

CLONALITY STUDY BY FLUORESCENCE IN SITU HYBRIDIZATION OF A PATIENT WITH REFRACTORY ANEMIA WITH RINGED SIDEROBLASTS AND MONOSOMY 7

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ABSTRACT

Myelodysplastic syndromes (MDS) are stem cell diseases but it is still controversial whether chromosomal abnormalities occurring in these disorders affect a multipotent stem cell or a committed progenitor. We studied a case of refractory anemia with ringed sideroblasts (RARS) and monosomy 7 in 100% of examined metaphases. Using the fluorescence *in situ* hybridization (FISH) technique with a probe specific for the centromeric region of chromosome 7, we demonstrated that 15% of BM cells fixed in acetic acid/methanol exhibited a normal diploid karyotype. Applying the FISH technique on PB cells smeared onto a slide, we observed that lymphocytes maintain two chromosomes 7, whereas other leukocytes exhibited monosomy 7.

Our study confirms that chromosomal abnormalities found in MDS can occur in cells capable of differentiation along granulocytic and monocytic lineages, but not along the lymphocytic lineage.

Key words: myelodysplastic syndrome, monosomy 7, clonality

Monosomy 7 is one of the most frequent numerical chromosomal abnormalities observed in both de novo and therapy-related myeloid disorders. Loss of chromosome 7 in primary myelodysplastic syndromes (MDS) is more frequent in refractory anemia with excess of blasts (RAEB), in RAEB in transformation and in chronic myelomonocytic leukemia, whereas it is less common in refractory anemia (RA) and RA with ringed sideroblasts (RARS).¹ Monosomy 7 is associated with an unfavorable prognosis because it indicates a high probability of transformation into acute phase of disease.

It has been shown that MDS result from the proliferation of a pluripotent stem cell capable of differentiating along myelomonocytic and lymphocytic lineages. The clonal origin of MDS has been demonstrated using biochemical,² cytogenetic³ and molecular techniques.⁴

Recently, with the advent of the fluorescence *in situ* hybridization (FISH) technique, it has become possible to study features of both mitotic dividing and interphase cells; this increases the number of cells that can be studied. Furthermore, FISH can be applied on peripheral blood (PB) cells fixed on a slide without any pretreatment.⁵ Using informative probes (i.e. the centromeric regions specific for the chromosome found to be monosomic or trisomic with conventional cytogenetics), it is therefore possible to verify which leukocytes carry the chromosomal abnormality.

In this article we report a typical case of RARS that exhibited monosomy 7 in all bone marrow (BM) metaphases examined at diagnosis. Utilizing a chromosome 7 centromeric probe, monosomy 7 was confirmed in a high percentage of BM cells, but not in all the cells analyzed. Applying FISH with a simplified tech-

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nique on PB smears we observed that polymorphonuclear cells and monocytes were monosomic, whereas all the lymphocytes maintained two chromosomes 7.

Case report

A 62-year-old male was first admitted to our ward in January 1994, because of pancytopenia. He had been working as a painter for several years and his past history was non-contributory, especially regarding exposure to myelotoxic drugs.

Physical examination revealed striking pallor of the skin and mucous membranes. There was no lymphadenopathy and the spleen was not enlarged. Laboratory findings showed severe normocytic, normochronic anemia (Hb 5.0 g/dL, MCV 94.2 fL, MCH 29.8 pg, retic. $30 \times 10^9/L$), leukopenia with absolute neutropenia (WBC $2 \times 10^9/L$, differential count N 28%, L 57%, M 15%), slight thrombocytopenia ($136 \times 10^9/L$). The serum iron concentration was 72 $\mu\text{g/dL}$, total iron binding capacity 201 $\mu\text{g/dL}$ and ferritin concentration 396 ng/mL. Serum cobalamin and folate levels were both in the normal range. Direct and indirect antiglobulin tests were negative and haptoglobin was 189 mg/dL. Bone biopsy disclosed increased marrow cellularity characterized by erythroid hyperplasia with marked dysplastic aspects, consisting principally of megaloblastoid maturation and nuclear fragmentation. The number of granulocytic and megakaryocytic precursors was reduced. More than 15% of marrow cellularity was identified as ringed sideroblasts by means of the Prussian blue reaction, and this observation was in agreement with the diagnosis of RARS.

Materials and Methods

Cytogenetic analysis

Cytogenetic analysis was performed with the Q-banding technique on BM cells routinely processed after 24 hours of culture. Four slides were prepared: two were utilized for conventional cytogenetics and two for interphase cytogenetics. At least 20 metaphases were examined,

whereas for the FISH analysis 1,000 nuclei were scored in areas of the slides where cells were not crowded.

Peripheral blood smears

PB samples were smeared onto glass slides, air dried, fixed with absolute methanol and used directly for FISH without any pretreatment.

Controls

As control we selected a patient with RA with a normal karyotype. We used the same probe α -7 on BM cells fixed in acetic acid/methanol and on PB cells smeared onto a slide to evaluate the efficiency and reproducibility of the methodology.

FISH

The DNA probe employed was specific for the α -satellite sequences of the centromeric region of chromosome 7 (D7Z1, Oncor Gaithersburg).

BM slides obtained from the cytogenetic procedure were treated with RNase and Proteinase K, as previously described.⁶ Denaturation, hybridization and post hybridization washings were carried out according to manufacturer's instructions.

