Cytogenetic oligoclonality resulting from jumping translocation and isodicentric Xq13 as evidenced by FISH

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Abstract

We describe a case of acute myeloid leukemia (AML) that had transformed from myelodysplastic syndrome (MDS) where cytogenetics revealed the presence of 5 unrelated clones in the bone marrow aspirate - 4 clones with chromosomal abnormalities and 1 which was karyotypically normal. Fluorescence in situ hybridization (FISH) using a combination of library - centromeric probes and library - library probes revealed jumping translocation as one of the causes of the cytogenetic oligoclonality. Different segments of the long arm of chromosome 1 (1q) had jumped onto the ends of three chromosomes namely 8q, 9q and 18q, resulting in the development of three cytogenetically unrelated clones. A fourth clone showed an isodicentric chromosome of Xq. This is an unusual case of cyrogenetic oligoclonality in MDS-AML shown explicitly by FISH to have arisen from two separate evenrs, namely jumping translocations of 1q and isodicentric formation involving Xq13.

Key words: oligoclonality; FISH; jumping translocation; isodicentric Xq13

Introduction

A clone is defined as a cell population having the same or closely related abnormal chromosome complements presumably derived from a single progenitor cell (ISCN 1995). Routine cytogenetics of patients with hematological malignances will usually show bone marrow metaphases with the same or obviously related clonal abnormalities, suggesting that these malignancies originate from a single cell. Occasionally however, co-existing clones with unrelated chromosomal abnormalities have been found in acute leukemias and myelodysplastic syndromes (MDS), either at diagnosis or relapse (Heim & Mitelman 1989; Kobayashi *et al.*, 1990; Musilova *et al.*, 1996). At times, the karyotypic changes in the clones detected at diagnosis were different from those at relapse. These findings have posed a challenge to the monoclonal origin theory of neoplastic hematological disorders and raised the possibility of an oligoclonal origin in some of these disorders.

We investigated here a case of cytogenetic oligoclonality in an elderly Malaysian woman with MDS-evolved acute myeloid leukemia (AML) using fluorescence in situ hybridization (FISH). We demonstrated that the unrelated abnormalities had resulted from 2 events, one from jumping translocations of chromosome 1q to 3 different chromosomes and the other from isodicenrric formation of Xq13.

Material and Methods

Case Report

A 63-year-old woman first presented in October 1993 with complaints of fever and headache of 8 months duration and easy bruising. A full blood count showed a hemoglobin level of 8.2 g/dL, total white cell count of 8×10^9/dL and a platelet count of 61×10^9/dL. The bone marrow aspirate showed features of dyserythropoeisis and numerous blasts (58%). The blasts were a heterogeneous population of large and small cells. Some blasts showed granules in the cytoplasm. Cytochemical studies were not informative. A diagnosis of myelodysplasia evolved acute myeloid leukemia was made. Before further investigative procedures could be taken, the patient discharged herself from the hospital against medical advice.

Cytogenetic study

Chromosome analysis was performed on trypsin-Giemsa banded bone marrow spreads harvested after 24 hours of culture. The analysis of the metaphases was done in accordance with the International System for Human Cyrogenetic Nomenclature (ISCN 1995).

FISH studies

FISH analysis was carried out on stored cell suspensions (-20°C) using a combination of library probecentromeric probe and library probe-library probe. WCP 1 SpectrumOrange DNA probe (Vysis, Inc.) was simultaneously hybridized with digoxigenin-labelled alpha satellite DNA probes (Boehringer Mannheim)(detected with fluorescein) for chromosomes 9 or 18 on meraphases. For identification of chromosome 8, a library probe for 8 (Boehringer Mannheim) was used. A digoxigenin-labelled probe for the centromeric region of the X chromosome (Boehringer Mannheim) was also used. Hybridization and detection were done according to the rapid method obtained from the Birmingham's Women Hospiral with some slight modifications. Slides were counterstained with 4', 6-diamidino-2-

phenylindole dihydrochloride (DAPI) and visualized on an Olympus fluorescence microscope. The FISH images were acquired, digitized and analyzed using the Cytovision Ultra (Applied Imaging).

