Cancer Genetics 205 (2012) 124–127

Cancer Genetics

BRIEF COMMUNICATION

The first case of Philadelphia chromosome-negative acute promyelocytic leukemia following imatinib for chronic myelogenous leukemia

Jad J. Wakim [a,*], Carlos A. Tirado [b], Jonathan Dowell [c,a], Weina Chen [d]
[a] *Division of Hematology and Oncology, University of Texas Southwestern Medical Center, Dallas, TX;* [b] *Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX;* [c] *Veterans Affairs, North Texas Healthcare System, Dallas, TX;* [d] *Ameripath/Quest Diagnostics, Dallas, TX*

In chronic myelogenous leukemia, chromosomal abnormalities in Philadelphia-negative cells are rare and usually transient, but can infrequently lead to myelodysplastic syndrome and/or acute myeloid leukemia. We report an 82-ear-old patient with an 11-year history of chronic myelogenous leukemia, in complete cytogenetic response, who developed Philadelphia-negative t(15;17)/PMLRARA acute promyelocytic leukemia. This isolated case reaffirms several important clinicopathologic and biologic aspects of chronic myelogenous leukemia, and sheds a unique light on its Philadelphia-negative hematopoiesis. It also underlines the importance of continued cytogenetic monitoring of patients in complete cytogenetic response for the emergence of new chromosomal abnormalities.

Keywords Chronic myeloid leukemia, chromosomal abnormalities, Philadelphia chromosome-negative, acute promyelocytic leukemia, imatinib

Chronic myelogenous leukemia (CML) is characterized by the reciprocal translocation t(9;22)(q34;q11.2) that results in the *BCR-ABL1* fusion gene and a chimeric protein with enhanced tyrosine kinase activity (1). Imatinib and other tyrosine kinase inhibitors (TKIs) have transformed the natural history of this typically triphasic disease (2).

Two categories of additional chromosomal abnormalities (CA) can occur in patients who receive imatinib for CML. CA that occur in Philadelphia (Ph)-positive cells define clonal evolution, a sign of disease progression to accelerated or blast phases (3). On the other hand, CA in Ph-negative cells are scarce and usually transient, but rarely associated with the development of myelodysplastic syndrome (MDS) and/or acute myeloid leukemia (AML) (4,5).

Herein, we present the first case of Ph-negative t(15;17)/*PML-RARA* acute promyelocytic leukemia (APL) in a patient who is in complete cytogenetic response (CCR) 11 years after the initial diagnosis of CML.

Received October 6, 2011; received in revised form January 10, 2012; accepted January 10, 2012.
* Corresponding author.
E-mail address: jad.wakim@phhs.org

Case report

An 81-year-old man presented to our institution in April 2010 with APL. He had been diagnosed 11 years earlier, in 1999, with t(9;22)(q34;q11.2)/*BCR-ABL1* CML, which was treated with interferon for 9 months, followed by imatinib 400 mg orally daily with complete hematologic and cytogenetic responses. He remained on imatinib until November 2007, when it was discontinued because of gastrointestinal intolerance. A bone marrow (BM) biopsy at that time showed no evidence of a myeloproliferative disorder and fluorescence in situ hybridization (FISH) showed no evidence of *BCR-ABL1*. The patient remained in CCR while off therapy, and was being monitored by FISH on peripheral blood every 4 months.

In April 2010, he presented with a white blood count of 3.8×10^9/L and 12% blasts. Immunophenotyping of BM aspirate showed 24–27% aberrant myeloblasts/promyelocytes. BM biopsy, cytogenetics, and FISH confirmed APL with the classic translocation t(15;17)(q24;q21.1)/*PML-RARA* (6), with no evidence of *BCR-ABL1* by FISH in 1,000 interphase nuclei examined. Before all-trans retinoic acid (ATRA) and idarubicin were started, the patient developed a left lower extremity deep vein thrombosis, received intravenous

2210-7762/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.cancergen.2012.01.006

heparin, and developed bilateral subdural hematomas. His platelet count was 67×10^9/L, fibrinogen 273 mg/dL (range: 200−633), D-Dimer >20 μg/mL (range: 0.01−0.55), partial thrombopolastin time (PTT) 34.3 seconds (range: 22.8−35.6), prothrombin time (PT) 20.8 seconds (range: 11.8−15.4), and International normalized ratio (INR) 1.7. His heparin was discontinued, but he died the following day.

