Discrepant Cytogenetic and Fluorescence In Situ Hybridization Results in a 26-Year-Old Male with Early T-Cell Acute Lymphocytic Leukemia

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ABSTRACT: Analyzable G-banded metaphases were normal in bone marrow from a 26-year-old male having 80% blasts. Fluorescence in situ hybridization (FISH) using the centromeric probe, D7Z1, revealed 85% of interphase cells with one signal for chromosome 7. Chromosome painting revealed a chromosome 7 rearrangement in a few metaphases that were otherwise unanalyzable. A repeat bone marrow confirmed 3 of 20 metaphases, by G-banding, to have multiple rearrangements and aneuploidy, including a large derivative chromosome involving a complex rearrangement of chromosomes 5, 7, and 9; that is, der(5)t(5;9)(q31;q13)ins(5;7)(p15;q731q34), with loss of most of chromosome 7 (7pter→7q31); one normal 7 was present. Immunophenotyping characterized the patient’s condition as an early T-cell acute lymphocytic leukemia (ALL), with a population of cells suggesting biphenotypic leukemia. He attained a complete clinical remission with chemotherapy. Six months after the initial presentation he received an allogeneic bone marrow transplant. Three months later a CNS relapse was followed by a bone marrow relapse. At this time, eight months after transplant, repeat study of his bone marrow revealed the majority of metaphases had structural and numerical chromosome abnormalities similar to the small clone in the earlier study, including der(5)t(5;9)ins(5;7), but with two normal 7s. FISH showed two 7-centromere signals in interphase. The patient expired one month later. © Elsevier Science Inc., 1998

INTRODUCTION

Fluorescence in situ hybridization (FISH) can be used to detect structural or numerical chromosome abnormalities in interphase as well as in metaphase. Monosomy 7 and deletion of 7q are common abnormalities in myelodysplastic syndromes (MDS) and acute myelocytic leukemias (AML) [1], but are uncommon in acute lymphocytic leukemias (ALL) [2]. The few cases of acute leukemias with monosomy 7 have been suggested to arise in multipotent or myeloid stem cell precursors rather than lymphoid stem cells [3]. On the other hand, structural abnormalities of chromosome 7 are known to occur in T-ALL, usually in cell lines which are particularly difficult to study by routine cytogenetics [4, 5].

We report cytogenetic and FISH results from a patient with early T-cell ALL who, in initial routine cytogenetic analysis, had a normal karyotype. However, interphase FISH and subsequent metaphase analysis by G-banding and FISH revealed an abnormal clone with multiple chromosome abnormalities, including a complex structural abnormality involving chromosome 7.

CASE REPORT

A 26-year-old male presented with a right mandibular mass and subcutaneous nodules in his forearm. He had a WBC of 33,000 cmm, a platelet count of 125,000 cmm, and a normal hematocrit of 41%. A peripheral blood smear showed atypical mononcytoid and lymphoid cells with 10–15% blasts. Further evaluation revealed a 3 × 4 cm right submandibular hard lymph node, mild bilateral axillary and inguinal lymphadenopathy, a palpable spleen tip, and numerous small subcutaneous nodules over both forearms. A bone marrow aspirate showed a hypercellular marrow, normal megakaryocytes and erythroid cell lineage, but with 80% blasts of lymphoid appearance. Immunophenotyping showing 95% CD5, 90% CD7, and 68%...
CD33 was indicative of a mixed cell lineage leukemia. A lumbar puncture showed central nervous system (CNS) involvement with lymphoblasts. A computerized tomography (CT) scan of the thorax revealed a 3–4 cm anterior mediastinal mass. The patient was treated with vincristine, Adriamycin, prednisone, and intrathecal Cytarabine. His cerebrospinal fluid cleared but his bone marrow, on day 28 of induction, still had 80% blasts. The patient was hospitalized with a perirectal abscess and was subsequently transferred to another hospital where he became a candidate for a bone marrow transplant. The patient was treated with a two-week course of L-asparaginase and attained a complete remission. On day 45 his WBC was 5,300, hematocrit 31% and platelet count 211,000. He had no circulating blasts and on day 55 his bone marrow demonstrated complete remission. He was continued on intensification therapy for ALL with three cycles of cytoxan, cytarabine, 6 mercaptosins, and prophylactic intrathecal mix, and showed a marked decrease in hematopoetic elements. Four months later he underwent an allogenic bone marrow transplant from his HLA-compatible sibling. He remained in clinical remission approximately 13 months after his initial visit.

