Case Report

Cytogenetic and Genome Research

Cytogenet Genome Res 322824
DOI: 10.1159/000322824

Accepted: September 28, 2010
by A. Geurts van Kessel
Published online: ■■■

© S. Karger AG, Basel
PROOF Copy for personal use only
ANY DISTRIBUTION OF THIS ARTICLE WITHOUT WRITTEN CONSENT FROM S. KARGER AG, BASEL IS A VIOLATION OF THE COPYRIGHT.

Novel Variant Ph Translocation t(9;22;11)(q34;q11.2;p15)inv(9)(p13q34) in Chronic Myeloid Leukemia Involving a One-Step Mechanism

C. Belli[a] M.F. Alú[a] G. Alfonso[b] M. Bianchini[a] I. Larripa[a]

[a]Departamento de Genética, Instituto de Investigaciones Hematológicas 'Mariano R. Castex' (IIHEMA), Academia Nacional de Medicina, y [b]Servicio de Hematología, Sanatorio Nuestra Señora del Pilar, Buenos Aires, Argentina

Key Words
Chronic myeloid leukemia · FISH · Mechanism · Variant Philadelphia chromosome

Abstract
Chronic myeloid leukemia (CML) is a clonal malignant disorder of a pluripotent hematopoietic stem cell characterized by the presence of a Philadelphia (Ph) chromosome. Less than 10% of patients present variant Ph chromosomes involving 1 or more additional chromosomes, other than chromosomes 9 and 22, with uncertain prognosis. There are mainly 1- or 2-step mechanisms proposed to explain the genesis of variant Ph chromosomes depending on whether the involved chromosomes are simultaneously broken and rejoined or if a standard t(9;22) occurs first. By combined standard cytogenetic and FISH analysis we detected a novel variant Ph translocation among chromosomes 9, 11 and 22 in a patient with CML without progression to an accelerated phase of the disease after 7 years, with the derivative chromosome 9 also having an acquired pericentric inversion. This novel case illustrates the use of FISH in metaphase to confirm a new rearrangement not previously described in variant Ph formation and that the present karyotype could have originated by a 1-step mechanism with 4 simultaneous breakages without deletion of *ABL1*. Copyright © 2011 S. Karger AG, Basel

Chronic myeloid leukemia (CML) is a clonal malignant disorder of a pluripotent hematopoetic stem cell characterized by the presence of a Philadelphia chromosome (Ph) in more than 90% of patients. The Ph is the product of a reciprocal translocation (9;22)(q34;q11.2) that results in a genetic recombination between the *ABL1* oncogene on chromosome 9 and the breakpoint cluster region *(BCR)* gene on chromosome 22. The resulting *BCR/ABL1* fusion gene encodes a cytoplasmic hybrid protein with abnormal tyrosine kinase activity important in the pathogenesis of this disease [Daley et al., 1990]. Less than 10% of the CML patients present variant or complex translocations of the Ph chromosome involving 1 or more additional chromosomes, other than chromosomes 9 and 22 [Giere et al., 2000; Bennour et al., 2009]. Nevertheless, the *BCR/ABL1* fusion gene can be detected by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR). The prognostic impact of such complex abnormalities is variable and recent reports have demonstrated that determining the genesis of variant Ph translocation by FISH could be important for prognosis assessment [Naumann and Decker, 2003; Reid et al., 2003; El-Zimaity et al., 2004].

C.B. and M.F.A. contributed equally to the manuscript.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2011 S. Karger AG, Basel
1424–8581/11/0000–0000$38.00/0

Accessible online at:
www.karger.com/cgr

Carolina Belli
Departamento de Genética, Instituto de Investigaciones Hematológicas 'Mariano R. Castex' (IIHEMA), Academia Nacional de Medicina, Pacheco de Melo 3081
Buenos Aires 1425 (Argentina)
Tel. +54 11 4805 8803, Fax +54 11 4803 9475, E-Mail cbelli@hematologia.anm.edu.ar

By combined standard cytogenetic and FISH analysis we detected a novel variant translocation among chromosomes 9, 11 and 22, the derivative chromosome 9 also showing an acquired pericentric inversion.

