

DETECTION OF MONOSOMY 7 BY FLUORESCENCE
IN SITU HYBRIDIZATION IN ACUTE
NONLYMPHOCYTIC LEUKEMIA AND
MYELODYSPLASTIC SYNDROME

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Summary Fluorescence *in situ* hybridization (FISH) with a chromosome 7 specific alpha satellite DNA probe was used to detect monosomy 7 in interphase and metaphase cells obtained from patients with myelodysplastic syndrome (MDS) and acute nonlymphocytic leukemia (ANLL). Chromosome analysis revealed monosomy 7, either alone or as part of a complex chromosome abnormality, in all cell samples. FISH analyses of 12 marrow samples and a blood sample using a chromosome 7 specific alpha satellite DNA probe revealed a single fluorescence spot in 80.5-97.5% of interphase cells indicating monosomy 7. In contrast, 83.5-92.0% of the same cells had two copies of chromosome 17 as two fluorescent spots were detected using a chromosome 17 specific alpha satellite DNA probe used as a positive control. The proportion of interphase cells with monosomy 7 did not correlated with the percentage of metaphase cells with monosomy 7 detected by conventional karyotyping or with the percentage of blast cells in the bone marrow.

Key Words monosomy 7, fluorescence *in situ* hybridization, myelodysplastic syndrome, acute nonlymphocytic leukemia

INTRODUCTION

Monosomy 7 is found as a single abnormal change or in association with complex chromosome abnormalities in patients with *de novo* acute nonlymphocytic leukemia (ANLL), therapy related leukemia, and myelodysplastic syndrome (MDS) (Sandberg, 1990). Previous studies revealed that monosomy 7 correlated with a poor prognosis in these patients (Rowley *et al.*, 1981; Le Beau *et al.*, 1986; Horiike *et al.*, 1988; Shiffer *et al.*, 1989).

Monosomy 7 can be detected in neoplastic cells by cytogenetic analysis of

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metaphase chromosomes. However, this method is limited to the study of metaphase cells, representative of mitotic cells, and not of all cells present in short-term cultures. Fluorescence *in situ* hybridization (FISH) using chromosome specific alpha satellite DNA probes has made it possible to detect numerical chromosome aberrations in interphase as well as metaphase cells. The satellite DNA probes recognize the centromeric region of each chromosome, thereby identifying numerical chromosome alterations in tumor cells (Cremmer *et al.*, 1988; Nederlof *et al.*, 1989; Dekken *et al.*, 1989; Poddighe *et al.*, 1991).

FISH was performed using a chromosome 7 specific alpha satellite DNA probe in six patients with MDS and three with ANLL. The results of FISH on interphase cells showed that a large population of cells had been replaced by a monosomy 7 clone, regardless of the proportion of blast cells at the time of diagnosis. Four MDS patients progressed to ANLL and incidence of monosomy 7 during the two phases of their disease was compared.

MATERIALS AND METHODS

Patients. Six patients with MDS and three with *de novo* ANLL were selected; all patients had cells with monosomy 7 and some had other complex chromosome aberrations as detected by ordinary karyotype analysis. Diagnosis was made according to the FAB criteria (Bennett *et al.*, 1976, 1982), and clinical data regarding these patients are summarized in Table 1. Four of the six MDS patients progressed to ANLL, and samples from the leukemic phase were also examined.

Chromosome analysis. Bone marrow cells were obtained from all patients with the exception of patient 9, from whom peripheral blood was obtained. Cells were cultured in RPMI 1640 with 15% fetal bovine serum for 24 hr at 37°C without mitogen and treated with colcemid for the last 1 hr of culture. Chromosome preparations were made according to standard procedures including treatment with a hypotonic solution and fixation with a methanol/acetic acid mixture (3:1). Chromosomes on slide glasses were banded by the trypsin-Giemsa method (Whang and Federoff, 1972). Well spread metaphase cells were photographed and karyotyped according to the International System for Cytogenetic Nomenclature (1991). A culture of lymphocytes from a normal female stimulated with phytohemagglutinin was used as a control. After chromosome preparations were made, the remainder of the cells were stored at -20°C in the same fixative.

