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Brief communication

Development of two cytogenetically abnormal clones from multipotential hematopoietic stem cells in a patient with juvenile myelomonocytic leukemia

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Abstract

We report a patient with juvenile myelomonocytic leukemia who had two cytogenetically independent clones at the time of diagnosis. Fluorescence in situ hybridization analyses showed that 42.5% of myeloperoxidase$^+$ cells and 27.3% of CD20$^+$ cells had three signals for chromosome 8, while 13.1% of myeloperoxidase$^+$ cells and 6.0% of CD20$^+$ cells had del (Y). However, a great majority of CD3$^+$ cells had no numerical aberration of the two chromosomes. The two karyotypically abnormal clones might have developed from multipotential hematopoietic stem cells with the potential to differentiate into myeloid and B-lymphoid lineages, but not T-lymphoid lineage.

Keywords: JMML; Clonality; FISH; Trisomy 8; Loss of Y chromosome

1. Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare myeloproliferative disorder that occurs in infancy and early childhood. Several studies have indicated clonality in myeloid or erythroid precursors on examining chromosomal abnormalities [1–3]. Using clonality assays based on the differential DNA methylation of X chromosomes and on the detection of a transcriptional polymorphism of the active X chromosome, Busque et al. [4] demonstrated the monoclonal origin of JMML CD34$^+$/CD38$^-$ cells, granulocytes, monocytes, erythroid cells and platelets. We previously reported a JMML patient who acquired monosomy 7 in B cells and NK cells as well as myeloid cells after intensive multiagent chemotherapy [5]. In addition, several patients with JMML who subsequently evolved B lineage acute lymphoblastic leukemia or T-cell lymphoma were reported [6–8]. Consequently, JMML is considered to be a clonal disease of pluripotent stem cell origin. However, there have been no reports of lymphoid involvement at the onset of this disorder.

Here we report for the first time a JMML patient who appeared to have biclonal chromosomal aberrations in multipotential hematopoietic stem cells with the potential to differentiate into myeloid and B-lymphoid lineages, but not T-lymphoid lineage.

2. Patient

A 31-month-old boy was admitted to our hospital on May 17, 2001 because of fever, petechiae and stomatitis. He had hepatosplenomegaly, lymphoadenopathy and skin rashes, but did not have neurological or developmental abnormalities.

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His white blood cell count was 76.3×10^9/l (49% segmented, 12% band forms, 5% metamyelocytes, 4% myelocytes, 1% promyelocyte, 1% myeloblast, 10% monocytes, 1% basophil, and 17% lymphocytes), hemoglobin level was 9.2 g/dl, and platelet count was 201×10^9/l. Serum LDH, lysozyme, and fetal hemoglobin levels were 363 IU/l, 19.1 μg/ml, and 19%, respectively. Viral infection including Epstein-Barr virus and cytomegalovirus could be eliminated on the basis of serological investigations. The bone marrow examination revealed myeloid hyperplasia with no excess of blasts and a decreased number of megakaryocytes. Neither Philadelphia chromosome nor bcr-abl rearrangement was detected. Spontaneous growth of GM colonies from the bone marrow cells was observed by semisolid colony assay [9,10]. Thus, the patient met the criteria proposed by the International JMML Working Group [11]. The patient had been treated with 6-mercaptopurine alone (6-MP, 1 mg/kg/day) from June 18. Leukocyte counts, hemoglobin levels, and platelet counts were maintained at approximately 20×10^9/l, 8 g/dl, and 50×10^9/l, respectively. Because of a bleeding tendency due to prominent thrombocytopenia and chromosomal aberration, he received an unrelated bone marrow transplantation on January 23, 2002 after a preparative regimen of busulfan, cytarabine, and cyclophosphamide. Graft-versus-host disease prophylaxis was provided with a combination of tacrolimus and a short course of methotrexate. Since hematologic reconstitution was not achieved, we performed an unrelated cord blood stem cell transplantation on March 27, 2002. Conditioning therapy consisted of fludarabine, melpharan and total lymphoid irradiation. Because of a second graft failure, peripheral blood stem cells mobilized with granulocyte colony-stimulating factor from the human leukocyte antigen-haploidentical father were infused after a combination of total body irradiation and chemotherapy (cyclophosphamide and fludarabine). However, the third transplantation resulted in only a temporary stabilization of the disease. He died 17 months after the diagnosis of JMML from disease progression and multiple organ failure.