Patient and Methods

Case History

In December 2002 a 68-year-old woman presented spontaneous hematomas without any other pathological signs in her physical examination. The hematological parameters were: hemoglobin 14.9 g/dl, platelets 600 $\times$ 10^3/μl and white blood cells (WBC) 34 $\times$ 10^3/μl with 5% myelocytes, 5% bands, 68% neutrophils, 2% eosinophils, 7% basophils and 13% lymphocytes. Bone marrow (BM) biopsy revealed hypercellular marrow, presence of 3 hematopoietic cell lines, predominantly represented by myeloid cells, consistent with chronic-phase CML. Molecular, cytogenetic and FISH studies were performed detecting the *BCR/ABL1* fusion gene (p210b2a2) and a Ph variant rearrangement among chromosomes 9, 22 and 11. Therapy was initiated with oral hydroxyurea (HU) (1 g/day) followed by interferon-γ (5 $\times$ 10^6 U/m^2/day). After 4 months of treatment, the patient had motor-sensorial neuropathies by electromyography. In October 2003, she started with imatinib (IM) (400 mg/day) that was discontinued after 7 months because of intolerance related to arthralgia and an extensive pemphigus-like dermatological reaction sensitive to IM administration. In July 2004 she returned to oral HU (1–1.5 g/day) with partial hematological response (hemoglobin 14.9 g/dl, platelets 650 $\times$ 10^3/μl and WBC 12.2 $\times$ 10^3/μl with 2% myelocytes, 84% neutrophils, 2% eosinophils, 2% basophils and 14% lymphocytes). When she was admitted to our center in August 2006 cytogenetic analysis confirmed the original karyotype and FISH analysis performed on BM revealed 98% nuclei positive for the *BCR/ABL1* fusion signal. Due to her intolerance background, a second generation TKI Dasatinib (50 mg twice/day) was proposed that was started in July 2008 [Hochhaus et al., 2007; Shah et al., 2010] without adherence to treatment for diarrhea and abdominal ache. Therefore, in February 2009 she decided to make a dose reduction to 50 mg/day, though she was advised about a non-response. After 8 months the patient shows complete hematological response (last control: hemoglobin 11.4 g/dl, platelets 109 $\times$ 10^3/μl and WBC 4.3 $\times$ 10^3/μl with 45% neutrophils, 2% eosinophils, 1% basophils, 3% monocytes and 49% lymphocytes), though she has not reached molecular response (*BCR-ABL1/ABL1* of 23%) quantified by real-time PCR (Molecular MD, TaqMan Method).

Chromosome Banding and FISH

G-, reverse DAPI and DAPI banding were done on metaphases from unstimulated BM cultures. FISH was performed with a locus-specific (LSI) BCR/ABL extra signal (ES) dual-color probe (Abbott-Vysis, Downers Grove, Ill., USA) and chromosome painting (WCP) probes for chromosomes 22 and 11 (Cambio, Cambridge, UK). Furthermore, probes for the telomere of 11p (Telvysion Probes, Abbott-Vysis) and LSI p16 (9p21)/CEP9 (Abbott-Vysis) were applied.

Results

G-, reverse DAPI and DAPI banding on metaphases from unstimulated BM cultures revealed the karyotype 46,XX,t(9;22;11)(q34;q11.2;p15)inv(9)(p13q34) (fig. 1). A normal G-banding karyotype found in 72-h stimulated lymphocyte cultures confirmed that the pericentric inversion is not constitutional (online suppl. fig. 1; for all online supplement material see www.karger.com/doi/10.1159/000322824).

