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Short communication

Secondary myelodysplastic syndrome after treatment for promyelocytic leukemia: clinical and genetic features of two cases

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Abstract Acute promyelocytic leukemia (APL) represents a biologic and clinically well-defined subtype of acute nonlymphocytic leukemia with specific morphologic and karyotypic characteristics. Although secondary leukemia and myelodysplastic syndromes (MDS) are the most frequent secondary neoplasms following chemotherapy for acute leukemia, their development after complete remission in patients with APL is uncommon. We describe the clinical and genetic features of two APL patients who achieved CR after chemotherapy and all-*trans* retinoid acid treatment and subsequently developed a MDS. Therapy-related MDS karyotype changes such as abnormalities of chromosomes 5 and 7 were found in the cytogenetic analysis. Since *TP53* alteration was detected in one case, possible implications of these findings in the onset of MDS are discussed.

1. Introduction

Secondary myelodysplastic syndrome (MDS) may result from the exposure to DNA-damaging drugs such as alkylating agents or those targeting DNA topoisomerase II. Each of these drug groups may induce secondary malignancies showing various characteristics in terms of morphology, latency period and cytogenetic abnormalities [1–3]. Although these drugs are included in several acute promyelocytic leukemia (APL) treatment regimens, secondary MDS developing in APL patients in complete remission (CR) are rare [4]. We describe the clinical and genetic features, emphasizing the study of *TP53*, in two APL patients in CR, who developed secondary MDS.

2. Case report

2.1. Case 1

A 70-year-old male was admitted due to asymptomatic pancytopenia. Blood count showed Hb 8.4 g/dL, white blood cell count 1.0×10^9/L with 40% abnormal promyelocytes, and platelet count 17×10^9/L. Bone marrow aspiration was hypercellular and showed 95% abnormal promyelocytes. G-banding analysis revealed two clones: 46,XY,t(15;17)(q21;q11) in 19 of 58 metaphases and 46,XY,inv(6)(p24q13),t(15;17)(q21;q11) in 39 of 58 metaphases.

Reverse transcriptase polymerase chain reaction (RT-PCR) detected the presence of *PML-RARA* transcript. Continuous CR with clearance of both cytogenetic and molecular aberrations was achieved after induction treatment with oral all-*trans* retinoic acid (ATRA) (45 mg/m^2/day $\times$ 35 days), idarubicin (12 mg/m^2/day $\times$ 4 days), and cytosine arabinoside (Ara-C) (100 mg/m^2/day $\times$ 8 days). Consolidation treatment included two idarubicin (at 5 mg/m^2/day $\times$ 4 days and 12 mg/ m^2/day $\times$ 1 day, respectively) and one mitoxantrone (10 mg/m^2/day $\times$ 5 days) courses that were followed by maintenance therapy for 2 years with daily oral 6-mercaptopurine (90 mg/m^2/day), weekly intramuscular methotrexate (15 mg/m^2/day), and oral ATRA (15-day courses every 3 months at 45 mg/m^2/day). Treatment was completed with persistence of CR.

However, 4 months later the patient showed Hb 8.4 g/dL, white cell count 3.3×10^9/L with 3% blast cells, and platelet count 104×10^9/L. Bone marrow aspiration was hypercellular, showing 12% blast cells and red-cell line hyperplasia with multiple dyserythropoyetic changes in erythroblasts

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(megaloblastoid features, internuclear chromatin bridging, abnormal mitosis, and lobulated nuclei). Flow cytometry of bone marrow cells showed an intense reactivity with CD34, CD13, CD33 and HLA-DR on blast cells. A new clone with monosomies of chromosomes Y and 7 (44,X,−Y,−7) was detected at the cytogenetic study of bone marrow in 41 of 50 metaphases. Based on hematologic, immunophenotypic, and cytogenetic changes, the patient was diagnosed with MDS (refractory anemia with excess blast) and he refused any treatment other than transfusion therapy. Disease evaluation after 6 months showed Hb 8.1 g/dL, white blood cell count of 4.2×10^9/L with 18% blast cells, and platelet count of 45×10^9/L. Bone marrow aspiration disclosed 25% blast cell infiltration with the same features showed at diagnosis of MDS. Cytogenetic analysis confirmed the presence of monosomies of chromosomes Y and 7. Conditions deteriorated further and the patient died one year after MDS diagnosis.

