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Short communication

Translocation (2;11)(q37;q23) in therapy-related myelodysplastic syndrome after treatment for acute promyelocytic leukemia

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Abstract Treatment of acute promyelocytic leukemia (APL) with a combination of anthracycline-based chemotherapy and all-*trans* retinoic acid (ATRA) leads to very high rates of complete remission and survival. There are only a limited number of publications on the development of therapy-related myelodysplastic syndrome (MDS) or acute myeloid leukemia during follow-up of APL. Although drugs targeting at DNA-topoisomerase II characteristically induce translocations involving 11q23, this was seldom seen in patients treated for APL. We report on a patient initially diagnosed with APL. Response to therapy was monitored by fluorescence in situ hybridization (FISH) and reverse-transcriptase polymerase chain reaction for the *PML-RARα* rearrangement. Consecutive samples showed a swift and complete reduction of *PML-RARα* rearranged cells. Twenty months after diagnosis, however, conventional cytogenetics revealed a complex karyotype with a translocation involving 11q23 and loss of chromosomes 7q and Xq. FISH analysis with the *MLL* probe identified 2q37 (harboring the *SEPT2* gene) as the translocation partner of chromosome 11. We consider the rather unique t(2;11)(q37;q23) as the primary event causing therapy-related MDS in our patient. This case stresses the importance of conventional karyotyping to be performed on a regular basis in all treated APL patients for the early detection of chromosomal aberrations that indicate the development of therapy-related MDS or acute myeloid leukemia.

1. Introduction

Acute promyelocytic leukemia (APL) is defined as an acute myeloid leukemia (AML) in which abnormal promyelocytes predominate [1]. The t(15;17)(q22;q21) is a typical marker of this disease [2], although variant translocations are also described [3]. Through this translocation, the promyelocytic leukemia (*PML*) gene and the retinoic acid receptor-α (*RARα*) gene are fused [4,5]. The fusion protein *PML/RARα* blocks the differentiation of cells at the promyelocyte stage. Patients with APL are particularly sensitive to treatment with pharmacologic doses of all *trans*-retinoic acid (ATRA), which overrules this differentiation block. The combination of ATRA and anthracycline-based chemotherapy results in very high rates of complete remission and survival in these patients [6].

There are only a limited number of APL cases reported in which therapy-related myelodysplastic syndrome (MDS) or AML occurred during follow-up [7–10]. Although translocations involving the *MLL* gene on 11q23 are commonly associated with drugs targeting at DNA-topoisomerase II–like anthracyclines [11,12], untill now, this was seldom seen after treatment for APL.

Here we report the identification of therapy-related MDS after APL by a clone harboring a rather unique translocation with cytogenetic breakpoints in 11q23 and 2q37.

2. Case report

A 56-year old female presented with a few weeks' history of multiple hematomas all over her body surface. She also had symptoms of dyspnea and fatigue. Routine blood analysis showed hemoglobin 64 g/L, platelets 18×10^9/L, and white blood cell count 0.4×10^9/L. Coagulation measured by activated prothrombin time and prothrombin time was within normal ranges.

Bone marrow examination revealed hypercellular marrow with dysplastic features, megaloblastoid erythropoiesis, 9% blast cells, and 26% promyelocytes. Fagots were

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present in both cell types. On the basis of morphology and immunophenotyping, a classification of acute promyelocytic leukemia was made. The results of karyotyping, fluorescence in situ hybridization (FISH), and reverse-transcriptase polymerase chain reaction (RT-PCR) confirmed this diagnosis.

Treatment was started with ATRA and idarubicin. Maintenance therapy involved ATRA and mitoxantrone. Morphologic findings, FISH, and RT-PCR analysis indicated that complete remission was achieved. One and a half years later, the patient developed new hematomas, and relapse of APL was suspected. Karyotyping performed 20 months after the initial diagnosis demonstrated a new clone with multiple cytogenetic abnormalities. The patient received an allogeneic sibling stem cell transplantation, which was unsuccessful, and 30 months after first presentation, she died from pneumonia.