Materials and methods

Cytogenetic analysis

The unstimulated aspirate sample was cultivated according to conventional cytogenetic techniques. A total of 20 G-banded (trypsin and Wright-Giemsa) metaphases were analyzed and described according to the International System for Human Cytogenetic Nomenclature (2009).

FISH analyses

FISH analysis using the Vysis LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe from Abbott Molecular (Des Plaines, IL) was performed on 1,000 interphase nuclei, which gives a sensitivity approaching that of polymerase chain reaction (PCR) studies. FISH using the Vysis LSI PML/RARA Dual Color, Dual Fusion Translocation Probe (Abbott Molecular) was performed to detect the translocation between the *PML* gene on chromosome 15q24 (previously assigned to band 15q22) and the *RARA* gene on chromosome 17q21.1 (6).

Morphology and immunophenotypic analysis

BM and peripheral blood smears were prepared and stained with Wright-Giemsa stain. The specimens were immunophenotyped using a 4-color FACSCalibur flow cytometer. Data were collected with CellQuest software (Becton Dickinson, San Jose, CA) and analyzed using cluster analysis with Paint-a-Gate Software (Becton Dickinson).

Results

In April 2010, the karyotype revealed a t(15;17) in all but one metaphase (Figure 1A). This was described as: 46,XY,t(15;17)(q24;q21.1)[19]/46,XY[1].

FISH analyses showed no evidence of *BCR-ABL1* in 1,000 interphase nuclei examined, while revealing a *PML-RARA* gene rearrangement in 94.5% of the interphase nuclei examined (Figure 1B).

Peripheral blood and BM aspirate examinations showed a predominant population of large myeloblasts/promyeloblasts with variably irregular nuclei-dispersed chromatin, variably prominent nucleoli, and scant to moderate amounts of cytoplasm. A few immature myeloid cells contained multiple Auer rods (Figure 2A).

Immunophenotypic analysis on marrow samples revealed a large population of aberrant myeloblasts/promyelocytes with the following immunophenotype: CD34(−), CD13(+), CD15(+), CD33(+), CD38(+), CD45(+), CD56(−), CD117(+), HLA-DR(−), myeloperoxidase(+), TdT(−), and other myeloid and lymphoid markers (predominantly −) (Figure 2B). These findings were consistent with APL.

Discussion

CA in Ph-negative cells are observed in less than 10% of CML patients following treatment with interferon or imatinib—the most common being monosomy 7, trisomy 8, and loss of chromosome Y (5). These abnormalities are usually transient, but approximately 2% of affected patients develop MDS and/or AML, especially those carrying monosomy 7 (5,7). We are the first to report the emergence of t(15;17)/*PML-RARA* APL in Ph-negative metaphases, which occurred 11 years after the initial diagnosis of CML. Although APL could conceivably occur de novo in any patient with CML, this has not been previously described in the literature, and more plausibly represents an additional chromosomal abnormality worthy of proper analysis. To our knowledge, this case is unique and reaffirms several important clinicopathologic and biologic aspects of CML.