2.2. Case 2

A 60-year-old female was admitted due to asymptomatic pancytopenia. Blood counts at admission were as follows: Hb 8.3 g/dL, white cell count 1.2×10^9/L with 28% neutrophils, platelet count 7×10^9/L. Peripheral blood smears showed the presence of abnormal promyelocytes. Bone marrow examination was consistent with APL, and diagnosis was confirmed by cytogenetic detection of t(15;17)(q22;q11) in 94% of studied cells, with presence of the *PML-RARA* transcript in the molecular analysis. Morphologic, cytogenetic and molecular CR was obtained through induction treatment consisting of daily oral ATRA (45 mg/m^2/day × 40 days) and intravenous idarubicin (12 mg/m^2/day × 4 days). Consolidation treatment included two idarubicin courses (at 5 mg/m^2/day × 4 days and 12 mg/m^2/day × 1 day, respectively) and a course of mitoxantrone (10 mg/m^2/day × 5 days). Maintenance therapy consisted of daily oral 6-mercaptopurine (90 mg/m^2/day), weekly intramuscular methotrexate (15 mg/m^2/day) and 15-d ATRA courses every 3 months at 45 mg/m^2/day.

Bone marrow analysis performed after 20 months of maintenance treatment detected a clone with 46,XX,del(5)(q13q33). *PML-RARA* mRNA was still negative. The bone marrow aspiration showed a decrease in red blood cell precursors, dysplastic changes in granulocytes, and increase in the number of megakaryocytes, having nonlobulated nuclei. The patient was diagnosed with secondary MDS (5q− syndrome) and is currently asymptomatic without treatment.

3. Materials and methods

3.1. Cytogenetic analysis

Cytogenetic studies were done on unstimulated short-term bone marrow cultures. G-banded karyotypes were described according to International System for Human Cytogenetic Nomenclature (ISCN 1995).

3.2. Molecular analysis

Genomic DNA was extracted following standard procedures from EDTA-anticoagulated peripheral blood and matched bone marrow samples. RNA extraction and RT-PCR for *PML/RARA* amplification were performed according to standard protocols [5].

Loss of heterozygosity (LOH) was tested through the analysis of the following variable number of tandem repeat (VNTR) markers: Rb1.20, a CTTT(T) repeat in intron 20 of the retinoblastoma (*RB1*) gene and RB1.2 (D13S153), a dinucleotide repeat marker located in intron 2 of the *RB1* gene (13q14.1) and P53IVS1, a dinucleotide repeat marker located in intron 1 of the *TP53* gene (17p13.1). In all of these cases, the analysis consisted in a PCR amplification with specific primers with a ^{32}P-end-labeled primer and subsequent analysis of the alleles in either 6% polyacrylamide (29:1, acrylamide: bisacrylamide) nondenaturing 35 × 45-cm gels or in 7% poliacrylamide Spreadex Polymer NAB gels (Elchrom Scientific AG, Cham, Switzerland).

The analysis of mutation at exons 5–8 of the *TP53* gene and homozygous deletion of the *p16INK4* gene were performed as previously described [6].

4. Results and discussion

Development of MDS after treatment for APL is rare [4]. Chemotherapy could contribute to it by several mechanisms. Our patients were exposed to topoisomerase II targeting agents, such as anthracyclines and mitoxantrone, but they did not show the typical chromosomal abnormalities at 11q23 or 21q22 associated with these secondary neoplasms. However, although they did not receive alkylating agents, their secondary cytogenetic abnormalities included losses at chromosomes 5 and 7, that is, aberrations often described in patients with MDS associated with exposure to alkylating agents. The explanation for this could be that methotrexate, 6-mercaptopurine or ATRA might modify anthracycline leukemogenesis [4].

Aside from proven effect of chemotherapy and ionizing radiation on development of secondary malignancies, some authors have suggested the contribution of a genetic predisposition to the onset of secondary MDS [2]. Studies performed on patients with secondary acute leukemias have demonstrated the presence of germline mutations of the *TP53* gene [7,8]. Since treatment with cytotoxic agents acting through a *p53*-dependent apoptotic cell death program seems to be the main risk factor for development of secondary leukemia, it has been hypothesized that mutations in DNA repair genes can lead to DNA instability, thus predisposing to secondary leukemia [9]. Moreover *TP53*, *RB* and the fusion protein APL-associated *PML-RARA* have been recently linked by showing that the last one could inactivate both *TP53* and *RB* pathways [10].