FISH techniques were applied to confirm the acquired karyotype (figs. 1, 2). The LSI BCR/ABL ES dual-color probe showed the ES on the inverted der(9) and a fusion signal on der(22) (fig. 1, 2A-ii, 2B-ii; online suppl. fig. 2A). Using whole chromosome painting probes for chromosomes 22 and 11 (fig. 2C-ii; online suppl. fig. 2B, C) confirmed the presence of chromosome 22 material at the end of 11p (fig. 2C-iii; online suppl. fig. 2C). Although there was no evidence of chromosome 11 material on the abnormal der(9) (online suppl. fig. 2B), a probe for the telomere of 11p hybridized on the remaining short arm of the inverted der(9) (fig. 2A-iv, 2C-iv; online suppl. fig. 2D). The pericentric inversion was confirmed using the LSI p16 (9p21)/CEP9 probe (fig. 2A-iii; online suppl. fig. 2E).

Discussion

Variant or complex Philadelphia chromosomes are present in 3–8% of the CML cases and more than 400 have been reported involving almost all chromosomes [Giere et al., 2000; Bennour et al., 2009; Mitelman et al., 2010]. The chromosomes most frequently involved are 1, 2, 3, 4, 7, 11, 12 and 17. Some studies have suggested that patients with variant Ph chromosomes may have an adverse prognosis, but others reported no prognostic effect [Naumann and Decker, 2003; Reid et al., 2003; El-Zimaity et al., 2004; Gorosu et al., 2007; Bennour et al., 2009].

Complex translocation t(9;22;11)(q34;q11.2;p15) has been previously published in 7 patients [Giere et al., 2000; Naumann and Decker, 2003; Mitelman et al., 2010] and 3 cases showed variant translocations involving the short arm of chromosome 9 [Loncarevic et al., 2002; Cianciulli et al., 2004; Gorusu et al., 2007; Mitelman et al., 2010]. Also, there is another report of a t(9;11;22)(p13;p15;q11) where the distal end of the long arm of chromosome 22 was translocated to the short arm of chromosome 9, and the short arm of chromosome 9 to the short arm of chromosome 11 [Yao et al., 1986]. Therefore, the present case

CGR322824.indd 2 09.12.2010 11:01:30

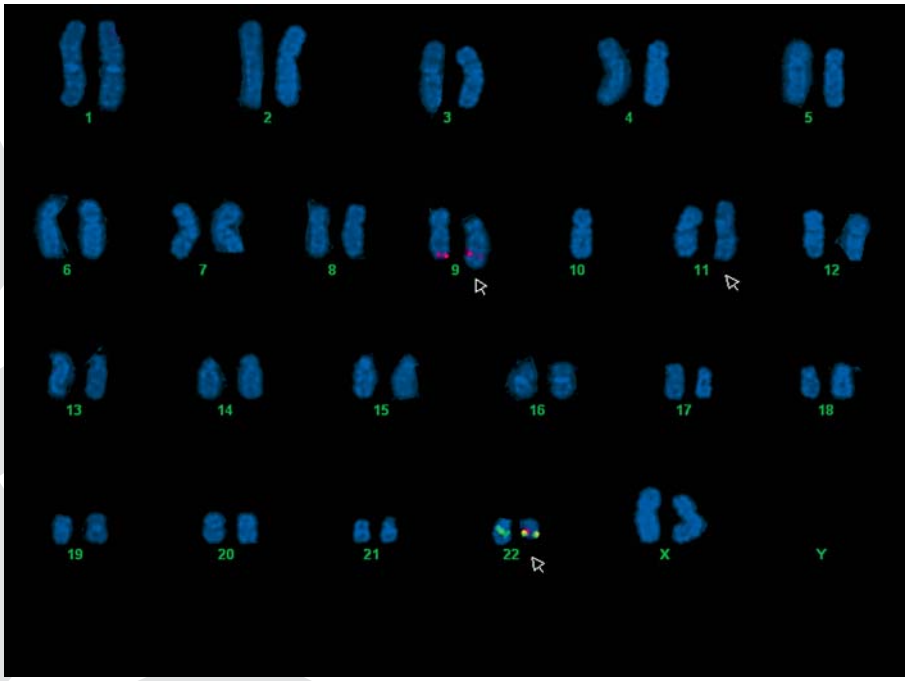

Fig. 1. Karyogram of the malignant clone after FISH with the LSI-ES BCR/ABL probe and counterstaining of chromosomes with DAPI. The 3 derivative chromosomes are indicated by white arrows. The *ABL1* signal (red) is located on the normal chromosome 9 and the ES ABL on the der(9), *BCR* signal (green) on the normal chromosome 22 and the *BCR/ABL1* fusion signal on der(22). In this metaphase one chromosome 10 is randomly missing.