Fluorescent in situ hybridization (FISH). Preparations were made from preserved materials in the same manner used for chromosome analysis. The slides were treated with proteinase K (60 µg/ml in PBS, pH 7.0) at 37°C for 7 min. After removal of proteinase K in PBS, slides were dehydrated in 70, 90, and 100% ethanol. Chromosome DNA was then denatured in 70% formamide in 2×SSC at 70°C for 90 sec, dehydrated again in ethanol at 4°C and dried.

Biotinylated chromosome specific alpha satellite DNA probes, p7t1 for chro-

mosome 7 and p17H8 for chromosome 17, were purchased from Oncor, USA. The p17H8 was used as a positive control. These probes were dissolved in formamide and denatured at 75°C for 10 min. *In situ* hybridization was performed according to the method previously described (Laurence *et al.*, 1988; Takahashi *et al.*, 1989) with modifications. Twenty microliters of hybridization buffer (50% formamide, 2×SSC, 400 µg/ml of salmon sperm DNA, 400 µg/ml of *E. coli* tRNA, and 10% dextran sulfate) containing 100 ng/ml of probe were placed on the slides, covered with parafilm, and incubated in a humidified chamber at 42°C for 10–16 hr. The slides were washed for 15 min in each of the following buffers; 50% formamide in 2×SSC, 2×SSC, and 1×SSC. Slides were washed at 45°C following incubation with the p7t1 probe and at 48°C for the p17H8 probe. Fluorescent signals were amplified as previously described with biotinylated goat anti-avidin antibody and fluorescein-avidin DCS (Pinkel *et al.*, 1986; Nakagawa *et al.*, 1992). The slides were then counter-stained with propidium iodide and mounted in antifade agent.

The number of fluorescent spots in each nucleus was counted under a fluorescence microscope with a filter combination Nikon B2-A according to reported methods (Cremmer *et al.*, 1988; Nederlof *et al.*, 1989; Dekken *et al.*, 1989). Two hundred single round nuclei were counted; not including cell clusters, broken cells, metaphase cells or cells with very weak or irregularly shaped signals.

RESULTS

Chromosome analysis

The results of chromosome analysis are summarized in Table 1. Monosomy 7 was found in all patients in greater than 50% of the metaphase cells analyzed. In five patients, monosomy 7 was either the sole abnormality or was associated with only one other anomaly. In four patients, the karyotypes were complex, and monosomy 7 was detected as one of the abnormalities. In patients 1–4, chromosome analysis was performed at the MDS and terminal leukemic phases. The population of cells with monosomy 7 increased in the leukemic phase in patient 4, disappeared in patient 2 (in whom only two mitotic cells were available for karyotyping), and remained essentially unchanged in patients 1 and 3.

FISH

In a control sample of PHA stimulated lymphocytes, 92 and 90% of interphase cells exhibited two fluorescent spots when hybridized to p7t1 and p17H8, respectively (Table 2). Representative metaphase and interphase cells are shown in Fig. 1.

Results of FISH in nine patients are summarized in Table 2. Following hybridization with the chromosome 7 specific p7t1 probe, a single fluorescent spot was observed in 70.5 to 97.5% of 200 interphase cells examined from each patient. In contrast, a single spot was detected in only 7.0 to 13.0% of cells probed with

Table 1. Clinical and cytogenetic features in nine patients with monosomy 7.