DNA samples from bone marrow and peripheral blood were available for case 1, both at the moment of PML diag-

nosis as well as at MDS development. The analysis of mutation of *TP53* in these samples showed an altered DGGE band pattern affecting the exon 7 of the gene in the MDS, but not in samples from the PML at diagnosis (Fig. 1). Sequencing of the PCR products revealed a G to A transition within exon 7 (TGT→TAT) that changes the residue number 238 of the protein from cysteine into tyrosine (C238T). Analysis of the VNTR marker located in the intron 1 of the *TP53* gene did not show LOH at this locus. In fact, the electrophoretogram of the sequencing of the blood sample showed the presence of the peaks corresponding to the normal (guanine) and mutant (adenine) strands with the same intensity, whereas in the bone marrow there was a clear overrepresentation of the mutant allele (Fig. 2). The presence of normal, non-leukemic cells in the bone marrow (residual normal hematopoiesis) and the peripheral blood of the patient, makes the analysis of LOH by comparison of the alleles for the P53IVS1 marker useless in this case. With the analysis of mutation of the conserved codons of the *TP53* gene that we performed in this study we cannot rule out the presence of alteration in the other allele outside this region.

This mutation has been frequently described in a wide variety of human tumors, including hematologic malignancies. Nevertheless, to our knowledge, it has never been described before in MDS cases following APL treatment. Although association between *TP53* mutation and 5q− deletion has been reported [11], suggesting cooperation between loss of both *p53* function and a putative tumor suppressor gene at 5q, we were not able to detect any mutation at *TP53* in our patient with 5q− (case 2). Because alterations on 13q14 have been found in a subgroup of acute leukemias resulting from MDS transformation and occasionally in therapy-related acute leukemia [3,12], our two cases were also screened for mutations at *RB1* and *p16INK4* genes. These genes act as negative regulators of cell proliferation by blocking cell-cycle transition from G1 into S phase through their protein products. Nevertheless, no abnormalities were detected at these genes in the samples from our patients.

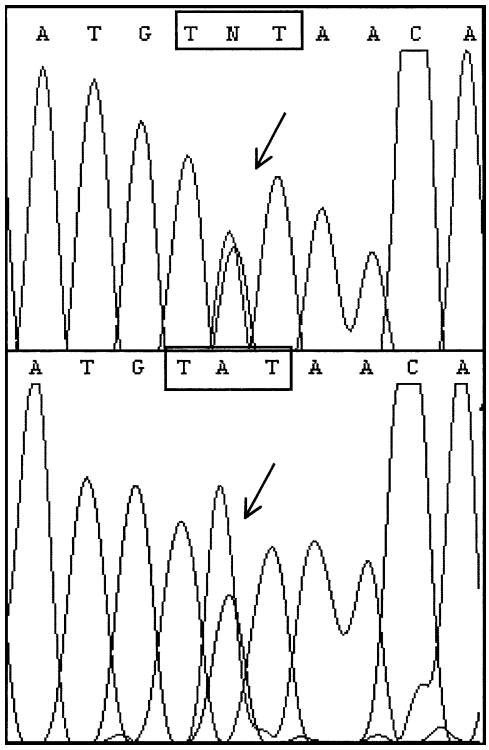

Fig. 2. Upper panel: Peripheral blood. Lower panel: Bone marrow. Electrophoretogram of the sequencing of the fragment of the exon 7 of the *TP53* gene containing the nucleotide change (TGT→TAT) in the peripheral blood and bone marrow of case 1 at MDS development.

In conclusion, we describe two new cases of MDS after treatment for APL. The presence of the *TP53* C238T mutation in both the bone marrow and blood samples at MDS development in one of the cases, but not at PML diagnosis, suggests that the pattern of molecular alterations changes with the development of MDS resulting in *TP53* pathway abrogation.

