Ring chromosome 7 in Down Syndrome with acute megakaryoblastic leukemia

Ten SK[1], Tan SK[1], Zubaidah Z[1], Khuzaiah RR[2], Roshida H[3] *[1]Division of Haematology, Institute for Medical Research, 50588 Kuala Lumpur; [2]Paediatric Institute, Hospital Kuala Lumpur; [3]Department of Pathology, Hospital Kuala Lumpur*

Abstract

Ring chromosome is an unusual cytogenetic aberration in hematological malignancies and diseases which acquire it rend to be aggressive and refractory to treatment. We presenr here a case report of a 2-year old Down Syndrome (DS) girl with AMKL/MDS who manifested a ring chromosome in all her bone marrow cells. Fluorescence in situ hybridization (FISH) analysis using chromosome-7-specific DNA probe verified the diagnosis of ring chromosome 7, r(7). To rhe best of our knowledge, this is the third reported case of r(7) in DS individuals with AMKL/MDS.

Key words: ring chromosome 7; acute megakaryoblastic; Down Syndrome

Introduction

Down Syndrome (DS) children are 10 - 20 times more likely to develop acute leukemia than the normal population (Zipursky *et al.*, 1987; Zipursky *et al.*, 1992). Ultimately, 1 % of DS children will be affected. About 50 % of the leukemia in DS children can be classified as acute megakaryoblastic leukemia (AMKL) and it occurs during the first 4 years of life. On the other hand, AMKL is a relatively rare form of childhood leukemia in the normal population accounting for 3 % of pediatric acute myeloid leukemia (AML) cases (Craig *et al.*, 1989).

Hyperdiploidy with numeric gains involving chromosomes 8, 19 and 21 are often observed in the leukemic cells of these DS patients (Kaneko *et al.*, 1981; Lu *et al.*, 1993). Structural abnormalities are relatively uncommon; even more so ring chromosomes which are rare events in leukemia.

Here we describe a case of a DS child presenting with AMKL with a 4-monrh history of myelodysplasia. Her bone marrow cells showed a ring chromosome 7, which was confirmed using flourescence in situ hybridization (FISH) techniques.

Materials and Methods

Case Report

A 2-year-old DS girl with atrial septal defect and a known history of myelodysplasia (4 months duration) was admitted to the Pediatric Institute, Hospital Kuala Lumpur on 11 March 1996 with fever, cough, pallor and gum bleeding. On admission, she was noted to have hepatosplenomegaly and bleeding tendency. Her haemoglobin was 5.0 g/dl, the white blood cell count was 14.2 x 10^9/ l, with >50 % blasts, and the platelet count was 19 x 10^9/ l. The bone marrow smear showed 63 % blasts which were fairly heterogeneous in nature - some blasts were large with basophilic cytoplasm whereas others were small with very high nuclear/cyroplasmic ratio, and condensed chromatin with indistinct nucleoli. Some of the blasts showed cytoplasmic blebbing. Flow cytometry revealed that 87 % of cells were positive for CD 33, 86% for CD 7 and CD 13, and 35% for CD 34 and CD 41. A diagnosis of acute megakaryoblastic leukemia was established and the patient was started on chemotherapy (iv daunorubicin and cytosine arabinoside) but died on the 6th day of treatment due to septicaemia.

Cytogenetic study

Chromosome analysis was performed on trypsin-Giemsa banded bone marrow spreads harvested after 24 hours of culture. Karyotyping was done according to the Guidelines for Cancer Cytogenetics (ISCN 1991).

FISH

The identity of the ring chromosome was confirmed using the WCP 7 SpectrumOrange DNA probe (Vysis, Inc.). In situ hybridization and detection were done according to the protocol outlined by the manufacturer. Slides were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and visualized on a Olympus fluorescence microscope. The FISH images were acquired and digitized using the Cytovision Ultra (Applied Imaging).

Results

Cytogeneric analysis of the bone marrow cells revealed monosomy 7 and a ring chromosome superimposed on the constitutional trisomy 21 in 20 metaphases analysed (Fig. 1). The karyorype was interpreted as : 47, XX, -7, +21c, +r. To verify whether the ring was derived from the apparently missing chromosome 7, some meraphases were hybridised wirh a WCP 7 DNA probe which hybridized to regions in the 7p arm, 7q arm and ro the cenrromere of human chromosome 7. Fig. 2 shows a bone marrow metaphase in which two chromosomes fluoresced after hybridization was performed: the ring chromosome exhibited one of the fluorescence signals indicating that it was derived from chromosome 7.