MATERIALS AND METHODS

Chromosome analyses from bone marrow aspirates were done from short-term cultures (24–48 hours) on three different occasions. Bone marrow was washed with Hanks’ balanced salt solution, and cultured in RPMI 1640 medium (GIBCO) supplemented with 20% FBS and antibiotics, without mitogen, for 24 to 48 hours. Cells were fixed in three changes of methanol:acetic acid (3:1) and dropped onto slides.Slides were stained with a modification of the trypsin-Giemsa banding method of Seabright [6]. For FISH, a modification of the method described by Pinkel et al. [7] was used. Slides were aged by incubation in 2 × SSC at 37°C for 30 min, and denatured in 70% formamide in 2 × SSC at 70°C for 4 minutes. Hybridizations were done using the alpha satellite probes, D7Z1 and D8Z2 (Oncor, Gaithersburg, MD) or painting library specific for chromosome 7 (Vysis, Downers Grove, IL). Probes were prepared and applied to the slides according to manufacturer’s instructions. The slides were incubated in a humidified chamber at 37°C overnight. Post-hybridization washes and probe detection were done using the manufacturer’s reagents and instructions.

RESULTS

A bone marrow was received by the Center for Human Genetics for cytogenetic study. Analysis of 20 cells by G-banding revealed a normal 46, XY karyotype. As a control for another study [8], FISH interphase analysis of chromosomes 7 and 8 was done using probes D7Z1 and D8Z2 (Oncor). The results revealed 85% of non-dividing nuclei from the patient’s sample to have a single signal for D7Z1 (Fig. 1). D8Z2, in comparison, showed a normal distribution of signals with >95% of nuclei showing the expected two signals. Because of the unexpected FISH result, an additional 30 metaphases were analyzed by G-banding, but analyzable cells still showed a normal 46, XY karyotype with no evidence of monosomy or structural abnormality involving chromosome 7. A repeat bone marrow sample, one month later, gave similar interphase FISH results with D7Z1 probes from both Oncor and Vysis. Study of interphase cells with a combination of a subtelomeric probe for 7q36-7qter and D7Z1 (Oncor) also confirmed loss of all or part of a chromosome 7 in 91% of interphase cells (Fig. 1B). No analyzable metaphases by G-banding were found in the second sample.

Metaphases from both samples were analyzed by FISH either using probe D7Z1 or a whole chromosome paint for chromosome 7 (Vysis). The majority of metaphases analyzed by FISH would not be analyzable by G-banding. Of 41 metaphases analyzed with D7Z1, 21 with poor morphology had one number 7: two of these appeared to have a ring chromosome (Fig. 1A). The remaining metaphases with fair-to-good morphology had equal-size signals on two G-group chromosomes. By painting, 17 of 20 metaphases had two chromosome 7s; three had one chromosome 7. One metaphase with very poor morphology had an apparent rearrangement of chromosome 7 (Fig. 1C); subsequent searching revealed three more cells with a similar rearrangement. Thus, this patient appeared to have a small population of metaphases with structural rearrangements, including at least two cells with a ring, that were not detected in metaphases analyzed by G-banding.

Independent study at New England Medical Center of a third bone marrow sample by G-banding revealed a small clone (3 of 20 cells) with multiple structural abnormalities (Fig. 2A), one of which was thought to involve a chromosome 7. A ring chromosome was also seen in one cell in this clone. A FISH study was not done. Approximately one month later, when the patient was in clinical remission, chromosome study of the patient’s marrow revealed that all cells had a normal karyotype. Interphase FISH at that time was also normal. Four months later the patient had an allogeneic bone marrow transplant. Five months later, chromosome and FISH studies of bone marrow revealed 80% of metaphases to have an abnormal karyotype with multiple chromosome abnormalities similar to the third sample but now with two apparently normal number 7s (Fig. 2B). FISH studies using D7Z1 now revealed a normal number of signals for chromosome 7 in interphase. By G-banding, a large A-size chromosome in both samples (Figs. 2A and 2B) appeared to involve a translocation between chromosomes 5 and 9, with an insertion of a piece of chromosome 7, der(5)(5;9)(q31;q13)ins(5;7)(p15;q31;q734). Chromosome painting confirmed a small piece of chromosome 7 to be present in a similarly large A-size chromosome (Figs. 1C and 1D). Absence of a telomere and centromere signals in
interphases from sample 1 (Fig. 1B) was consistent with an interstitial insertion of 7q material in the derivative chromosome.