Results

Conventional cytogenetic analysis detected the presence of 5 cytogenetically distinct clones: 4 unrelated abnormal clones viz., 46, XX, add (18)(q23) [50]; 46, XX, add (8)(q24) [33]; 47, XX, add(9)(q34), +21 [8] and 46, X, idic(X)(q13) [3] / 47, X, idic(X)(q13)x2 [3]; and a normal 46, XX [15] clone.

Three abnormal clones showed respectively additional (add) material on chromosomes 18q, 8q and 9q. Dual colour FISH with paint for chromosome 1 (Spectrum Orange) and library or satellite centromeric probes for the respective derivative chromosomes showed that the additional materials originated from chromosome 1 (Fig 1a-c). Based on this identification, the derivative chromosomes were thus assigned as der (18) t (1; 18)(q32; q23), der (8) t (1; 8)(q25; q24) and der (9) t (1; 9)(q21; q34) respectively.

The idic (X)(q13) was verified using the alpha satellite probe for centromeric chromosome X and the FISH signals are as shown in Fig 1d.

Discussion

Karyotypically unrelated clones are relatively rare events in hematological malignancies. These unrelated clones were more frequently reported in myeloid diseases, usually MDS or acute nonlymphocytic leukemia (ANLL), although it may be seen in acute lymphoblastic leukemia and chronic lymphocytic leukemia (Kobayashi *et al.*, 1990, Heim & Mitelman, 1989). Heim and Mitelman (1989) in their review of 912 cases of MDS and 2,506 cases of ANLL reported that the frequency of unrelated clones in these diseases were 4.3% and 1.1% respectively. Unlike some other tumour types like squamous cell carcinoma where polyclonal tumorigenesis may be the rule, unrelated clones in hematological disorders