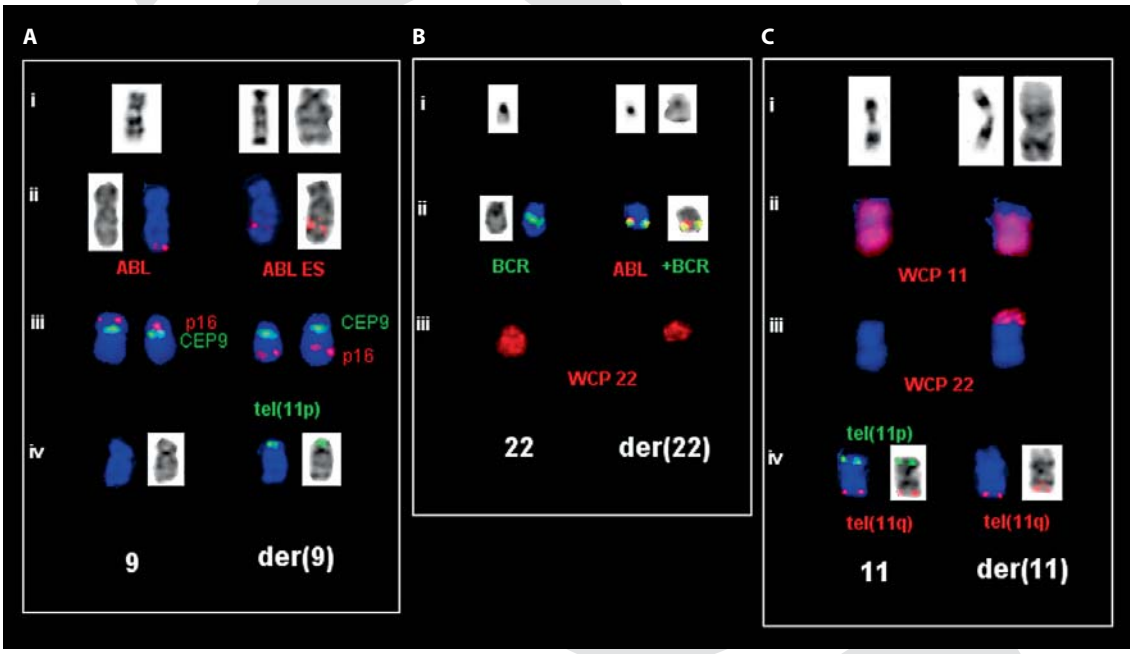

Fig. 2. Image of conventional cytogenetic, FISH and reverse DAPI banding analysis. **A** Normal chromosome 9 (left) and der(9)inv(9)(p13q34)t(9;22;11) (right): (i) G-Banding; (ii) FISH with ABL (red) on the normal chromosome 9 and the ES ABL on der(9); (iii) FISH with LSI p16 (red) and CEP9 (green) showing the pericentric inversion on the right side; (iv) FISH using telomere 11p probe (green) confirmed its presence on the remaining short arm of the der(9). **B** Normal chromosome 22 (left) and der(22)t(9;22;11) (right): (i) G-banding; (ii) *BCR* signal (green) on the normal chromosome 22 and the fusion signal *BCR/ABL1* on der(22); (iii) WCP 22 analysis revealed the normal chromosome 22 and the der(22) Ph chromosome. **C** Normal chromosome 11 (left) and der(11)t(9;22;11) (right): (i) G-banding; (ii) WCP 11 analysis revealed the normal chromosome 11 and a WCP 11-negative region on der(11); (iii) WCP 22 analysis revealed the presence of chromosome 22 material at the end of 11p; (iv) FISH using telomere probes for 11p (green) and 11q (red) confirmed both signals on normal chromosome 11 and only the tel11q on the der(11).