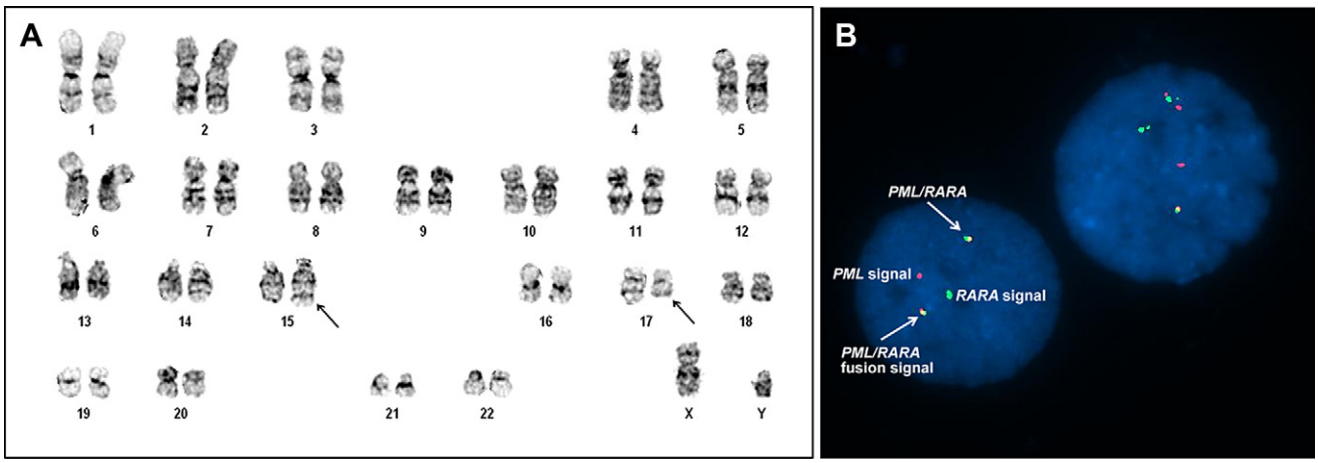

Figure 1 (A) Karyogram depicts a typical translocation t(15;17). (B) Fluorescence in situ hybridization (FISH) shows *PML-RARA* rearrangement in 94.5% of the interphase nuclei examined.

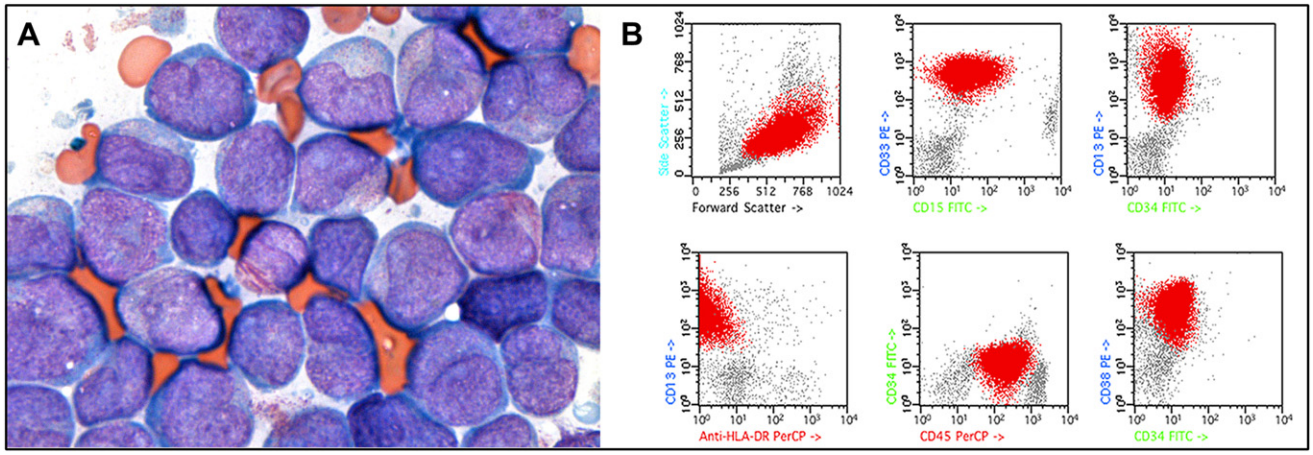

Figure 2 (A) Abundant myeloblasts and promyeloblasts present in BM aspirate. Rare promyelocytes containing multiple Auer rods. (B) Flow cytometric analysis reveals immature myeloid cells/promelocytes (in red) expressing CD13, CD15, CD33, CD38, CD45, and lacking CD34 and HLA-DR.

The possibility of promyelocytic blast crisis of CML was first ruled out when FISH showed no evidence of *BCR-ABL1* fusion in 1,000 interphase nuclei examined, which gives a sensitivity approaching that of PCR studies. The t(15;17)/*PML-RARA* clearly represented a new chromosomal abnormality in Ph-negative cells instead of a clonal evolution of CML into a myeloid blast phase.

