

Transposition of *c-abl* oncogene in a case of masked Ph chromosome duplicated in blastic phase

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Summary. A female with chronic myelocytic leukemia (CML) in blastic phase (BP) showed a masked Ph chromosome that had originated by a translocation between chromosomes 8 and 22, with no obvious involvement of chromosome 9. A duplication of the masked Ph and trisomy 13 were present as additional anomalies. The karyotype on peripheral blood unstimulated cultures was 48,XX,t(8;22)(p12;q11),+13,+der(22)t(8;22)/47,XX,t(8;22)(p12;q11),+der(22)t(8;22). While the duplication of the Ph is a frequent finding in BP of CML, we did not find any other case in the literature with duplication of a masked Ph. In situ hybridization with *c-abl* and *bcr* probes showed that a 3' *bcr* sequence was translocated to the der(8) chromosome, while the *c-abl* oncogene was transposed to the masked Ph.

Introduction

The Philadelphia (Ph) chromosome of chronic myelocytic leukemia (CML) originates from a reciprocal translocation between a chromosome 9 and a 22 in more than 95%, and from variant and complex rearrangements in less than 5% of the cases (Sandberg 1980). The morphology of the Ph is the same in the standard t(9;22) and in most variant translocations, but there are a few cases in which the chromosome derived from the 22 is longer than the normal 22. These are called masked Ph because the Ph is not obvious, but the pattern of the rearrangement is similar to that of complex Ph translocations.

We present a case of masked Ph due to an apparently simple translocation (8;22) in a patient with CML in blastic phase (BP). The Ph was duplicated, and we performed a study of the rearrangement by means of in situ hybridization with probes of the "breaking cluster region" (*bcr*) of chromosome 22 and of the human *c-abl* oncogene.

Case report

M.L.L., a female, was born in 1927. In 1977 an intraductal carcinoma of the breast was diagnosed and she underwent surgical treatment. In 1980 a high WBC count was found incidentally, and she was admitted to the hospital. In November 1980 her clinical examination showed enlargement of the spleen (4 cm below the costal margin). Laboratory data showed a

hemoglobin of 14.7 g/100 ml and WBCs 55 000/mm³ with a differential count of 60% neutrophils, 3% eosinophils, 0 basophils, 12% lymphocytes, 1% monocytes, 9% metamyelocytes, 12% myelocytes, and 3% promyelocytes; the platelet count was 278 000/mm³. The leukocyte alkaline phosphatase (LAP) score was 7 (normal 90 ± 50). The bone marrow was hypercellular and a diagnosis of CML was made. The patient was treated with busulfan with immediate improvement and kept in stable chronic phase until June 1984.

In June 1984 the patient complained of recurrent vomiting and the splenomegaly had reappeared. Her WBC count was 76 000/mm³ and her platelet count 950 000/mm³. The differential count showed 45% neutrophils, 1% eosinophils, 0 basophils, 1% lymphocytes, 0 monocytes, 19% metamyelocytes, 8% myelocytes, 14% promyelocytes, and 12% myeloblasts. The LAP score was 455. She was then treated with several courses of hydroxyurea and 6-mercaptopurine with good results: In September 1984 her spleen was palpable at the costal margin and the WBC were 15 700/mm³ with a normal differential count. However, the thrombocytopenia persisted and reached 240 000 platelets/mm³ in April 1985. In June 1985 a relapse of the blastic phase of the disease was observed with the spleen again enlarged (10 cm below the costal margin), and no improvement was obtained with hydroxyurea and 6-mercaptopurine therapy: the WBC were 300 000/mm³ in July 1985. The patient died of cerebral hemorrhage in August 1985.

Materials and methods

The chromosome analyses were performed with QFQ- and GTG- banding techniques on peripheral blood cultures without mitogens after 24 and 48 h. PHA-stimulated cultures were set up to determine the constitutional karyotype.

