Cell Lineage Involvement in Four Patients with Myelodysplastic Syndrome and t(1;7) or Trisomy 8 Studied by Simultaneous Immunophenotyping and Fluorescence In Situ Hybridization

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ABSTRACT: Four patients with myelodysplastic syndrome (MDS), one with t(1;7) and three with trisomy 8, were studied by immunophenotyping and fluorescence in situ hybridization (FISH) to assess cell lineage involvement. The t(1;7) was detected using a biotin-labeled chromosome 1 centromere-specific DNA probe. This aberration was present in CD34-positive stem cells, the erythroid cell lineage (GPA +), and the granulocytic/monocytic (CD13 + and CD64 +) cell lineages. We were not able to demonstrate the abnormality in the lymphoid cell lineages. In the patients with trisomy 8, the aberration was detected with chromosome 8 centromere-specific DNA probe or by chromosome in situ suppression hybridization (CISS) with a chromosome 8-specific library probe. The trisomy was detected in stem cells, erythroid precursor cells, megakaryocytes, and granulocytes/monocytes. In these MDS patients, the chromosome aberrations appear to occur only in cells of myeloid lineage.

INTRODUCTION

Myelodysplastic syndrome (MDS) comprises a group of clonal stem cell disorders characterized by dysplastic changes in bone marrow (BM) morphology. In MDS, BM dysfunction usually leads to some degree of anemia, leukopenia, and/or thrombocytopenia. A clonal population of neoplastic stem cells in the hematopoietic lineage may progress to leukemic proliferation: 20–40% of patients will develop acute myeloblastic leukemia (AML) [1].

Nonrandom chromosome aberrations are observed in more than 50% of MDS patients; some of these cytogenetic abnormalities are also frequently observed in AML. The karyotypic features are useful as both prognostic and diagnostic indicators [2]. An unbalanced translocation between chromosomes 1 and 7, t(1;7)(q10;p10), often associated with therapy-related MDS, leads to trisomy of the long arm of chromosome 1 and monosomy of the long arm of chromosome 7. The patients often progress to AML [3–5]. Trisomy 8 (+8), one of the most frequently occurring chromosome abnormalities in MDS, also is often observed in AML patients [6]. These chromosome aberrations serve as genetic markers for identification of neoplastic cells.

DNA in situ hybridization (ISH) with chromosome-specific probes detects the abnormalities in both metaphases and interphase nuclei. Combining ISH with immunophenotypic classification of cells, allows the neoplastic clones to be identified and assigned to a specific hematopoietic cell lineage [7]. This information is valuable for understanding of the differentiation pathways of hematopoietic cells. Investigation of the cell lineage involvement of chromosome aberrations in preleukemias may lead to better understanding of the process of leukemogenesis.

We report four patients with MDS, of whom one had t(1;7) and three who had trisomy 8. By combining immunophenotypic analysis with fluorescence ISH (FISH) using centromere-specific DNA probes or a chromosome library probe, we analyzed clonal characteristics of different hematopoietic neoplastic cell lineages in MDS.
MATERIALS AND METHODS

Patients
We studied four patients with MDS, three females and one male (Table 1). The diagnosis of MDS was made according to the criteria of the French-American-British (FAB) Cooperative Study Group [8].

Cytogenetic Studies
BM aspirates were obtained from all patients; peripheral blood (PB) samples were also obtained from patients 1–3. Karyotype analysis was performed on unstimulated 24-hour BM cell cultures and on phytohemagglutinin (PHA, 0.5 mg/ml) (Difco Laboratories, Detroit, MI) stimulated PB cultures. RPMI 1640 medium was supplemented with fetal calf serum (20%), L-glutamine (0.29 mg/ml), penicillin (100 U/ml), and streptomycin (100 µg/ml) (all GIBCO, Grand Island, NY). Cell cultures were incubated at 37°C in 5% CO2. Harvesting, slide preparation, and G-banding were performed by standard techniques. At least 15 metaphases per patient sample were analyzed.

Immunophenotyping
Mononuclear cells from PB and BM cells were isolated by one-step density gradient centrifugation in Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) and cultured for 1–3 days. Cytospin preparations were made as described earlier [9]. The immunological phenotype of the cells was characterized with a modified alkaline phosphatase-antialkaline phosphatase method (APAAP) [10] with the following monoclonal antibodies. Anti-CD34 (My10) (Becton Dickinson, San Jose, CA) recognizes hematopoietic precursor cells; anti-CD13 (My7) (Coulter, Hialeah, FL) recognizes the granulocytic and monocytic lineages, and antiglycophorin A (anti-GPA; provided by Dr. Griffin, Boston, MA) is specific for the erythroid cell lineage. Anti-CD68 (Y-2/51) (Becton Dickinson, Erembodegem, Belgium) identifies T cells, and a mixture of anti-CD20 and anti-CD22 (Coulter and Jansen Biochimica, Beerse, Belgium) identifies B cells. Anti-CD64 (Serotec, Oxford, England) is a monocyte-specific antibody; anti-CD56 (Leu 19) and anti-CD16 (Leu 11a, b, and c) (Becton Dickinson) are specific for large granular lymphocytes (LGL). After immunophenotyping was completed, the cells were counterstained with Giemsa. The positive mitotic and interphase cells were scored and photographed on Kodak Ektachrome EPY-64T film.