CGR322824.indd 3
09.12.2010 11:01:31

is, to our knowledge, the first report of a variant Ph among chromosomes 9, 22 and 11 where the distal end of the short arm of der(9) was rearranged to its long arm, resembling a pericentric inversion of the der(9).

There are mainly 1- or 2-step mechanisms proposed to explain the genesis of variant Ph chromosomes depending on whether the involved chromosomes are simultaneously broken and rejoined or if a standard t(9;22) occurs first [Naumann and Decker, 2003; Gorusu et al., 2007]. Also, a multi-step mechanism was described for more complex translocations [Bennour et al., 2009].

Gorusu et al. [2007] used the double fusion (DF) BCR/ABL probe (Abbott-Vysis) to establish the genesis of variant Ph chromosomes by FISH. However, using the LSI-ES BCR/ABL probe, we can conclude that the rearrangement reported here arose by a 1-step mechanism because the ES for *ABL1* was on the inverted der(9), instead of being on the der(11). Naumann and Decker [2003] observed this last *ABL1*-ES pattern in a t(9;22;11) by a 2-step mechanism. The breakage sites of the pericentric inversion of der(9) were evidenced by the ABL-ES probe and by the tel11p probe. The inversion could have occurred independently or simultaneously. However, the absence of WCP11 signal on the inverted der(9) next to the remaining ES-ABL (online suppl. fig. 2B) suggests that the translocation and the inversion could have occurred simultaneously.

One-step rearrangements are predominant in different series [Gorusu et al., 2007; Richebourg et al., 2008] and are associated with better prognosis and a lower prevalence of *ABL1* deletions [Gorusu et al., 2007; So et al., 2008; Bennour et al., 2009]. Deletions of the *ABL1* or *BCR* locus are more prevalent in variant translocations and are related to worse therapeutic response even when treated with a superior regimen such as IM [Reid et al., 2003; El-Zimaity et al., 2004; Gorusu et al., 2007]. Four-break rearrangements in the formation of complex translocations were also related to worse prognosis, similar to 2-step mechanisms, and resemble additional genetic changes to the Ph chromosome [Gorusu et al., 2007; So et al., 2008]. However, others authors suggest that these mechanisms of genesis do not correlate with an *ABL1* or *BCR* deletion status, clonal evolution or response to IM therapy [Richebourg et al., 2008]. The observed differences among these published series could be related to the use of WCP probes or different LSI FISH probes for *BCR/ABL1*, the retrospective characteristic of these studies including a low number of analyzed patients or different regimens of treatments.

This novel case illustrates the use of FISH to confirm a new rearrangement not previously described in variant Philadelphia chromosome formation. The present karyotype could have involved a 1-step mechanism with 4 simultaneous breakages without deletion of *ABL1*. Therefore, the acquired karyotype might not be considered as a cytogenetic 'warning' feature different from the standard translocation (9;22)(q34;q11.2) and similar to another reported case involving the same 9p13 band in a variant translocation [Cianciulli et al., 2004]. The patient is intolerant to IM (the gold-standard first-line treatment), not suitable for hematopoietic stem cell transplantation, intolerant to interferon-γ (which are the recommended options in case of IM intolerance by Baccarani et al. [2006]), partially responder to HU and also intolerant to the recommended doses of dasatinib [Shah et al., 2010]. However, she continues in a chronic phase of the disease without progression after 7 years of diagnosis with complete hematological response.

In conclusion, the use of FISH in metaphase cells can be crucial to investigate the particular mechanism of development of complex translocations in CML and whether variant translocations are or are not accompanied by *ABL1* deletion. These analyses performed in a larger number of cases with available clinical data will provide insights into tumor development in CML to access clinical implications.

Acknowledgements

The authors would like to thank Fernanda Bergonzi for providing probes for FISH analyses. This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT).