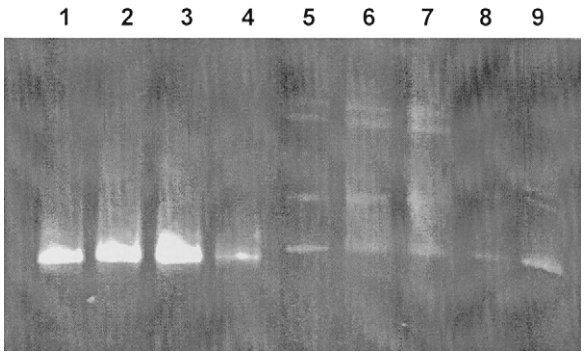

Fig. 1. DGGE showing the altered band patterns in peripheral blood and bone marrow samples of case 1. Lanes 1, 2, and 3: normal control; lane 4: bone marrow of case 1 at the time of PML diagnosis; lane 5: bone marrow of case 1 at the time of MDS diagnosis; lanes 6 and 7: peripheral blood samples of case 1 at the time of MDS diagnosis; and lanes 8 and 9: mutant controls.

References

[1] Alessandrino EP, Amadori S, Cazzola M, Locatelli F, Mecucci C, Morra E, Saglio G, Visani G, Tura S. Myelodysplastic syndromes: recent advances. Haematologica 2001;86:1124–57.

[2] Pagano L, Pulsoni A, Tosti ME, Avvisati G, Mele L, Mele A, Martino B, Visani G, Cerri R, Di Bona E, Invernizzi R, Nosari A, Clavio M, Allione B, Coser P, Candoni A, Levis A, Camera A, Melillo L, Leone G, Mandelli F for the GIMEMA. Clinical and biological features of acute myeloid leukemia occurring as second malignancy: GIMEMA archive of adult acute leukemia. Br J Haematol 2001;112:109–17.

[3] Pedersen-Bjergaard J, Pedersen M, Roulston D, Philip P. Different genetic pathways in leukemogenesis for patients presenting with therapy-related myelodysplasia and therapy-related acute myeloid leukemia. Blood 1995;86:3542–52.

[4] Pecci A, Invernizzi R. A therapy-related myelodysplastic syndrome with unusual features in a patient treated for acute promyelocytic leukemia. Haematologica 2001;86:102–3.

[5] Borrow J, Goddard AD, Gibbons B, Katz F, Swirsky D, Fioretos T,

Dube I, Winfield DA, Kingston J, Hagemeijer A. Diagnosis of acute promyelocytic leukemia by RT-PCR detection of PML-RARA and RARA-PML fusion transcripts. Br J Haematol 1992;82:529–40.

[6] Patiño-García A, Sierrasesúmaga L. Analysis of the p16INK4 and p53 tumor suppressor genes in bone sarcoma pediatric patients. Cancer Genet Cytogenet 1997;98:50–5.

[7] Felix CA, Megonigal MD, Chervinsky DS, Leonard DGB, Tsuchida N, Kakati S, Block AMW, Fisher J, Grossi M, Salhany KI, Jani-Sait SN, Aplan PD. Association of germline p53 mutation with MLL segmental jumping translocation in treatment-related leukemia. Blood 1998;91:4451–6.

[8] Panizo C, Patiño A, Calasanz MJ, Rifón J, Sierrasesúmaga L, Rocha E. Emergence of secondary acute leukemia in a patient treated for osteosarcoma: implications of germline TP53 mutations. Med Pediatr Oncol 1998;30:165–9.

[9] Levine AJ. p53: the cellular gatekeeper for growth and division. Cell 1997;88:323–31.

[10] Gottifredi V, Prives C. P53 and PML: new partners in tumor suppression. Trends Cell Biol 2001;11:184–7.

[11] Christiansen DH, Andersen MK, Pedersen-Bjergaard JP. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol 2001;19:1405–13.

[12] Nagai M, Fujita M, Ikeda T, Ohmori M, Kuwabara H, Yamaoka G, Tanaka K, Kamada N, Taniwaki M, Inoue T, Irino S, Takahara J. Alterations of p53 and Rb genes in a novel human GM-CSF-dependent myeloid cell line (OHN-GM) established from therapy-related leukaemia. Br J Haematol 1997;98:392–8.