| No. of patients* | Age/Sex | FAB | BM blast (%) | Survival | Karyotype | Cells with -7/ karyotyped (%) |
|------------------|---------|--------|--------------|----------|--|----------------------------------|
| 1a** | 29/F | BAEB | 15.2 | 30M | 45, XX, -7[24] | 24/24 (100) |
| b | | M2 | 74.2 | | 45, XX, -6, +der(6)t(1;6)(q2;p2), -7[1]/45, XX, -7[5] | 6/6 (100) |
| 2a | 65/M | CMML | 17.4 | 10M | 45, XY, -7[12]/46, XY[2] | 12/14 (85.7) |
| b | | M4 | 55.6 | | 46, XY[1]/45, Y, -X[1] | 0/2 (0.0) |
| 3a** | 37/F | RAEB | 16.8 | 2M | 45, XX, -7[21]/46, XX[3] | 21/24 (87.5) |
| b | | M4 | 36.0 | | 45, XX, -7[10]/46, XX[2] | 10/12 (83.3) |
| 4a** | 70/M | RAEB-T | 20.6 | 7M | 44, XY, -3, del(5)(q31q35), -7, -12, +mar[1]/46, XY[4] | 11/15 (70.3) |
| b | | M2 | | | 44, XY, -3, del(5)(q31q35), -7, -12, +mar[8] | 8/8 (100) |
| 5 | 43/M | CMML | 8.0 | 4M | 45, XY, -7[3]/46, XY[3] | 3/6 (50.0) |
| 6** | 67/M | RAEB-T | 20.8 | 1M | 43, XY, del(5)(q13q33), -7, -17, +der(17)t(17;22)(q11;p11), -18, -20[29]/44, idem, +mar[3] | 32/32 (100) |
| 7 | 41/M | M4 | 31.6 | 12M | 45, XY, -7[21]/46, XY[10] | 21/31 (67.7) |
| 8 | 61/M | M2 | 62.0 | 5M | 45, XY, del(3)(q21q24), -7[13]/46, XY[2] | 13/15 (86.7) |
| 9** | 32/F | M4 | 75.0*** | 2M | 45, XX, -7, t(1;7;8)(q31.2;q36;q21), t(3;3)(q21;q26.2)[21] | 21/21 (100) |

* Samples were obtained at MDS phase (a) and at leukemic phase (b). Intervals between samples a and b are 15M, 7M, 1M, and 3M, in case 1, 2, 3, and 4, respectively. ** Karyotypes were reported by Horike *et al.* (1988). *** Percent of blasts in peripheral blood. RAEB, refractory anemia with excess of blasts; CMML, chronic myelomonocytic leukemia; RAEB-T, RAEB in transformation; M2, acute myeloblastic leukemia with maturation; M4, acute myelomonocytic leukemia.

Table 2. Number of fluorescent spots detected in control lymphocytes, MDS and ANLL interphase cells hybridized with chromosome specific biotinylated probes.

| Patient No. | Chromosome 7* | | | | Chromosome 17* | | | |
|-------------|---------------|------|-----|-----|----------------|------|-----|-----|
| | 1 | 2 | 3 | ≥4 | 1 | 2 | 3 | ≥4 |
| Control | 7.0 | 92.0 | 0.0 | 1.0 | 8.0 | 90.0 | 1.0 | 1.0 |
| 1a | 70.5 | 28.5 | 0.5 | 0.5 | 10.0 | 86.0 | 2.5 | 1.5 |
| b | 83.0 | 16.5 | 0.0 | 0.5 | 7.0 | 92.0 | 1.0 | 0.0 |
| 2a | 81.0 | 16.0 | 2.0 | 1.0 | 10.0 | 86.0 | 2.5 | 1.5 |
| b | 81.0 | 18.0 | 0.5 | 0.5 | 13.0 | 84.0 | 2.0 | 1.0 |
| 3a | 83.0 | 17.0 | 0.0 | 0.0 | 13.0 | 84.0 | 2.0 | 1.0 |
| b | 97.5 | 2.5 | 0.0 | 0.0 | 9.5 | 90.0 | 0.0 | 0.5 |
| 4a | 77.5 | 21.0 | 0.5 | 1.0 | 10.0 | 86.0 | 3.0 | 1.0 |
| b | 95.0 | 5.0 | 0.0 | 0.0 | 11.0 | 86.0 | 1.5 | 1.0 |
| 5 | 89.5 | 10.0 | 0.5 | 0.0 | 13.0 | 85.0 | 1.0 | 1.0 |
| 6 | 88.5 | 11.5 | 0.0 | 0.0 | 7.0 | 83.5 | 2.0 | 2.5 |
| 7 | 90.0 | 10.0 | 0.0 | 0.0 | 9.0 | 89.5 | 0.5 | 1.0 |
| 8 | 92.0 | 8.0 | 0.0 | 0.0 | 11.0 | 88.5 | 0.5 | 0.0 |
| 9 | 70.5 | 29.5 | 0.0 | 0.0 | 13.0 | 86.0 | 1.0 | 0.0 |

* Numerals represent number of fluorescent spots. a, Samples obtained at MDS phase. b, Samples at terminal leukemic phase. Numbers of fluorescent spots are expressed as a percentage of the 200 cells analyzed in each group.

the chromosome 17 specific p17H8 DNA (Fig. 2). In patients 1, 3, and 4, there were fewer cells with a single spot in MDS phase as compared to the leukemic phase; from 70.5 to 83.0%, 83.0 to 97.5%, and 77.5 to 90.0%, respectively (Statistically significant with Chi square test, $p < 0.05$). In patients 2 and 5, we noticed only one signal on polymorphonuclear cells or monocytes determined from the shape of the nuclei using p7t1 (Fig. 3).