3. Materials and methods

3.1. Conventional cytogenetics

Bone marrow samples were cultured in medium supplemented with 15% fetal calf serum for 24 or 72 hours and harvested according to standard protocols. Metaphase chromosomes were analyzed using a routine Q-banding method and defined according to the International System for Human Cytogenetic Nomenclature (1995) [13].

3.2. FISH

For FISH analysis, fixed suspensions remaining from the conventional cytogenetic analysis or obtained from uncultured samples were used. The commercially available LSI *PML/RARA* dual-color translocation probe (on 15q22 and 17q12~q21), LSI *MLL* dual-color, break-apart rearrangement probe (on 11q23), LSI Williams probe (on 7q31), and LSI *ATM* probe (on 11q22.3) were applied. The manufacturer's recommendations were followed for the hybridization and post-washing procedures (Abbott Molecular/Vysis, Des Plains, IL, USA).

4. Results

Results of conventional cytogenetics, FISH, and RT-PCR analysis on bone marrow and blood samples are summarized in Table 1. Upon diagnosis, 24 metaphases were analyzed and the t(15;17)(q22;q21) was present in 10 of them. No other cytogenetic abnormalities were observed. Interphase FISH demonstrated a *PML-RARα* rearrangement in 65% of the nuclei. RT-PCR for t(15;17) revealed a *PML-RARα* transcript with the breakpoint in breakpoint cluster region 2 (bcr2).

The presence of the *PML-RARα* rearrangement was monitored by FISH and RT-PCR. At day 21, the percentage of cells exhibiting the *PML-RARα* rearrangement was decreased to 35% (FISH). In all samples obtained on day 35 or later, FISH and RT-PCR showed (near) complete reduction of the abnormal clone. These data corresponded with morphologic findings.

Bone marrow obtained at day 601 yielded only two metaphases of poor quality for conventional karyotyping. Since an aberrant chromosome 11 was suspected, additional FISH analysis was performed with a probe for

Table 1
Summary of morphological, cytogenetic and molecular findings

Day	Morphology bone marrow	46,XX	46,XX,t(15;17)(q22;q21)	46,X,del(X)(q22q28), t(2;11)(q37;q23), del(7)(q22q36)	46,XY *(donor)*	FISH P/R[a]	FISH MLL[a]	RT-PCR t(15;17)
0	Hypercellular, 26% promyelocytes, 9% blast cells, Auer rods	14	10	0	0	65%	normal	pos
21	Aplasia	-	-	-	-	35%	nd	nd
35	Hypocellular, <2% blast cells, 9% promyelocytes	-	-	-	-	6%	nd	neg
65	Complete remission	-	-	-	-	nd	nd	neg
127	Complete remission	-	-	-	-	7%	nd	neg
181	Complete remission	-	-	-	-	4%	nd	neg
307	Complete remission	-	-	-	-	2%	nd	neg
440	nd *(blood sample)*	-	-	-	-	nd	nd	neg
503	nd *(blood sample)*	-	-	-	-	nd	nd	neg
601	Complete remission	0	0	2[b]	0	7%	76%	neg
689	Hypercellular, 7% promyelocytes 0.6% blast cells, no Auer rods	0	0	9	0	4%	75%	neg
755	Hypocellular, normal blast cell count	5	0	9	0	nd	nd	nd
832	Complete remission	9	0	12	0	nd	nd	neg
930	Complete remission	0	0	10	13	8%	26%	neg
955	9% blast cells	-	-	-	-	nd	nd	nd

Abbreviations: P/R, PML/RARA; nd, no data; pos, positive; neg, negative.

[a] FISH results are given as percentage of cells with aberrant signal pattern. For LSI PML/RARA and LSI MLL: translocation [1F1O1G].

[b] Only 2 metaphases of poor quality were obtained, therefore no adequate karyotype could be obtained at that moment. Aberrations matched those found in later samples.