In situ hybridization was carried out according to Bartram et al. (1983) using two DNA probes: a human *c-abl* oncogene sequence of 0.65 kb and a human *bcr* sequence of 1.2 kb, which recognizes the 3'-terminal region of *bcr* (Oncogene Science, Inc.). The specific activities of the two probes labeled with tritiated nucleotides by nick translation were 1.3×10^7 cpm/μg for the *c-abl* and 1×10^7 cpm/μg for the *bcr*.

Autoradiography was done with liquid emulsion (Kodak NTB2) and the slides were analyzed after 10–15 days of exposure. Staining for QFQ bands was performed, and the metaphases were observed in double light to analyze the distribution of the grains and the banding pattern simultaneously.

Results

The first chromosome analysis, performed in November 1980 at an early stage of the disease, showed a masked Ph due to a translocation between the short arm of a chromosome 8 and the long arm of a 22, with the breakpoint at band 22q11 as in standard and nonmasked variant Ph chromosomes. No in-

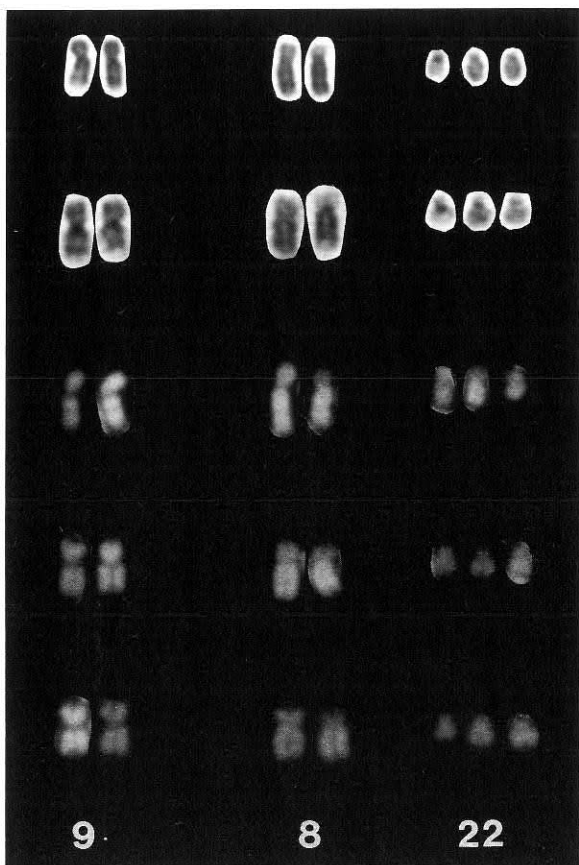


Fig. 1. Cut-out of chromosomes 9, 8, and 22 from five metaphases stained for GTG- and QFQ- bands (June 1985)