DISCUSSION

Acquired loss of chromosome 7 has frequently been found in patients with MDS and ANLL (Le Beau *et al.*, 1986). This chromosome abnormality can be detected either as the sole cytogenetic abnormality or in association with trisomy 8, whole or partial monosomy 5, or other complex abnormalities (Sandberg, 1990). The prognosis of all patients with monosomy 7 is uniformly poor and most patients die of leukemia or related complications within months to a few years (Yunis *et al.*, 1986a, b).

Nine patients with ANLL or MDS were studied, who exhibited monosomy 7 in 50 to 100% of metaphase cells karyotyped by routine chromosome analysis. Then, FISH was utilized with a chromosome 7 specific alpha satellite DNA probe

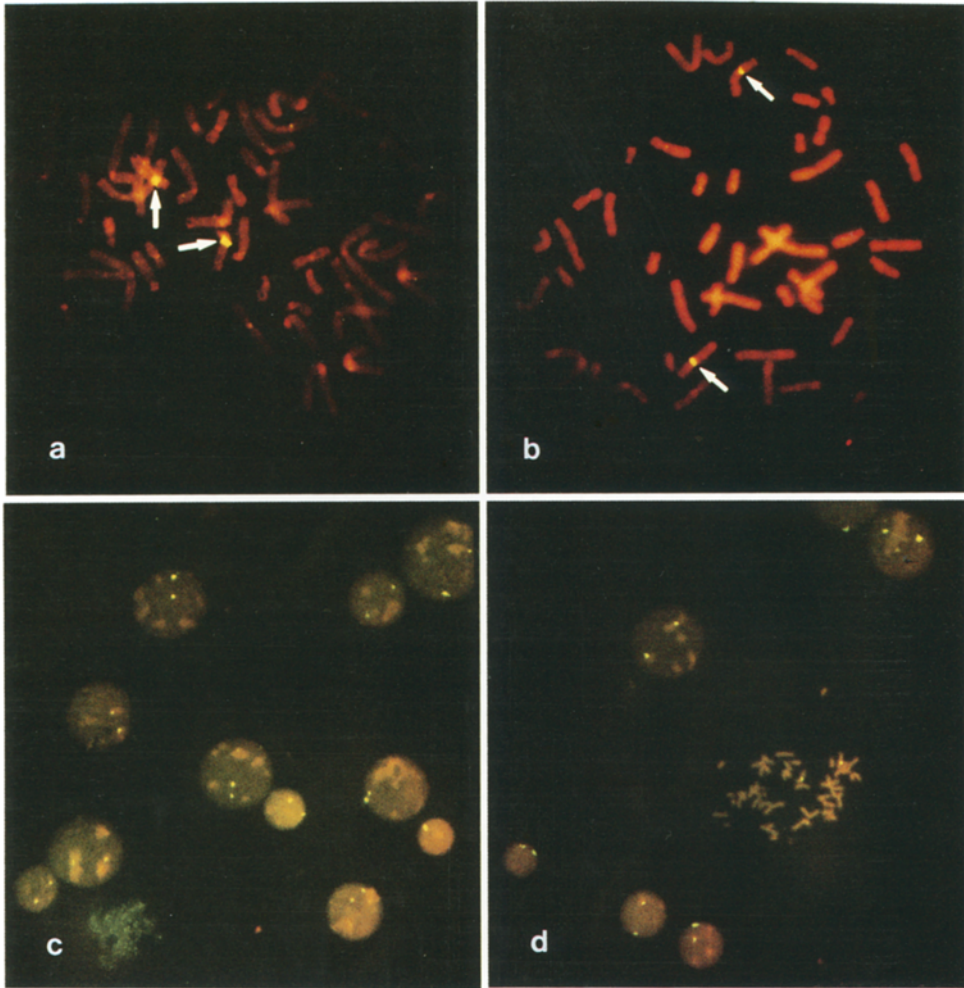


Fig. 1. Results of FISH with p17H8 (a, c) and p7t1 (b, d) probes on metaphase and interphase cells obtained from PHA-stimulated lymphocyte culture. Arrows indicate fluorescent spots at centromeric lesions of chromosome 17 and 7 on a metaphase cell, respectively (a, b). Two fluorescent spots are shown in the majority of interphase nuclei (c, d).