FISH
For FISH, Giemsa stain and APAAP were dissolved in methanol:acetic acid (3:1) for at least 1 hour and the samples were air-dried. The cytoplasm was removed from the cells by treatment with pepsin (0.1 mg/ml in 0.01 N HCl) for 6 minutes at 37°C. The slides were rinsed in distilled water and dehydrated in an ethanol series. All hybridizations were performed within 4 weeks of APAAP staining.

DNA Probes and Nonradioactive Labeling.
For FISH of chromosome 1, we used a repeat-sequence satellite DNA probe for the heterochromatin region at 1q12 (pUC1.77) [11]. Cells with the t(1;7) show three signals per nucleus, two from normal chromosome 1 and one from the translocation derivative. For detection of chromosome 8 trisomy, we used a centromere-specific α-satellite DNA probe generated by poly-
Figure 1  Bone marrow (BM) cells from patient 1 showing (A) glycophorin A (GPA)-positive cell of the erythroid lineage (arrow). (B) Same cells after fluorescence in situ hybridization (FISH) with a chromosome 1-specific repeat-sequence DNA probe. Arrows show signals of the hybridized probe on chromosome 1 and der(1;7)(q10; p10). (C) BM cells from the same patient showing CD64-positive monocytes. (D) Same cells after FISH with the probe for chromosome 1. Arrows show one of the positive cells and signals in the interphase nucleus.

The chromosome 1 centromere-specific probe (#1) and the chromosome 8 library probe were labeled by nick-translation using biotin-11-dUTP (Sigma, St. Louis, MO) and a nick-translation kit (Nick Translation Kit, Bethesda Research Laboratories, Bethesda, MD) in the manner recommended by the manufacturer. The centromere-specific probe for chromosome 8 (#8) was labeled with digoxigenin-11-dUTP (Boehringer Mannheim GmbH, Mannheim, Germany) during PCR.

merase chain reaction (PCR) with oligonucleotide primers for conserved regions of the α-satellite monomer [12]. The source of DNA amplification was a chromosome 8 plasmid library probe (Library LL08NS02, American Type Culture Collection, ATCC). Chromosome in situ suppression hybridization (CISS) of chromosome 8 metaphase cells was performed with the library probe described above. Cells with trisomy 8 show three signals with these probes.
We performed FISH as described by Pinkel et al., with modifications [13]. After labeling, the biotinylated probe #1 was dissolved in a hybridization mixture containing 60% deionized formamide ( Gibco BRL, Gaithersburg, MD), 10% dextran sulfate ( Sigma, 2 x SSC and 0.5 mg/ml herring sperm DNA ( Sigma). The hybridization mixture for #8 contained 65% formamide in 2 x SSC. We performed CISS as described elsewhere [14, 15].

Slides hybridized with probe #1 were washed three times in 60% formamide, 2 x SSC (pH 7.0) and Tween 0.05% at 44°C for 5 minutes each, followed by three 5-minute washes in 0.1 x SSC at room temperature. Slides hybridized with #8 were washed in three changes of 50% formamide, 2 x SSC (pH 7.0) at 43°C for 5 minutes each and finally three times in 0.1 x SSC for 5 minutes each. Slides hybridized with the chromosome 8 library probe were washed three times in 50% formamide for 5 minutes each, and again three times for 5 minutes in 0.1 x SSC at 58°C.

**Probes Detection**

After being washed, the slides were incubated in bovine serum albumin (Boehringer Mannheim) at 37°C for 30 minutes. The biotinylated probe #1 was detected using avidin conjugated with FITC (1:200, Vector Laboratories, Burlingame, CA) followed by biotinylated goat antiavidin (1:200, Vector) and avidin-FITC, as described by Pinkel et al. [13]. For detection of #8, we used a sequential layer with mouse anti-digoxigenin (1:500), FITC-conjugated sheep anti-mouse antibody (1:200), and FITC-conjugated donkey anti-sheep antibody (1:200) [all Sigma] [16]. Cells were counterstained with propidium iodide (0.001 mg/ml, Sigma) and 4, 6-diamidino-2-phenylindole dihydrochloride (DAPI) (5 mg/ml, Sigma), and mounted in fluorescence antifading buffer [17].

Microscopic evaluation was made with a Leitz Laborlux D fluorescence photomicroscope equipped with filter sets Leitz I2/3 (FITC) and A (DAPI). Analysis of metaphase cells and interphase nuclei was made by two observers according to the criteria described by Hopman et al. [18]. Photographs were taken on Kodak Ektachrome 400 ASA color slide film.

**RESULTS**

Immunophenotyping combined with DNA FISH was performed on BM specimens and PB cells from patients 1 and 2 and on BM from patient 4. FISH without immunophenotyping was performed on BM from patient 3. Results are shown in Table 1.

