ELSEVIER

Cancer Genetics and Cytogenetics 161 (2005) 70–73

Cancer Genetics and Cytogenetics

Short communication

Variant acute promyelocytic leukemia translocation (15;17) originating from two subsequent balanced translocations involving the same chromosomes 15 and 17 while preserving the *PML/RARA* fusion

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Received 7 December 2004; received in revised form 5 January 2005; accepted 10 January 2005

Abstract Fluorescence in situ hybridization (FISH) analysis of the bone marrow of a 24-year-old man diagnosed with acute promyelocytic leukemia (APL) revealed a variant pattern with one fusion signal instead of the typical two fusions expected with the probe set used. The combined FISH and conventional chromosome analyses suggested that two subsequent translocations had occurred in this patient involving the same chromosomes 15 and 17. As the prognostic outcome in APL is strictly associated with the presence of a *PML/RARA* fusion, it is useful and necessary to perform both cytogenetic and FISH analyses of a variant t(15;17) to determine the status of the *PML/RARA* fusion.

1. Introduction

Acute promyelocytic leukemia (APL) is a myeloid disorder characterized by the specific t(15;17)(q22;q21) or by its variants [1]. The disease presents with less dramatic leukocytosis and thrombocytopenia than other acute leukemias; however, it has a lower remission rate and higher morbidity than most of the other acute leukemias when treated with conventional chemotherapy [2].

We present the case of a 24-year-old man diagnosed with APL. Fluorescence in situ hybridization (FISH) analysis of his bone marrow revealed a variant fusion pattern showing two green *PML* signals, two red *RARA* signals, and only one yellow PML/RARA fusion signal. To better understand the origin of this variant pattern, we performed a G-banded chromosome study, which showed the following complex karyotype: 46,XY,del(15)(q15q22),der(17)t(15;17)(q22;q21)t(15;17)(q15;q21). The combined FISH and conventional chromosome analyses suggested that two subsequent translocations had occurred in this patient involving the same chromosomes 15 and 17. The first event resulted in the typical t(15;17)(q22;q21). The second event caused the separation of the *RARA/PML* fusion, which typically occurs on the derivative chromosome 17 while preserving the *PML/RARA* fusion on the derivative chromosome 15.

Although conventional cytogenetics did not reveal any cells with the typical t(15;17), FISH detected the two-fusion signal pattern typically seen in the reciprocal t(15;17) in 5% of interphase cells, supporting the occurrence of two subsequent translocation events. According to previous reports in the literature, the prognostic course of complex and variant t(15;17), in which the *PML/RARA* fusion on the derivative chromosome 15 is still intact, as in our present case, does not differ from the course observed for the typical t(15;17) [2–4]. In fact, the patient responded well to the all-*trans*-retinoic acid (ATRA) treatment and is presently in remission.

2. Case report

A 24-year-old man presented with pancytopenia and severe headaches before hospitalization. Computed tomography scan diagnosed multiple intracranial hemorrhages. A bone marrow biopsy was performed, and the diagnosis of APL was made.

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0165-4608/05/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cancergencyto.2005.01.005

The patient received three doses of chemotherapy with idarubicin at dose of 60 mg/m^2 daily, as well as ATRA. He was placed into a poor prognosis category because of his low platelet count and his high white blood cell count at presentation. However, he responded well to treatment and is presently in consolidated chemotherapy with idarubicin. He will also receive maintenance therapy, which will include ATRA, with methotrexate, and 6-mercaptopurine.

3. Materials and methods

3.1. FISH

FISH studies were performed using the dual-color, dual-fusion probe *PML/RARA* (Vysis, Downers Grove, IL) following the manufacturer's instructions. Cells were analyzed using a BX51/BX52 Olympus fluorescence microscope (Exfo America; Olympus, Richardson, TX) equipped with CytoVision Probe Software (Applied Imaging, Santa Clara, CA). Selected images were captured using a CCD camera (Sensys; Photometrics, Tucson, AZ).

3.2. Chromosome analysis

To better characterize the variant pattern obtained with FISH, a G-banded chromosome study was also performed. The bone marrow sample was processed using standard cytogenetic techniques. Slides were prepared and stained using G-banding (Giemsa Trypsin Wright), and 20 metaphases were analyzed. Cells were imaged and karyotypes were prepared using CytoVision Computer-Assisted Karyotyping System (Applied Imaging). The karyotypes were described according to the International System for Human Cytogenetics Nomenclature [5].