Fig. 2. FISH of patient 7 reveals one fluorescent spot in 90% of interphase cells hybridized with p7t1 (a) and two fluorescent spots in 89.5% of cells hybridized with p17H8 (b).

Fig. 3. A metaphase and two interphase cells from a CMML patient (patient 2) showing a fluorescent spot with p7t1 (a). Polymorphonuclear granulocytes or monocytes of the same patient also exhibit one signal with p7t1 (b) (filter combination Nikon B2-E).

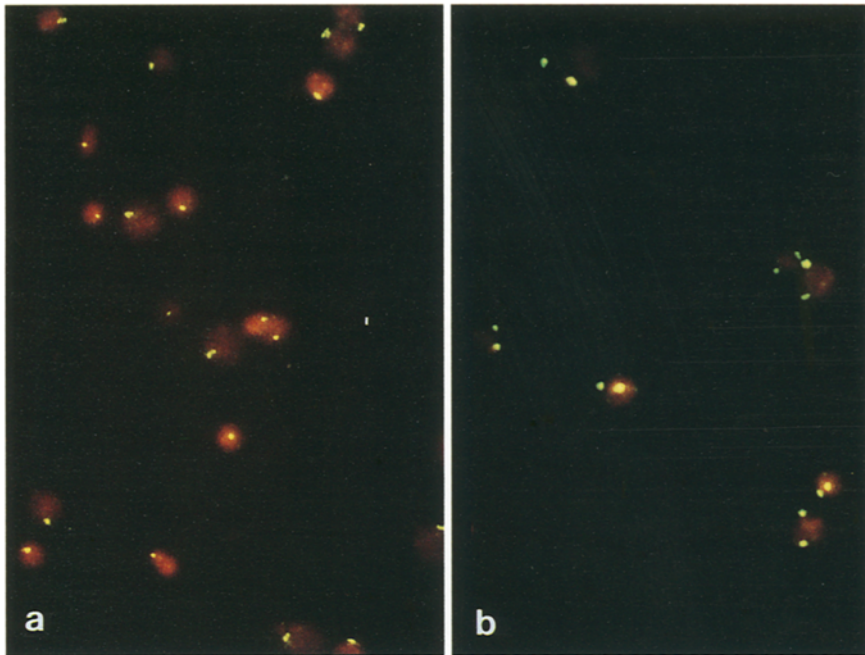


Fig. 2.