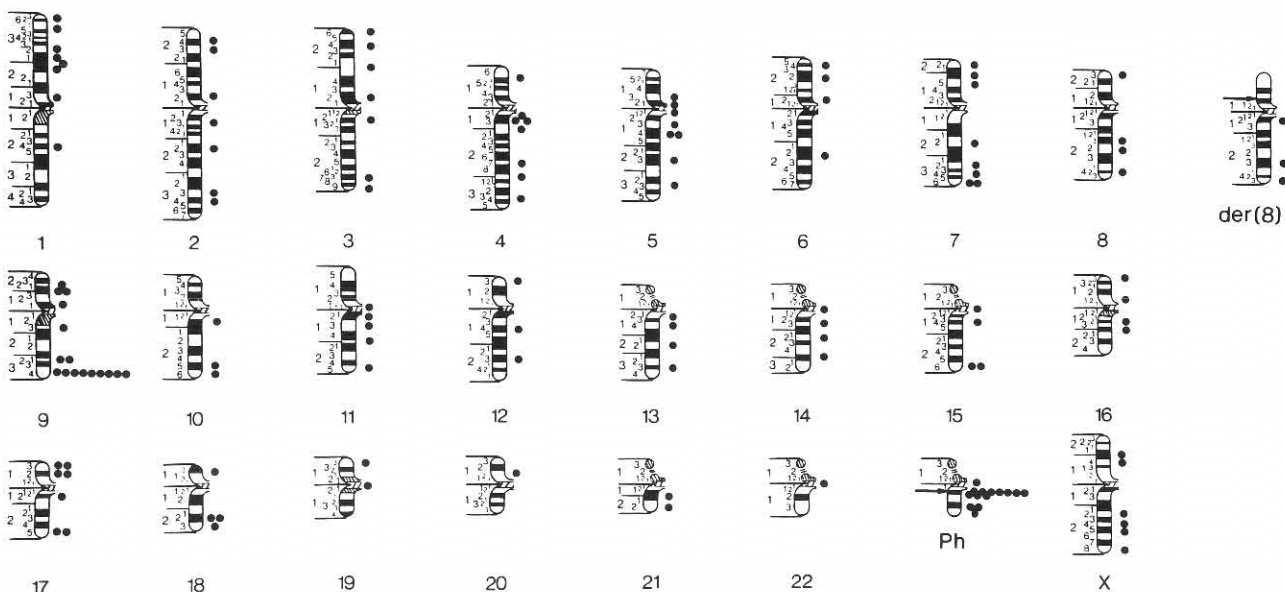


Fig. 2. In situ hybridization with a probe of human *c-abl* oncogene: distribution of the grains along the chromosomes (21 mitoses; background < 2/3 of the grains counted on the genome)

volvement of chromosomes 9 was visible and the karyotype was 46,XX,t(8;22)(p12;q11) in 30 mitoses analyzed. Cells from PHA-stimulated cultures showed a normal karyotype.

In June 1985 unstimulated blood cultures showed two cell lines with the masked Ph and additional anomalies. A trisomy 13 and a duplication of the Ph were present in 35 of 45 mitoses, while the duplication of the Ph alone was present in the other 10 cells: 48,XX,t(8;22)(p12;q11),+13,+der(22)t(8;22)/47,XX,t(8;22)(p12;q11),+der(22)t(8;22) (Fig. 1).

The result of in situ hybridization with the *c-abl* probe was obtained analyzing 21 mitoses with a total of 134 grains. The distribution observed is shown in Fig. 2. Two sites on the chromosomes 9 and der(22) are significantly involved. One of them corresponds to the band 9q34, where the *c-abl* sequence is normally located; the other one is on the long arm of the masked Ph at the level of the junction point with the transposed part of the chromosome 8.

For analysis of the hybridization with the *bcr* probe, 30 mitoses with 66 grains were taken into account. The distribution of these is shown in Fig. 3, and in this case also, two sites are preferentially involved: the band q11 of the normal chromosome 22 and a site near the junction point on the der(8) chromosome.

Discussion

In the literature we found 25 cases of masked Ph (Sasaki et al. 1983; K. Ohyashiki et al. 1987; Coates et al. 1987); 15 of these showed a translocation pattern in which chromosomes 9 and 22 were involved at the same bands of the standard translocation, together with one or more other chromosomes. In the other 10 cases the translocation pattern did not reveal any involvement of chromosome 9, but appeared to be that of a simple translocation between a 22 and another chromosome.

Duplication of the Ph is one of the typical anomalies of the BP of CML (First International Workshop on Chromosomes in Leukaemia 1978). Among the 25 cases of masked Ph from the literature 8 were in BP: 7 of these were reviewed by Ohyashiki K. et al. (1987), and 1 was reported by Sasaki et al. (1983) with the diagnosis of Ph-positive acute myelogenous