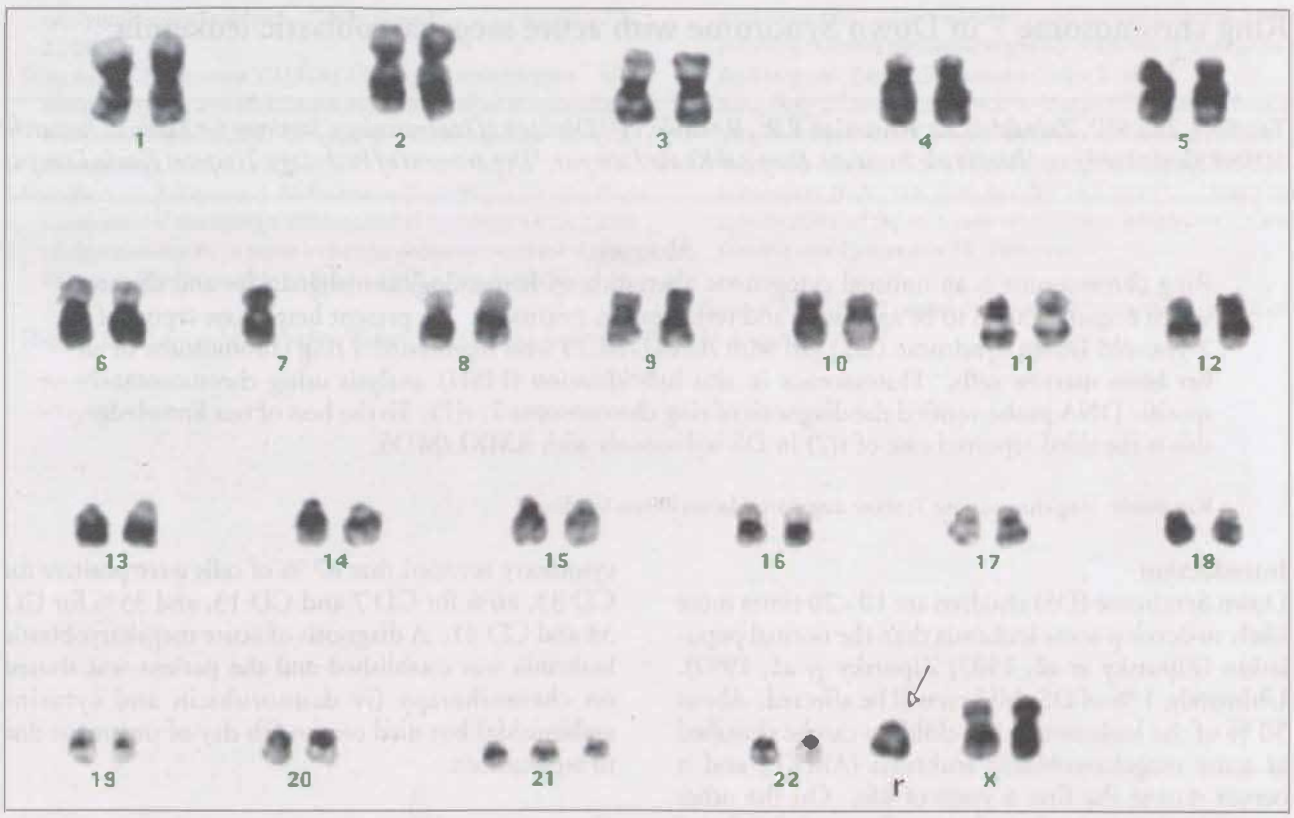

Fig. 1. Karyotype of patient showing monosomy 7, trisomy 21 and a ring chromosome (arrow).