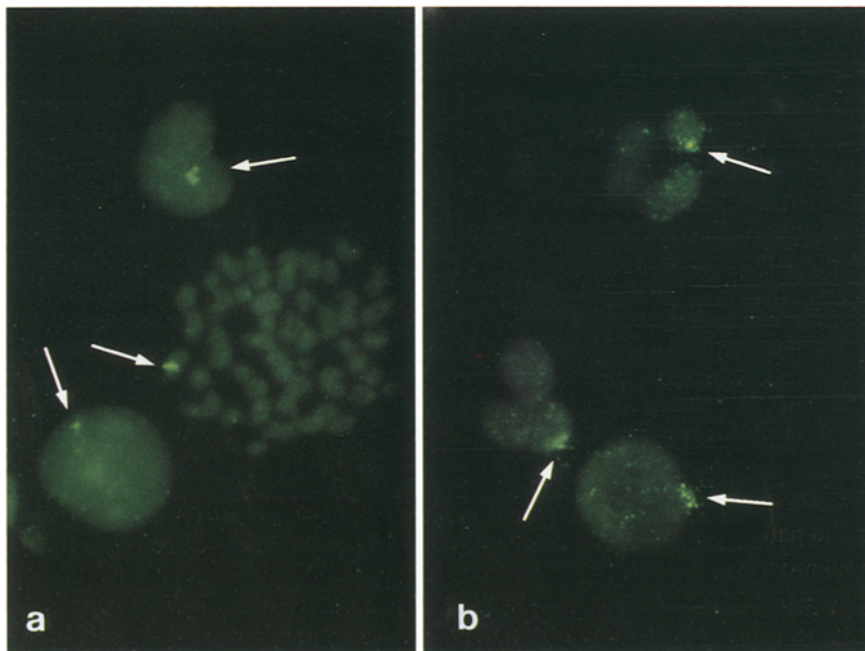


Fig. 3.

p7t1 to detect monosomy 7 in interphase cells. A single fluorescent spot confirms the diagnosis of monosomy 7, and 70–90% of cells from all patients were positive for a single fluorescent spot. A chromosome 17 alpha satellite probe was used as a positive control in FISH analysis and two fluorescent spots, indicative of two intact centromeric regions of chromosome 17, were detected in greater than 84% of interphase cells examined from all patients. In contrast, interphase cells obtained from normal lymphocytes were positive for two fluorescent spots in greater than 90% of cells following hybridization with both the p7t1 and p17H8 probes.

High stringency condition was employed in order to minimize cross-hybridization of the probes to other alpha satellite DNA families. Fluorescent signals were amplified by biotinylated anti avidin and fluorescein-avidin DCS. A reduced signal is hypothesized to be due to misintegration of the probe or overlapping of two different signals on the same plate. Additionally, in the absence of stringent conditions, cross-hybridization of the probe to homologous alpha satellite DNA might increase the number of fluorescent spots detected (Nederlof *et al.*, 1989). Similar findings have been reported by other investigators who performed FISH with chromosome specific DNA probes (Dekken *et al.*, 1989; Kiechle-Schwartz *et al.*, 1991; Poddighe *et al.*, 1991).

The proportion of monosomy 7 cells detected with FISH did not correlate with that in routine karyotypic analysis in our 13 samples ($r = -0.17$). Since FISH is used to analyze interphase cells and karyotyping is performed on metaphase cells, it is possible that the proportion of monosomy 7 cells differs in these populations. However, the number of cells analyzed by karyotyping may be too small to be conclusive; less than ten metaphases were karyotyped in four samples. In a sample from the leukemic phase of patient 2, only two mitoses were karyotyped and monosomy 7 could not be detected. We utilized FISH on 200 bone marrow cells from this patient, and monosomy 7 was present in a large proportion of the cells. In such cases, conclusive cytogenetic information can only be obtained by performing interphase cytogenetics.

The proportion of myeloblasts in bone marrow smears varied from 8.0 to 74.2% among the samples examined. Monosomy 7 cells was detected in greater than 70% of the cells examined with FISH regardless of the percent blasts in the sample. These results indicate that a replacement of bone marrow cells with monosomy 7 cells is independent of the percentage of blast cells. In two chronic myelomonocytic leukemia patients, one fluorescent spot was observed with the chromosome 7 specific probe in mature neutrophils and monocytes (Fig. 3).

In the patients who progressed from the MDS to leukemia stage of their disease, the population of interphase cells with monosomy 7 tended to increase. Monosomy 7 clones existed in the morphological blast cell population as well as differentiated cells. Kere *et al.* (1987) detected a loss of heterozygosity of the chromosome 7 genome in granulocytes and monocytes of MDS patients. Tachibana *et al.* (1988) reported that the monosomy 7 genotype was detected in erythroid as well as gran-

ulocyte/macrophage progenitors in patients with MDS using the colony assay technique. Thus, the target cell of clonal neoplastic change is hypothesized to be a primitive hemopoietic progenitor cell capable of differentiating into granulocyte/monocyte, erythrocyte, and megakaryocyte lineages (Michiels *et al.*, 1986).

FISH allowed a more sensitive and specific analysis of interphase chromosome than conventional karyotype technique (Jenkins *et al.*, 1992). The use of FISH may also enable the detection of a residual monosomy 7 cell clone in ANLL and MDS patients who have achieved complete remissions. Thus, this tool may provide information which can be invaluable in assessing the efficacy of various chemotherapies.

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