To understand this rare event, we examined our patient's findings in light of previously proposed explanations for the emergence of CA in Ph-negative cells of treated CML patients. The first proposition entails a two-step pathogenesis of CML where the Ph-negative stem cell accumulates CA and the Ph chromosome represents a "second hit." The selective suppression of the Ph-positive clone by imatinib allows the Ph-negative clones expressing the "first hit" to emerge. This theory is supported by the presence of *BCR-ABL1* transcripts in non-leukemic individuals, stressing that a "first hit" is needed in order for *BCR-ABL1* to cause CML (8). The second explanation describes a genetically damaged hematopoiesis that produces multiple abnormal clones, which remain masked until the eradication of the dominant Ph-positive clone by imatinib (9). In our patient, these theories would imply the presence, from the start, of two competing clones: a t(9;22) clone suppressing a t(15;17) Ph-negative clone, suggesting that CML inhibits the development of APL, which is unlikely. The third and perhaps most plausible proposition implicates imatinib in the emergence of CA, because its target, *ABL1*, has been shown to be involved in DNA repair (10). In fact, loss of chromosome Y, a common chromosome abnormality in Ph-negative cells, seems to be an indicator of genetic instability (11). In this scenario, the hematopoiesis of a patient with imatinib-induced CCR would rely on a restricted group of Ph-negative stem cells riddled with genetic instability and prone to the emergence of new CA. This would be especially true in patients with long-standing CCR, such as our patient.

In conclusion, the age of TKIs has opened a window of opportunity for scientists to study and better understand Ph-negative hematopoiesis in CML patients. Those in CCR should still be monitored by cytogenetics, because they risk developing additional CA. These events are usually transient, but they rarely lead to MDS and/or AML. The occurrence of APL is so far unique to our case, but with the contemporary existence of CML patients in long-standing CCR, other cases can be expected.

Acknowledgment

The authors thank Rolando Garcia (Cytogenetics Laboratory, University of Texas Southwestern Medical Center, Dallas, TX) for his technical support with the figures.

References

1. Faderl S, Talpaz M, Estrov Z, et al. The biology of chronic myeloid leukemia. N Engl J Med 1999;341(3):164–172.
2. Goldman JM, Melo JV. Chronic myeloid leukemia—advances in biology and new approaches to treatment. N Engl J Med 2003; 349(15):1451–1464.
3. Kantarjian HM, Dixon D, Keating MJ, et al. Characteristics of accelerated disease in chronic myelogenous leukemia. Cancer 1988;61(7):1441–1446.
4. Kovitz C, Kantarjian H, Garcia-Manero G, et al. Myelodysplastic syndromes and acute leukemia developing after imatinib mesylate therapy for chronic myeloid leukemia. Blood 2006;108(8): 2811–2813.
5. Jabbour E, Kantarjian HM, Abruzzo LV, et al. Chromosomal abnormalities in Philadelphia chromosome negative metaphases appearing during imatinib mesylate therapy in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Blood 2007;110(8):2991–2995.
6. Stock AD, Dennis TR, Spallone PA. Precise localization by microdissection/reverse ISH and FISH of the t(15;17)(q24;q21.1) chromosomal breakpoints associated with acute promyelocytic leukemia. Cancer Genet Cytogenet 2000;119(1):15–17.
7. Schafhausen P, Dierlamm J, Bokemeyer C, et al. Development of AML with t(8;21)(q22;q22) and RUNX1-RUNX1T1 fusion following Philadelphia-negative clonal evolution during treatment of CML with Imatinib. Cancer Genet Cytogenet 2009;189(1): 63–67.

8. Bose S, Deininger M, Gora-Tybor J, et al. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. Blood 1998;92(9): 3362–3367.
9. Bumm T, Muller C, Al-Ali HK, et al. Emergence of clonal cytogenetic abnormalities in Ph– cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. Blood 2003;101(5): 1941–1949.
10. Gong JG, Costanzo A, Yang HQ, et al. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature 1999;399(6738):806–809.
11. Wiktor A, Rybicki BA, Piao ZS, et al. Clinical significance of Y chromosome loss in hematologic disease. Genes Chromosomes Cancer 2000;27(1):11–16.