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MISLEADING CYTOGENETIC EVIDENCE OF RELAPSE IN DONOR CELLS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION CORRECTED BY FLUORESCENT IN SITU HYBRIDIZATION

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The possibility of a leukemic relapse in donor cells after allogeneic bone marrow transplantation (BMT) has been recognized since 1971,[1] and it is usually demonstrated by means of karyotypic or molecular studies. This phenomenon has also been reported in chronic myelogenous leukemia (CML).[2]

Recently, FISH (*fluorescent in situ hybridization*) analysis has been employed to characterize hematopoietic cells in mixed chimeras,[3] to obtain further evidence about the suspected donor origin of leukemic relapse after BMT,[4] and even to demonstrate the donor origin of secondary neoplasms after solid allografts.[5]

We report here the case of a transplanted patient with CML, in whom FISH analysis allowed us to disprove a suspected leukemic relapse in donor cells that was suggested by conventional cytogenetics.

Case report

A 30-year-old female was diagnosed as having Ph1-negative, bcr/abl-positive (b2a2, p210) CML in October 1992. The patient was treated effectively with hydroxyurea, followed by α-interferon until April 1993, when she successfully underwent BMT from a matched male sibling. In April 1994, the patient relapsed with morphological and immunological features of common acute lymphoblastic leukemia. The growth fraction of leukemic cells, as determined using the APAAP technique and Ki67 monoclonal antibody, was found to be extremely low (< 1%). The blood group was still that of the donor. In this phase, repeated karyotype analyses of marrow cells (blasts 95%) demonstrated the presence of only six evaluable normal male (46, XY) metaphases, thus suggesting the possibility of a relapse in the donor cells.

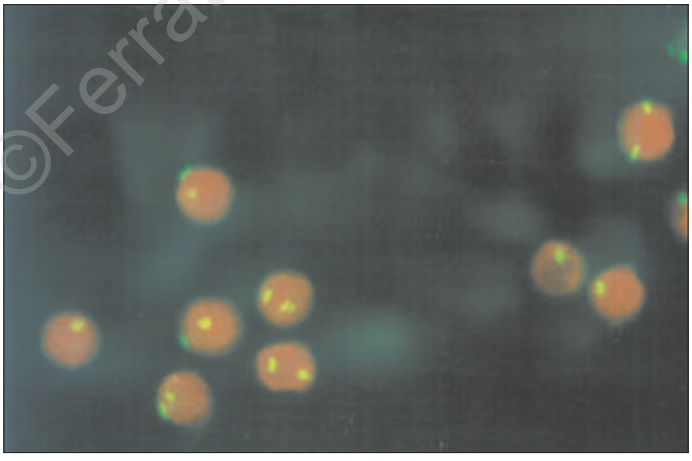

Figure 1. Bone marrow at relapse after BMT: in situ hybridization with the DXZ1, X-specific probe shows two signals (female cells) in the majority of interphase nuclei.

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However, FISH analysis performed with the α-satellite DXZ1 (Oncor), X chromosome-specific probe clearly evidenced the female origin of the leukemic cells by showing the presence of two or one fluorescent signals, respectively, in 78% and 15% of 500 interphase nuclei examined (Figure 1); 7% of cells showed no hybridization. The rare evaluable mitoses observed in the FISH preparations had a modal number of 46 chromosomes and carried a single fluorescent signal. In normal male and female controls (peripheral blood lymphocytes), the percentage of single or double hybridization in interphase nuclei with the DXZ1 probe was 87% and 83%, respectively. We concluded that relapse had occurred in the recipient cells.

After a new complete remission had been achieved by administration of vincristine, doxorubicin and prednisone, both cytogenetic and FISH techniques revealed only normal male metaphases in the bone marrow, with the occasional presence of interphase female cells (about 1 every 350 male cells). The patient is currently undergoing a second BMT.

Conclusions

Although the small number of metaphases available at relapse after BMT (likely due to the very low proliferative activity of leukemic cells) does not allow us to exclude the presence in this patient of a 47,XXY clone or a duplicated centromere from the X chromosome in 46,XY cells, our results indicate that when specific chromosomal markers are lacking, simple cytogenetic analysis of sex chromosomes may not be enough to confirm a diagnosis of relapse in donor cells in sex-mismatched bone marrow transplants. In such cases, FISH may provide useful additional information.

References

1. Fialkow PJ, Thomas CD, Bryant JI, Neiman RE. Leukemic transformation of engrafted human marrow cells in vivo. Lancet 1971; i:251-6.
2. Marmont A, Frassoni E, Bacigalupo A, et al. Recurrence of Ph1 positive leukemia in donor cells after marrow transplantation for chronic granulocytic leukemia. N Engl J Med 1984; 310:903-6.
3. Przepiorka D, Thomas ED, Durnam DM, Fisher I. Use of a probe to repeat sequence of the Y chromosome for detection of host cells in peripheral blood of bone marrow transplant recipients. Am J Clin Pathol 1991; 95:201-6.
4. Mouratidou M, Sotiropoulos D, Deremotzaki K, et al. Recurrence of acute leukemia in donor cells after bone marrow transplantation: documentation by in situ DNA hybridization. Bone Marrow Transplant 1993; 12:77-80.
5. Hegele RG, Bicknell SG, Bailey DJ, Cameron RG. In situ hybridization for the Y chromosome reveals a donor origin for a post-transplant lymphoproliferative disorder in a sex- mismatched hepatic allograft. Arch Pathol Lab Med 1994; 118: 795-6.