4. Results

4.1. FISH

FISH analysis showed a variant fusion pattern with two *PML* signals, two *RARA* signals, and only one *PML/RARA* fusion signal in 84% of the cells examined (Figs. 1 and 2). FISH also detected the typical dual-fusion pattern of one *PML*, one *RARA*, and two *PML/RARA* fusions in 5% of cells (data not shown).

According to our validation studies for this probe, our normal cut-off value is 99%, with 98% confidence.

4.2. Chromosome results

By conventional cytogenetics, a complex variant translocation involving the same chromosomes 15 and 17 was detected. The karyotype was described as follows: 46,XY,del(15)(q15q22),der(17)t(15;17)(q22;q21)t(15;17)(q15;q21) (Fig. 3).

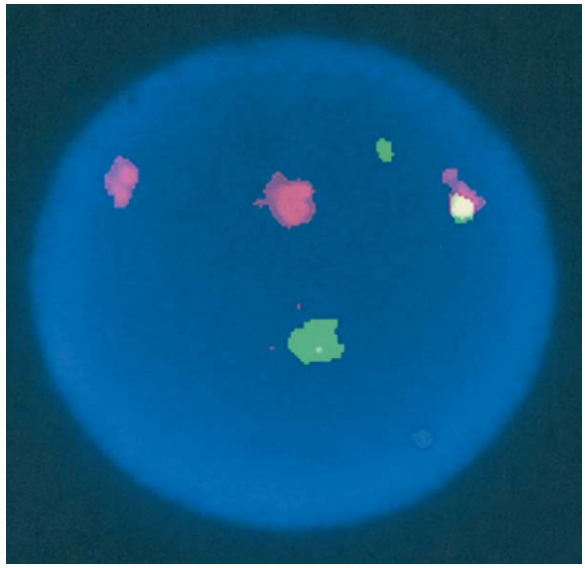

Fig. 1. Interphase nuclei showing two green *PML* signals, two red *RARA* signals, and one yellow *PML/RARA* fusion signal.

5. Discussion

The t(15;17)(q22;q21) is specific to the APL, M3 phenotype, and the resultant *PML-RARA* fusion can be demonstrated in 98% of APL cases. The typical t(15;17) FISH pattern for the Vysis dual-color, dual-fusion probe is one *PML*, one *RARA*, and two fusion signals. In this particular case, however, the pattern was two *PML* and two *RARA* signals and one fusion signal, suggesting most likely a variant translocation. In fact, the concurrent chromosomal analysis showed that only one of the chromosomes 15 and one of the chromosomes 17 was involved in the translocation, but the end result was different from that expected with a typical t(15;17). The combination of FISH and G-banding studies suggested that two subsequent translocations had occurred involving the same chromosomes 15 and 17. The first translocation resulted in the typical t(15;17)(q22;q21), and the second event caused the separation of the *RARA/PML* fusion, which typically occurs on the derivative chromosome 17. The *PML/RARA* fusion on the derivative chromosome 15 was preserved. Although we did not see cells with the typical t(15;17) by conventional cytogenetics, FISH detected the two-fusion pattern in 5% of nuclei, suggesting the existence of a typical t(15;17) and the occurrence of a subsequent translocation.

Variant translocations have been reported previously [4–12]. The majority of them show cryptic *PML-RARA* fusions that were only detected by further FISH or other molecular analyses. The three simple variant translocations that have been characterized at the molecular level include the *PLZF-RARA* fusion in t(11;17)(q23;q21), *NuMA-RARA* fusion in t(11;17)(q13;q21), and *NPM-RARA* fusion in t(5;17)(q32;q12) [1]. The common involvement of the *RARA* gene in all these variant types emphasizes its importance