References

Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, et al: Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European Leukemia Net. Blood 108:1809–1820 (2006).

Bennour A, Sennana H, Laatiri M, Elloumi M, Khelif A, Saad A: Molecular cytogenetic characterization of variant Philadelphia translocation in chronic myeloid leukemia: genesis and deletion of derivative chromosome 9. Cancer Genet Cytogenet 194:30–37 (2009).

Cianciulli AM, Marzano R, Merola R, Orlandi G, Petti MC, et al: Complex variant Philadelphia translocation involving the short arm of chromosome 9 in a case of chronic myeloid leukemia. Haematologica 89:ECR37 (2004).

CGR322824.indd 4 09.12.2010 11:01:32

Daley G, Van Etten R, Baltimore D: Induction of chronic myelogenous leukemia in mice by the P210*bcr/abl* gene of the Philadelphia chromosome. Science 247:824–830 (1990).
El-Zimaity MM, Kantarjian H, Talpaz M, O'Brien S, Giles F, et al: Results of imatinib mesylate therapy in chronic myelogenous leukaemia with variant Philadelphia chromosome. Br J Haematol 125:187–195 (2004).
Giere I, Migliorini AM, Bengió R, Arias D, Slavutsky I, Larripa I: Cytogenetic and molecular studies of variant Ph' translocations. Haematologica 85:435–437 (2000).
Gorosu M, Benn P, Li Z, Fang M: On the genesis and prognosis of variant translocation in chronic myeloid leukemia. Cancer Genet Cytogenet 173:97–106 (2007).
Hochhaus A, Kantarjian H, Baccarani M, Lipton JH, Apperley JF, et al: Dasatinib induces notable hematologic and cytogenetic responses in chronic-phase chronic myeloid leukemia after failure of imatinib therapy. Blood 109: 2303–2309 (2007).
Loncarevic I, Römer J, Starke H, Heller A, Bleck C, et al: Heterogenic molecular basis for loss of *ABL1-BCR* transcription: deletions in der(9)t(9;22) and variants of standard t(9;22) in *BCR-ABL1*-positive chronic myeloid leukemia. Genes Chromosomes Cancer 34:193–200 (2002).
Mitelman F, Johanson B, Mertens F (eds): Mitelman Database of Chromosome Aberrations in Cancer. http://www.cgap.ncbi.nih.gov/Chromosomes/Mitelman (2010).
Naumann S, Decker HJ: Genesis of variant Philadelphia chromosome translocations in chronic myelocytic leukemia. Cancer Genet Cytogenet 147:18–22 (2003).
Reid AG, Huntly BJ, Grace C, Green AR, Nacheva EP: Survival implications of molecular heterogeneity in variant Philadelphia-positive chronic myeloid leukaemia. Br J Haematol 121:419–427 (2003).
Richebourg S, Eclache V, Perot C, Portnoi MF, Van den Akker J, et al: Mechanisms of genesis of variant translocation in chronic myeloid leukemia are not correlated with *ABL1* or *BCR* deletion status or response to imatinib therapy. Cancer Genet Cytogenet 182: 95–102 (2008).
Shah NP, Kim DW, Kantarjian H, Rousselot P, Llacer PE, et al: Potent, transient inhibition of BCR-ABL with dasatinib 100 mg daily achieves rapid and durable cytogenetic responses and high transformation-free survival rates in chronic phase chronic myeloid leukemia patients with resistance, suboptimal response or intolerance to imatinib. Haematologica 95:232–240 (2010).
So CC, Wan TS, Yip SF, Chan LC: A dual colour dual fusion fluorescence in situ hybridisation study on the genesis of complex variant translocations in chronic myelogenous leukaemia. Oncol Rep 19:1181–1184 (2008).
Yao E, Sadamori N, Tomonaga Y, Matsunaga M, Tagawa M, et al: A new complex translocation in chronic myelogenous leukemia with monoblastic crisis. Cancer Genet Cytogenet 19:357–359 (1986).

PROOF

CGR322824.indd 5 09.12.2010 11:01:32