Patient 1 had t(1;7) as the only cytogenetic abnormality. With FISH, the translocation was detected in the CD34-positive stem cells and in the CD13-positive granulocytic/monocytic cell lineage. CD64 + monocytes had the abnormality (Fig. 1C and D). The translocation was further shown in the GPA-positive erythroid lineage (Fig. 1A and B). CD61-positive megakaryocyte metaphase cells yielded three signals (frequencies not estimated). The frequency of T- and B-cells showing three signals was 1.7% and 2.5%, respectively; 1.6% of the CD56-positive LGL-cells (NK cells and T-cell subset) and 1.6% of the CD16-positive LGL-cells yielded three signals.

Patient 2 had trisomy 8 in about 5% of metaphases studied by CISS. The trisomy was evident in the CD34-positive stem cells and in the erythroid lineage. The CD13 + granulocytic/monocytic lineage did not contain the aberration. The lymphocytic lineage was not studied.

Patient 3 showed trisomy 8 in only about 1% of metaphases at diagnosis, as analyzed by CISS with the chromosome 8 library probe. Because of the low frequency of trisomic cells, cell lineage specificity could not be studied. After BM transplantation, no trisomy was observed by FISH or CISS.

Trisomy 8 in patient 4 was studied with the chromosome 8 repeat-sequence probe in BM interphase cells: 9% of all cells showed three signals. Chromosome painting of metaphases using the library probe showed the trisomy in 18%. After immunophenotyping, cells of the GPA-positive erythroid lineage and CD61-positive megakaryocytic cells were shown to be trisomic. The CD64 + monocytic lineage, studied with chromosome painting, also had the trisomy.

**DISCUSSION**

DNA FISH performed on immunophenotyped metaphase and interphase cells enables detection of specific chromosome anomalies in different cell types. In this study, we analyzed lineage specificity of cytogenetic alterations in three patients with MDS. We were able to show involvement of the erythroid lineage in all three patients, one of whom had the unbalanced t(1;7) and two of whom had trisomy 8. We also detected these aberrations in CD34+ stem cells and the megakaryocytic, monocytic, and granulocytic cell lineages. We report the first evidence that the t(1;7) is present in erythroid cells as well as in the granulocytic/monocytic lineage.

Trisomy 8 was detected in patient 4 in the erythrocytic, monocytic, and megakaryocytic lineages and in patient 2 in CD34-positive stem cells and in cells of the erythroid lineage. The CD13 + granulocytic/monocytic lineage in patient 2 was not involved, evidence that the mutation had occurred in a stem cell capable of differentiating only into erythrocytes. The aberration may have occurred in a stem cell capable of differentiating into both erythrocytes and granulocytes/monocytes, but did not confer any growth advantage on granulocyte/monocyte lineages. Our results are consistent with those of Kibbelaar et al. [19], who studied lineage involvement in MDS patients with trisomy 8 and t(1;7) by combined immunophenotyping and DNA ISH. Their results also indicated that the chromosome aberrations are limited to myeloid lineages.

The results and our unpublished data on cell lineage involvement in two patients with AML carrying the chromosome aberration trisomy 8 indicate that trisomy 8 may not be restricted to any specific cell lineage. In one patient, the trisomy was detected in CD64+ monocytes but not in GPA-positive cells of the erythrocyte lineage. On the other hand, all myeloid lineages were affected in the second AML patient, who also had trisomy 8. Whether the specific chromosome aberration leads to a growth advantage in a given cell lineage may depend on the genetic history. Patient 2 had a low frequency of trisomy 8 cells but morphologically dysplastic features in all myeloid lineages in BM, suggesting that trisomy 8 was not an early event in the malignant transformation.
To investigate whether the lymphoid cell lineages were affected, we performed FISH on immunophenotyped B, T, and LGL cells from the patient showing t(1;7). The frequency of lymphoid cells with three hybridization signals was less than 3%, indicating that most cells in the lymphoid lineages did not contain the abnormality. A small abnormal subpopulation of lymphoid cells may have existed; because of the background in FISH, the sensitivity of the technique does not allow detection of a cell population that consists of 3% of the whole.

Our results are consistent with those of Gerritsen et al. [20], who used FISH to investigate involvement of monosomy 7 in myeloid and lymphoid lineages in eight MDS patients. The monosomy was not detected in established B- or T-cell lines or in separated PB lymphocytes, but was present in the myeloid cell compartment. According to van Kamp et al. [21], MDS is a disorder with clonal hematopoiesis in cells of myeloid origin, whereas circulating T and B lymphocytes and NK cells in their material were not involved.

In our study t(1;7) was present in all myeloid lineages. We also showed that trisomy 8 may be present in all myeloid lineages, or in only a single cell lineage, which suggests that the mutations may occur in a stem cell with the ability to develop into either mature myeloid cells or in a stem cell committed to only one cell lineage. Our results do not exclude involvement of lymphoid cells in MDS.

This work was supported by the Sigrid Jusélius Foundation, the Finnish Cancer Society, the Academy of Finland and the National Council of Scientific and Technological Research (CONICET) from Argentina. The authors thank Heloisa Markkanen for skillful technical assistance.

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