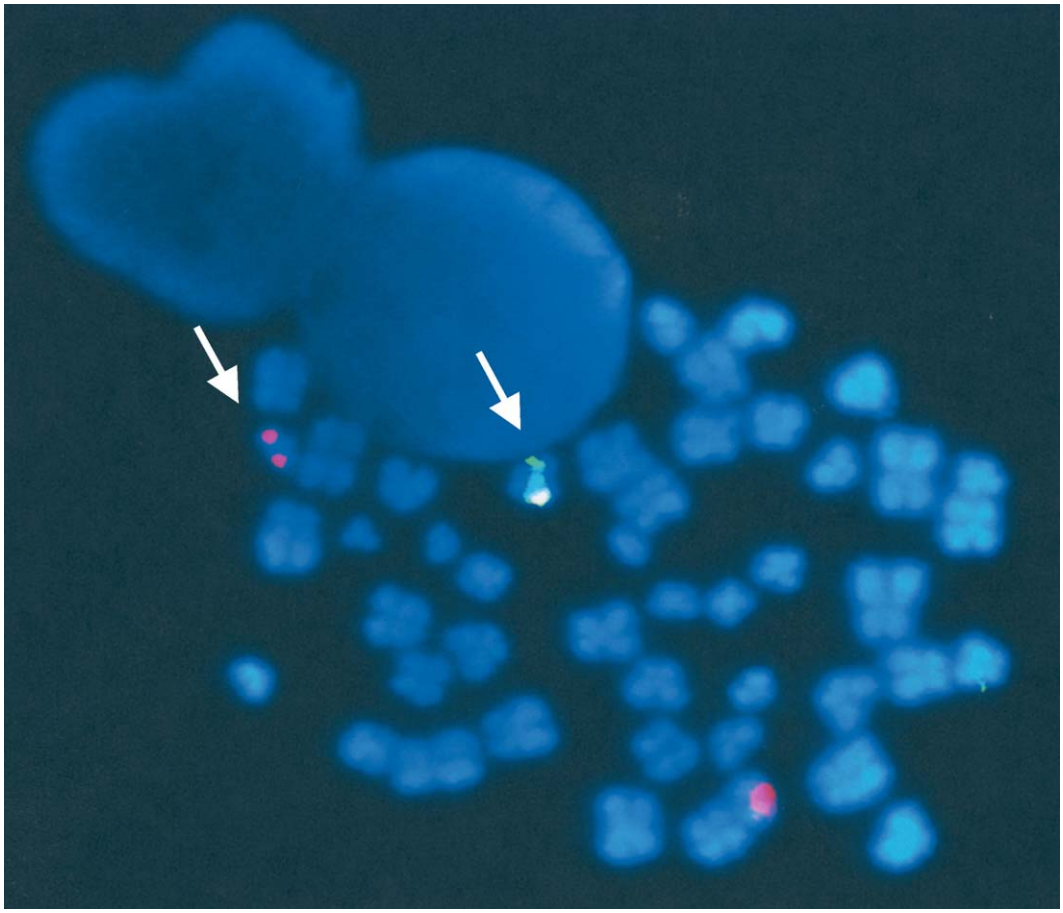

Fig. 2. Metaphase showing the same pattern: two green *PML* signals, two red *RARA* signals, and one yellow *PML/RARA* fusion signal.

in APL leukemogenesis [4]. These variant translocations, however, respond poorly, if at all, to the ATRA treatment.

Complex variant t(15;17)s are increasingly recognized in APL. Wan et al. [10] reviewed 22 cases with different three-way translocations. In their study, they reported a novel t(X;17;15)(q13;q12;q21), where Southern blot analysis showed a rearrangement of the *RARA* gene at intron 2. In the literature there are 21 cases of three-way translocations involving chromosomes 15 and 17 [7,9,10].

Tirado et al. [7] described an unusual case of APL with a t(15;17;17)(q22;q23;q21) in a patient that responded well to ATRA. FISH analysis of this patient's sample showed a positive fusion signal pattern in 100% of cells, confirming the presence of a *PML/RARA* rearrangement. Multicolor FISH confirmed the cytogenetic interpretation of the rearrangement. Their additional studies using the *RARA* break-apart DNA probe showed that it was not rearranged on the derivative chromosome 17, which received the q22~qter segment from chromosome 15. The *RARA* locus on the smaller derivative 17 was the allele involved in the fusion in this three-way rearrangement. Molecular studies corroborated the presence of the *PML-RARA* fusion but the complementary *RARA-PML*, which was usually detectable, was absent.

Fujita et al. [8] reported 10 APL patients treated with ATRA and/or chemotherapy, all of which achieved complete remission. Their cytogenetic analysis revealed the classic t(15;17) in 9 of 10 patients, and the remaining patient had an apparently normal karyotype. Metaphase FISH of the patient with normal karyotype revealed a juxtaposed *PML-RARA* fusion signal on one chromosome 17 homologue, one *RARA* signal on the other chromosome 17 homologue, and one *PML* signal on each chromosome 15 homologue.