DISCUSSION

Monosomy 7 is a common finding in MDS, and is associated with a poor prognosis, with usually rapid transformation to AML [1]. Several studies of MDS suggest that monosomy 7 clones may be detected in interphase by FISH that are not detected by conventional cytogenetics [8–11]. In fact, only a small number of cases (3/117) in these studies were confirmed by conventional cytogenetics. One was shown to precede the onset of acute leukemia [10]. Paradoxically, in both MDS and AML, the percent of cells with one signal in interphase is frequently lower than the percent of metaphases with monosomy 7 [10–14]. Among cases with AML and CMML, no cases of mono-
Figure 2  (A) Karyotype of abnormal clone from patient’s bone marrow prior to transplant: 44,XY,t(1;?)(q23;?), +der(4)t(4;11)(q12;q21),der(5)t(5;9)(q31;q13)ins(5;7)(p15;q31q34),−7,−9,der(9)t(9;11)(p13;q21),−11,der(16)t(16;?) (q13;?),−21,del(22)(q13),+mar1[3]/46,XY[17]. (B) Karyotype of abnormal clone after bone-marrow relapse: 47,XY, t(1;?)(q23;?),der(3)t(3;?),+der(4)t(4;11)(q12;q21),der(5)t(5;9)(q31;q13)ins(5;7)(p15;q31q34),−9,der(9)t(9;11)(p13;q21), der(11)(11;?)p13;?,+12,der(16)t(16;?)q13;?,−21,−22,+mar1,+mar2[16]/46,XY[4]. Arrowheads point to chromosome aberrations that are the same in both karyotypes. Small arrows point to chromosome abnormalities that are new in (B) or are involved in a structural or numerical change from (A) to (B). Large arrow points especially to numerical change in chromosome 7 from (A) to (B). Der(5) in both karyotypes involves segment of chromosome 7 that was detected by chromosome 7 painting probe (see Fig. 1). Numerical changes in chromosome 7 were detected by FISH studies of interphase nuclei using alpha satellite probe, D7Z1 (see Fig. 1). Karyotype designations are according to ISCN 1995 [22].
Monosomy 7 by FISH, undetected by cytogenetics, are reported [10, 14]. In contrast, observation of monosomy 7 by conventional cytogenetics in ALL is rare. In 21 consecutive cases of childhood ALL studied by Kadam et al. [9], five had apparent monosomy 7, detected solely in interphase by FISH, in 12–43% of cells. However, none of these five cases was demonstrated to have monosomy or structural abnormalities of chromosome 7 in metaphase. Five additional cases had abnormalities of 7 detected by both cytogenetics and FISH. In contrast to MDS or AML, the percent monosomy 7 in the latter was higher in interphase than in metaphase. In MDS, the monosomy 7 clones were shown in several studies to be derived from myeloid cells, but not from the lymphoid cells [15–18]. In the study by Kadam et al. [9], the majority of cases had pre B-ALL markers, but monocyte populations were not specifically targeted in the FISH studies. Of seven cases with monosomy 7, one had T-ALL.

The present case represents the second with T-ALL, with an abnormality of chromosome 7 detected initially by interphase FISH but undetected by conventional cytogenetics. We also demonstrated the utility of metaphase FISH to analyze the very poor metaphases, by which we determined that apparent monosomy 7 in interphase was probably due to a translocation between a chromosome 7 and an A-size chromosome, subsequently shown by G-banding to involve chromosomes 5, 7, and 9. It was also evident from metaphase FISH that a significant subclone had lost an entire chromosome 7.

Although considerable effort has been expended in using interphase probes to detect monosomy 7 in MDS, with results being of somewhat uncertain clinical significance, studies of B- and T-ALL in children and young adults have been under-represented. In contrast to MDS and AML, where cells with monosomy 7 appear to have a proliferative advantage [15], chromosome abnormalities involving 7 in ALL appear to be more frequently masked [3]. Approximately 26% of childhood T-cell ALL patients reportedly have normal karyotypes [19].

Monosomy 7 is probably rare in ALL, although it has been associated with mixed cell lineages [2]. As we have demonstrated, some cases with apparent monosomy 7 by interphase FISH may, in fact, represent structural abnormalities of 7 rather than simple aneuploidy. Yamada et al. [4] reported one B-cell patient with a very small number of cells with i(7q). However, Southern blot analysis determined i(7q) was present in 70% of cells. Chromosome re-arrangements involving bands 7p15 and 7q34, in particular, have been associated with T-cell leukemia-lymphomas [5, 20–21]. The use of a telomere probe, as we have done, or of cosmid probes distal to these bands in the long and short arms of chromosome 7, in addition to the alpha-satellite probe, therefore, might be an effective interphase FISH approach for detecting structural abnormalities of chromosome 7. This could be of particular benefit for early detection in the high risk, poor prognosis group of patients in whom routine cytogenetic detection of abnormal clones is typically difficult.

REFERENCES

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