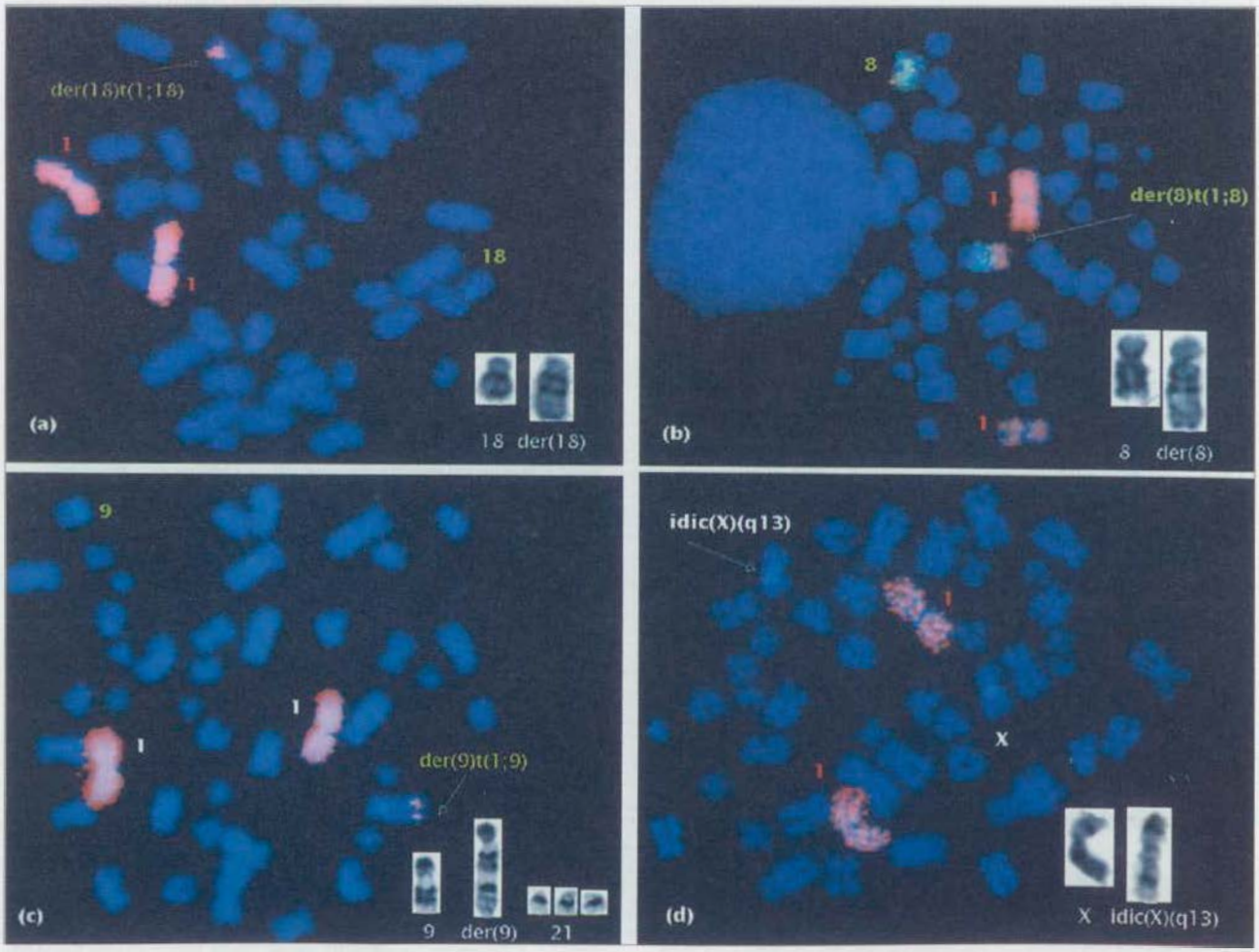

Fig 1. (a) FISH analysis using chromosome 1 paint (red) and centromeric 18 probe (green), confirming chromosome 1 material at the end of the long arm of one chromosome 18q. Inset : Partial G-banded karyotype showing der(18)t(1;18). (b) Chromosome 1 paint (red), chromosome 8 paint (green), showing chromosome 1 material at 8q. Inset : der(8)t(1;8) (c) Chromosome 1 paint, centromeric 9 probe (green), confirming chromosome 1 material at 9q. Inset : der(9)t(1;9) and +21. (d) Centromeric X probe (green). Inset: idic(X)(q13).

were believed to be derived from clonal evolution of a single neoplastic clone (without microscopic chromosomal changes) (Sandberg *et al.*, 1996; Kobayashi *et al.*, 1990; Heim *et al.*, 1988). MDS, a stem cell disorder, is proposed to evolve from a multistep pathogenesis, involving at least two important events, one causing proliferation of a clone of genetically unstable pluripotent stem cells, the other inducing chromosomal abnormalities in the daughter cells (Raskind *et al.*, 1984). Although the stem cell initially involved is capable of both myeloid and lymphoid differentiation, it has been reported that the acquired chromosomal abnormalities are selectively expressed in myeloid-derived cells but not in lymphoid cell lineages (Fugazza *et al.*, 1995; Weimar *et al.*, 1994).

Jumping translocation (JT) is a rare phenomenon that is increasingly reported in leukemias and lymphomas (Ten *et al.*, 1997, *Najfeld et al.*, 1995, Wlodarska *et al.*, 1994). The occurrence of JT is associated with stabilising cytogenetically abnormal clones, and is not specifically related to the pathogenesis of malignancies. Structural abnormalities of the X chromosomes are also rarely described in haematological malignancies. Of the cases reported, isodicentric Xq13 occurs more frequently and may play a significant pathogenetic role affecting exclusively females, typically of advanced age, and deriving predominantly from early progenitor cells namely, in myelodysplastic syndromes and myeloproliferative disorders (Dewald *et al.*, 1982; Dewald *et al.*, 1989; Mackinnon *et al.*, 1988, Dierlamm *et al.*, 1995). In the present study, we were able to verify jumping translocations of 1q as a cause of the cytogenetic oligoclonality using a combination of centromeric probe-library probe, and library probe-library probe hybridizations.

Such a combination of jumping translocations of 1q and idic (X)(q13) in unrelated clones has not been reported previously. No plausible explanation has been given for unrelated clones in haematologic disorders. The history of myelodysplasia and the pattern of the karyotypic changes in our patient, suggest that the unrelated chromosomal aberrations reflect secondary changes acquired late by different proliferating subclones of a common mutated pluripotent stem cell. It is probable that these secondary abnormalities may confer some proliferative advantages to the malignant clones.

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