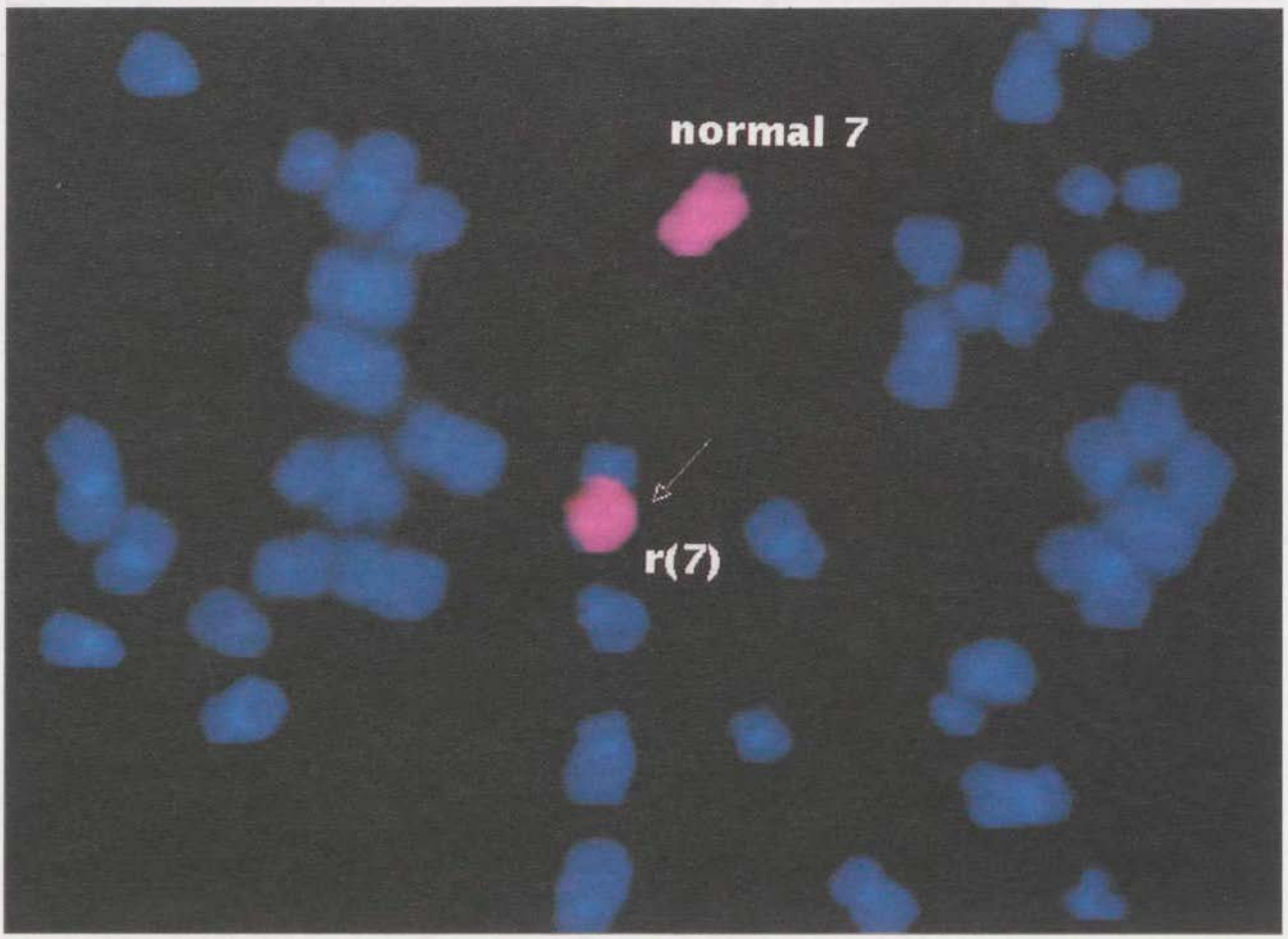

Fig. 2. A bone marrow metaphase showing two WCP-7 signals after in situ hybridization. The r(7) is indicated by arrow.

Discussion

Our report illustrates a distinct form of AMKL associated with MDS found in patients with Down Syndrome. AMKL in DS children is described as a unique disease characterized by a preceding phase of myelodysplasia, presence of bone marrow fibrosis and unusual chromosomal findings (Zipursky *et al.*, 1992; Lu *et al.*, 1993). The progenitor cell in MDS/AMKL in DS differs from the leukemia cell in MDS in normal individuals in that it has the potential of forming cells of both megakaryocytic and erythroid lineages while the latter forms only myeloid precursors (Zipursky *et al.*, 1994a).

Trisomy 8 and monosomy 7 are frequent chromosomal changes in MDS in normal individuals and are similarly observed in DS AMKL and its preleukemic state of MDS (Hecht *et al.*, 1986; Lu *et al.*, 1993; Zipursky *et al.*, 1994b). Ring chromosomes on the other hand are unusual cytogenetic events in hematological malignancies and are more frequently described in erythroleukemia and chronic myeloid leukemia in blast crisis (Sandberg 1990; Lewis *et al.*, 1988). It was suggested that diseases associated with acquired ring chromosomes tend to be aggressive and refractory to therapy (Lewis *et al.*, 1988). Theoretically a ring may arise from the loss of telomeric regions from both arms of a chromosome with subsequent fusion of the open ends with or without subtelomeric deletion, or it may arise from the telomeric association of a single chromosome without the loss of telomeric repeats (Fugazza *et al.*, 1996). Fugazza *et al.* (1996) using a probe specific for all human telomeres, reported on the loss of telomeric sequences in a ring chromosome 8 in a case of refractory anemia with excess of blasts in transformation. However the cause of ring chromosome formation in malignant cells is still not fully understood.