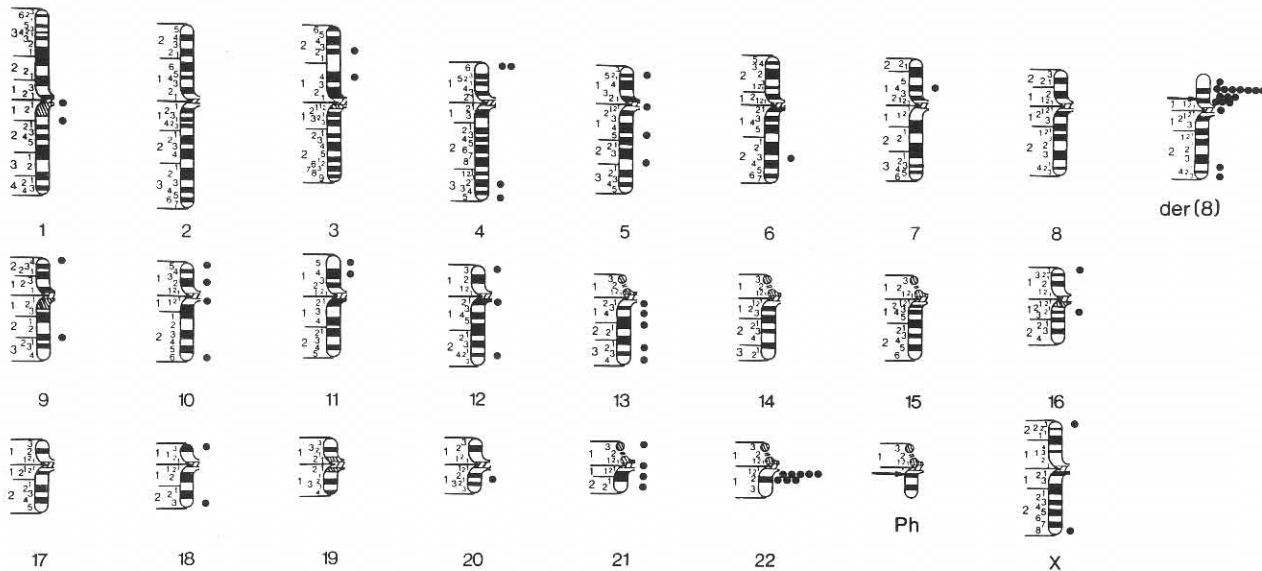


Fig. 3. In situ hybridization with a probe which recognizes the 3' part of human *bcr*: distribution of the grains along the chromosomes (30 mitoses; background < 2/3 of the grains counted on the genome)

leukemia. Numerical and structural anomalies additional to the Ph were present in these eight cases, but in none was there a duplication of the masked Ph. The case reported here shows that duplication of the Ph may be associated with BP also in cases of masked Ph. The translocations that give origin to non-masked Ph chromosomes involve attachment of the terminal segment of the long arm of chromosome 9, containing the *c-abl* oncogene sequence, to the 5' part of the *bcr* region both in standard (9;22) and in variant translocations (Hagemeyer et al. 1984).

Few molecular data on the rearrangements of masked Ph chromosomes are available in the literature. Ohyashiki J. H. et al. (1987) obtained some evidence for the disruption of the *bcr* in one case of masked Ph due to translocation (5;9;22), while a more complete analysis of the fate of the *bcr* and of the *c-abl* oncogene sequences was offered by Hagemeyer et al. (1985) in two cases of masked Ph. One of these was a complex translocation (1;3;5;9;22), while the other one was apparently due to a simple translocation (6;22) with a pattern similar to that of our case. Both cases showed the same *bcr-c-abl* rearrangement on the masked Ph as in standard t(9;22).

Our case is similar to those of the other ten masked Ph chromosomes in the apparent two-chromosome translocation, and our results agree with those obtained by Hagemeyer et al. (1985) in the only other case of this type studied at the molecular level. In fact a 3' *bcr* sequence was translocated onto the der(8) chromosome, while the *c-abl* oncogene was transposed to the masked Ph, thus supporting the view that a *bcr-c-abl* recombination is always required for the development of CML.

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Note added in proof. Since this paper was prepared, molecular data recorded in two further cases of masked Ph chromosome have been reported, by Ohyashiki et al. (Cancer Genet Cytogenet 26:105–115, 1987) and by Alimena et al. (Cancer Genet Cytogenet 27:21–26, 1987). This first case demonstrates that the *bcr* region is disrupted, while the second, which is in fact one of standard translocation (9;22), simply confirms the *bcr-c-abl* rearrangement.

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