Kurian et al. [9] reported on a 69-year-old woman who had developed APL 10 months after receiving adjuvant cyclophosphamide, doxorubicin, and paclitaxel for breast cancer. Cytogenetic studies revealed a t(15;17) with atypical breakpoints for APL. FISH paints and *RARA/PML* cosmid probes verified that the breakpoints on chromosomes 15 and 17 were proximal to both the *PML* and *RARA* genes: t(15;17)(q13;q12). Although the patient received induction chemotherapy and a several month trial of ATRA, there was no clinical improvement or hematologic remission. We suspect that this patient developed post-chemotherapy secondary APL with an atypical t(15;17), which rendered her leukemic cells unresponsive to ATRA therapy.

Kurian et al. [9] and other previous reports [11] point out that the prognostic course of complex and variant t(15;17)

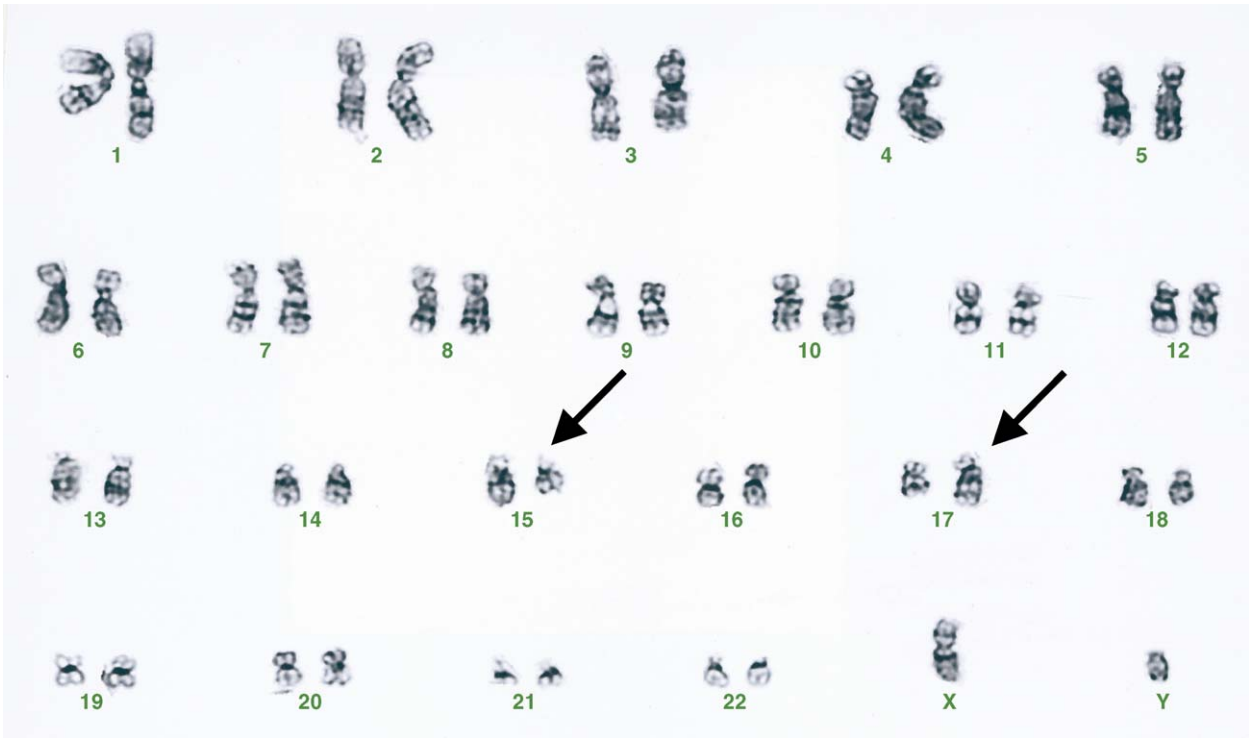

Fig. 3. The karyotype of this case is as follows: 46,XY,del(15)(q15q22),der(17)t(15;17)(q22;q21)t(15;17)(q15;q21).

in which the *PML/RARA* fusion on the derivative chromosome 15 is intact, such as in the present case, does not differ from that of the typical t(15;17). In fact, our patient is presently in remission and doing well after chemotherapy with daunorubicin and ATRA treatment. As the prognostic outcome in APL is associated with the presence of a *PML/RARA* fusion, it is useful and necessary to perform both cytogenetic and FISH analyses to better characterize a variant and complex t(15;17).

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