Despite the purportedly rare occurrence of ring chromosomes, the detection of r(7) in 4 cases of DS with AML, that is, 3 cases with AMKL/MDS (Hayashi *et al.*, 1988; Bunin *et al.*, 1991; present data) and one case with AML-M2 (Alimena *et al.*, 1985), suggests that the presence of r(7) could well delineate a subtype of this unique haematological disorder. While monosomy 7 and deletion of 7q are known poor prognostic factors in MDS among nonDS children, the significance of r(7) in this special group of patients particularly its effect on treatment outcome with chemotherapy remains to be clarified with further studies. This is noteworthy in the light of recent studies which have indicated a superior outcome in DS children with AML placed on current treatment regimes containing high-dose cytosine arabinoside (Ara-C) compared to the reportedly low cure rate with chemotherapy in pediatric AML cases (Taub & Ravindranath, 1996). The superior response appears to be due to the increased sensitivity of AML blasts with trisomy 21 to both Ara-C and daunorubicin on account of certain enzymes localized in chromosome 21. Notwithstanding that, our present data supports the contention of a frequent association of chromosome 7 abnormalities with DS AMKL/MDS (Bunin *et al.*, 1991).

Acknowledgements

The authors thank the Director, Institute for Medical Research, Kuala Lumpur, for permission to publish the findings and the Head, Department of Clinical Pathology, for his encouragement and advice. This project was supported by an IRPA grant (06-05-01-0042) from the Ministry of Science, Technology & Environment, Malaysia.

References

Alimena G, Billstrom R, Casalone R, Gallo E, Mitelman P & Pasquali F (1985). Cytogenetic pattern in leukemic cells of patients with constitutional chromosomal anomalies. *Cancer Genetics and Cytogenetics* 16, 207-218.

Bunin N, Nowell PC, Belasco J, Shah N, Willoughby M, Farber PA & Lange B (1991). Chromosome 7 abnormalities in children with Down Syndrome and preleukemia. *Cancer Genetics and Cytogenetics* 54, 119-126.

Craig AJ, Benjamin DR, Lampkin B & Hammond D (1989). Acute nonlymphocytic leukemia of childhood: Inter-observer variability and problems in the use of the FAB classification. *Cancer* 63, 295-301.

Fugazza G, Bruzzone R & Sessarego M (1996). Loss of telomeric sequences in a ring derived from chromosome 8 in refractory anemia with excess of blasts in transformation. *Cancer Genetics and Cytogenetics* 89, 31-33.

Hayashi Y, Eguchi M, Sugita K, Nakazawa S, Sato T, Kojima S, Bessho F, Konishi S, Inaba T, Hanada R & Yamamoto K (1988). Cytogenetic findings and clinical features in acute leukemia and transient myeloproliferative disorder in Down's Syndrome. *Blood* 72, 15-23.

Hecht F, Hecht BK & Morgan R (1986). Chromosome clues to acute leukemia in Down's Syndrome. *Cancer Genetics and Cytogenetics* 21, 93-98.

Kaneko Y, Rowley JD, Variakojis D, Chilcote RR, Moohr JW & Patel D (1981). Chromosomal abnormalities in Down's Syndrome patients with acute leukemia. *Blood* 58, 459-466.

Lewis J, Jenks H & Walling P (1988). Ring chromosomes in chronic myelogenous leukemia: an ominous finding. *Leukemia Research* 12, 379-383.

Lu G, Altman AJ & Benn PA (1993). Review of the cytogenetic changes in acute megakaryoblastic leukemia: one disease or several? *Cancer Genetics and Cytogenetics* 67, 81-89.

Sandberg AA (1990). The chromosomes in human cancer and leukemia. 2nd Ed. New York, Elsevier, pp. 299-302.

Taub JW & Ravindranath Y (1996). Treatment of childhood acute myeloid leukemia. *Bailliere's Clinical Haematology* 9, 129-146.

Zipursky A, Peeters M & Poon A (1987). Megakaryoblastic leukemia and Down's Syndrome: A review. *Pediatric Hematology and Oncology* 4, 211-230.

Zipursky A, Poon A, & Doyle J (1992). Leukemia in Down Syndrome: A review. *Pediatric Hematology and Oncology* 9, 139-149.

Zipursky A, Wang H, Brown EJ & Squire J (1994a). Interphase cytogenetic analysis of in vivo differentiation in the myelodysplasia of Down Syndrome. *Blood* 84, 2278-2282.

Zipursky A, Thorner P, de Harven E, Christensen H & Doyle J (1994b). Myelodysplasia and acute megakaryoblastic leukemia in Down's Syndrome. *Leukemia Research* 18, 163-171.

Received 3 June 1997; revised 2 September 1997; accepted for publication 4 September 1997.