3. Materials and methods

3.1. Cell preparation

To prepare CD3- or CD20-positive peripheral blood (PB) cells, PB mononuclear cells (MNCs) that had been frozen with liquid nitrogen were rapidly thawed, passed through a 200-μm monofilament nylon filter, and suspended in alpha-medium consisting of Ca^{2+}- and Mg^{2+}-free cold phosphate-buffered saline, 1 mmol/L EDTA 2 Na, and 2.5% fetal bovine serum, as described previously [5,12]. Dead cells were eliminated from the samples, using a MACS dead cell removal kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Thereafter, MNCs (1×10^6) were treated with 100 μl of FcR blocking reagent, mixed with fluorescein isothiocyanate (FITC)-conjugated anti-CD3- or anti-CD20 monoclonal antibody, and incubated with anti-FITC Microbeads (Miltenyi Biotec). The magnetically labeled cells were separated with a MS^+/RS^+ column in the magnetic field of a MACS separator (VarioMACS). More than 98% of the isolated cells were CD3- or CD20-positive, according to a flow cytometric analysis (data not shown).

3.2. Fluorescence in situ hybridization (FISH) analysis

FISH analysis was performed according to a modification of a procedure described previously [5,12]. We obtained probes specific for the centromere of chromosome 8 (CEP 8), and of chromosome X (CEP X) from Vysis (Downers Grove, IL).

3.3. Combination of myeloperoxidase (MPO) staining with FISH

The combination of MPO staining with FISH was performed as described elsewhere [12].

4. Results

On May 21, 2001, a G-banding analysis showed 47,XY,+8 in 13 of 22 bone marrow (BM) cells and 46,XY in the remaining 9 cells. On the other hand, all 27 peripheral blood MNCs stimulated with phytohemagglutinin for 3 days had a normal karyotype. Additionally, 97.6% of buccal mucosal cells had two signals per nucleus for the centromere of chromosome 8 according to the FISH analysis (98.6% in normal control). These results indicate that the trisomy 8 was not constitutional, but an acquired chromosomal aberration specific to hematopoietic cells except probably T cells. Because of a gradual decrease in the platelet count, we re-examined the cytogenetics on June 7, 2001. Twenty BM cells analyzed consisted of 11 cells with 47,XY,+8, 7 cells with 46,XY, and 2 cells with 45,X,−Y. FISH analysis with the simultaneous application of probes specific to chromosomes 8 and Y revealed that 214 (42.8%) of 500 marrow cells had three spots for chromosome 8 and one spot for chromosome Y, and 56 cells (11.2%) had two signals for chromosome 8 and no signal for chromosome Y, as presented in Table 1. Approximately 45% of cells displayed normal patterns in the copy numbers of the two chromosomes. The incidence of cells sharing the two chromosomal abnormalities was negligible. Thus, these results indicate the coexistence of karyotypically independent clones in this case. The frequency of BM cells with extra copies of chromosome 8 markedly decreased 6 months after treatment with 6-MP alone (1.2% of 500 BM cells analyzed). Reversely, a loss of the sex chromosome became a predominant cytogenetic abnormality (83.4% of 500 BM cells).

To examine whether the two cytogenetically unrelated clones originated from different hematopoietic stem cells, we performed a cytogenetic clonality analysis of circulating myeloid, B and T cells obtained before treatment with 6-MP.