). Only one normal chromosome 22 is present. Karyotype is 45,XX,der(21)t(21;22)(q22;q11), -22. *Bottom*: patient #2. Chromosomes 8,9,10 and 22 involved in the masked Ph translocation. The chromosome 22 on the right is the masked Ph. The rearrangement may be indicated as: t(8;22;10;9)(q22;q11;q22;q34).

confirmed the presence of chromosome 8 material on the der(22), of chromosome 22 on the der(10), and of chromosome 10 material on the der(9) (not shown).

The dual color FISH with BCR/ABL and α -8 probes showed that the BCR/ABL hybrid gene was located on the der(8) (Figure 3). The der(8) can be interpreted as: 8pter→8q22 :: 22q11 :: 9q34→9qter.

Molecular analysis by RT-PCR showed a b3a2 rearrangement in both cases.

Discussion

A small number of CML patients do not show any evidence of a Ph chromosome because complex rearrangements can produce a *masked Ph*. In these cases classic cytogenetic analysis cannot provide complete information concerning the chromosomal aberrations that have occurred. The two patients we report were cytogenetically Ph negative but at the molecular level they showed a typical b3a2 configuration. In the first case only one chromosome 22 was

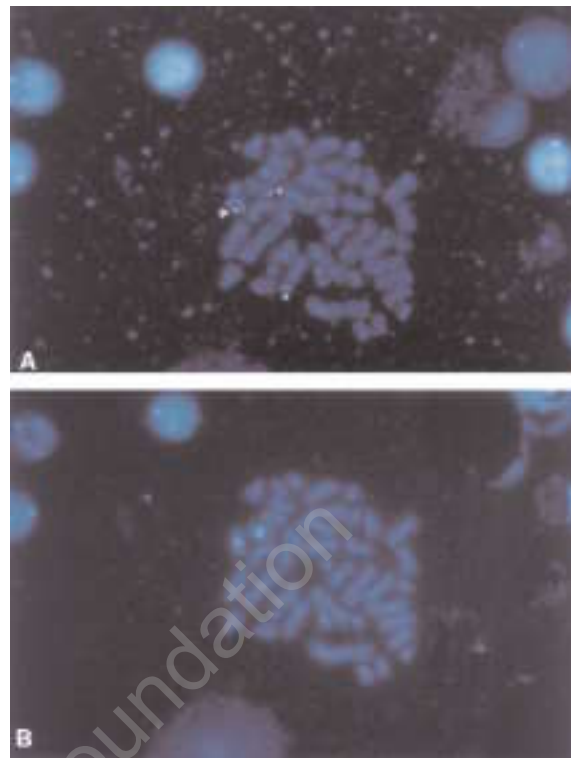


Figure 2. The same metaphase from patient #1 counterstained with DAPI and hybridized with BCR and ABL probes (A) and with centromeric specific sequences of chromosome 9 (B) showing that the BCR/ABL fusion gene is located at the 9q34 band (large arrowhead) whereas the second BCR and ABL alleles are located on the normal chromosomes 9 (small arrowhead) and 22 (small arrowhead) respectively.

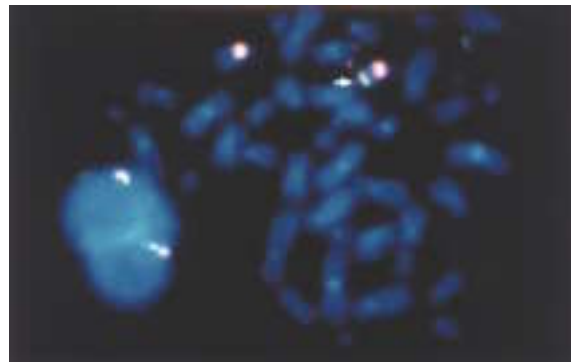


Figure 3. DAPI counterstained metaphase from patient 2 hybridized with chromosome 8 α -satellite probe (large red signal), BCR probe (green signal) and ABL probe (red signal). The arrow shows the der(8) carrying the BCR/ABL fusion gene.

present. FISH revealed that the BCR/ABL hybrid gene was present and located on 9q34 instead of the usual 22q11 band. In the second patient the BCR/ABL gene translocated to the chromosome 8 at the breakpoint involved in the formation of the masked Ph.

The finding of a BCR/ABL gene on 9q34, as

occurred in our first case, was first reported by Hagemeyer *et al.*¹² and afterwards by other authors.¹³⁻²³ It was found both in Ph⁻ patients with apparently normal karyotype and in Ph⁺ patients with standard t(9;22) or with unusual translocations. The BCR/ABL hybrid gene was observed even more rarely on chromosomal bands other than 22q11 or 9q34²⁴⁻²⁸ as in our second case.

The formation mechanisms of these two types of rearrangements are not clear. In the first case we can hypothesize that BCR sequences were inserted within the 5' ABL region giving rise to the typical BCR/ABL hybrid gene. This event requires two breakpoints in the 22q11 band; the 22q11→22qter telomeric segment moves to 21q22, whereas the der(22) carrying the centromeric region, the 21q22 telomeric sequences and the classical Ph appearance, get lost. It is not clear whether these rearrangements arise from one or from two successive events.

As far as patient #2 is concerned we can consider that two successive events occurred. First the variant translocation (9;22;10)(q34;q11;q22) took place leading to the formation of the typical Ph, of the der(9) with additional material translocated from chromosome 10 to 9q34 band and of the der(10) with the 22q11→22qter segment translocated to the 10q22 band. The second event can be considered as an apparently reciprocal translocation between a chromosome 8 and the der(22)t(9;22;10) with a 22q11 breakpoint centromeric to the first one. As a consequence of this second translocation, the BCR/ABL hybrid gene was translocated on the der(8) in the q22 band and the Ph became *masked*. From a clinical point of view, the few reported cases with unusual locations of the BCR/ABL gene seem to indicate these patients have a poor prognosis.¹⁵ Patient #1 showed unusual hematologic features without leukocytosis and with marrow fibrosis and progressive anemia. Early marrow fibrosis in CML usually represents a poor prognostic factor because its occurrence is predictive of the blastic phase.²⁹ Currently (43 months from diagnosis) the patient's general conditions have worsened without hematologic features of blastic transformation. Patient #2 received classic CML therapy (hydroxyurea followed by α -interferon) with satisfactory clinical but not cytogenetic response. Considering that both patients showed a b3a2 rearrangement it can be hypothesized that other events may influence prognosis, i.e. the t(21;22)(q22;q11), the monosomy 22pter→22q11 in the first case; the breakpoints other than 9q34 and 22q11 in the second.

In conclusion, we confirm that the BCR/ABL hybrid gene may be located on chromosomal bands other than 22q11 even if the typical BCR/ABL mRNA is expressed. FISH analysis alone, is able to demonstrate this event which represents a rare Ph variant.

Further cytogenetic, molecular and clinical studies are needed to specify the real frequency of this event and the correlation with prognosis.

Contributions and Acknowledgments

MS formulated the design of the study and wrote the paper. GF performed FISH experiments. RB performed the cytogenetic analyses. ABal and ABac were the clinicians involved in

following the patients and collected the data. MM performed PCR experiments.

The order of the authorship indicated in the paper was a joint decision of the authors.

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Disclosures

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Potential implications for clinical practice

- ◆ Different formation modalities of the Ph may be correlated with different modalities of evolution of the disease.

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