Detection and amplification of the biotinylated probes were achieved using fluorescein isothiocyanate (FITC)-labelled avidin (Vector laboratories) (5 $\mu\text{g/mL}$ in 4 x SSC, 0.05% tween, 1% BSA) and biotinylated goat anti-avidin (Vector laboratories) (5 $\mu\text{g/mL}$ in PBS). An antifade solution containing 1 $\mu\text{g/mL}$ propidium iodide was used to counterstain BM slides, whereas for PB smears no counterstain was employed.

Results

Table 1 summarizes cytogenetic and FISH results from the control and from the patient. The patient showed monosomy 7 without any structural abnormality in all 20 examined metaphases. With an α -7 probe the karyotypically normal control showed two signals, as expected, in 94.5% of BM cells and in 93% of PB leukocytes fixed on a slide. FISH performed on the same patient cellular sample utilized for the

Table 1. FISH hybridization spots per nucleus expressed in percentages.

| Cytogenetics (metaphases) | BM cells | | | | PB cells | | | |
|------------------------------|----------|------|------|-----|----------|----|----|---|
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| Control 46,XY, (30) | 2.1 | 3.2 | 94.5 | 0.2 | 5 | 2 | 93 | - |
| Patient 45,XY,-7 (20) | 8.7 | 75.8 | 15.5 | - | 6 | 50 | 44 | - |

cytogenetic study demonstrated that more than 75.8% of cells exhibited one signal (indicative of monosomy 7), 15.5% showed two signals (diploid cells) and 8.7% displayed very weak hybridization signals or no signal at all (methodological error). Examination of PB leukocytes fixed on a slide and hybridized with the same α -7 probe allowed us to observe that the 44 lymphocytes tested showed two signals, whereas 50 polymorphonucleated cells and monocytes exhibited one signal only (Figure 1). Lastly, 6 leukocytes showed a diffused hybridization signal or no hybridization signal at all (methodological error).

Discussion

The FISH technique may be applied in numerous circumstances and offers certain advantages: it can be performed not only on metaphases but also on interphase nuclei from the same cytogenetic preparation, allowing examination of a high number of cells in cell cycle periods other than the mitotic phase. Furthermore, it can be applied to whole cells fixed on a slide without culture or specific pretreatment. Several studies have suggested that FISH is capable of detecting numerical chromosomal abnormalities in hematological disorders.⁷

In this report we present a patient with RARS who exhibited monosomy 7 in all metaphases examined at diagnosis. When submitted to FISH analysis, these BM cells were found to be less than 100% monosomic because of the persistence of diploid cells (more than 15%) not identified with conventional cytogenetics. This discrepancy suggests that cells carrying monosomy 7 have a shorter replication time than dis-

omic ones and/or that they are the only ones actively proliferating. In addition to BM cells, we analyzed a PB sample smeared directly onto a slide, air dried, fixed in methanol and immediately used for FISH. From a technical point of view, we observed that PB cells treated in this way exhibited clear signals, that their cellular structure was not damaged, that cell morphology remained sufficiently intact and that leukocytes could be recognized by the size and shape of their nuclei.

PB cells from the patient demonstrated that all the lymphocytes examined maintained a normal karyotype, whereas all the other nucleated cells (i.e. granulocytes and monocytes) were monosomic (Figure 1). This finding suggests that a chromosomal aberration occurred in a pluripotent cell capable of granulocytic and monocytic but not lymphocytic differentiation. This observation is not necessarily in contrast with the origin of the neoplastic clone in MDS, which take place in a stem cell that is also capable of lymphocytic differentiation.⁸ The chromosomal abnormality in our patient can be considered as a later event involving a genetically unstable subclone not capable of lymphocytic differentiation. Similar results were recently reported using FISH⁹⁻¹⁰ and other techniques;¹¹⁻¹² lymphocytes are not involved in chromosomal abnormalities in MDS. This observation may explain the fact that MDS can only evolve into acute non lymphocytic leukemias: the clone

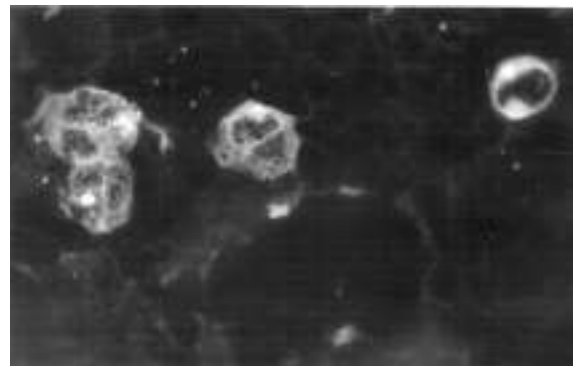


Figure 1. Four peripheral blood leukocytes fixed with absolute methanol and hybridized with a probe specific for the centromeric region of chromosome 7. A lymphocyte with two well-defined hybridization signals is present at the right; the other three leukocytes are polymorphonuclear cells showing a single hybridization signal indicative of monosomy 7.

capable of acquiring the chromosomal abnormality is predisposed to progression into overt leukemia.

In conclusion, our findings suggest that interphase FISH together with a centromeric probe for chromosome 7 is a sensitive method not only for confirming the monosomy of BM cells, but also for verifying which mature PB cells carry the aberration. This represents a further clinical application of FISH.¹³

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