The c-sis Gene Expression in Cells From a Patient With Acute Megakaryoblastic Leukemia and Down's Syndrome

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The c-sis gene expression in leukemia cells from a patient with acute megakaryoblastic leukemia and Down’s syndrome was studied. The leukemia blasts were identified as megakaryoblasts by the platelet peroxidase reaction and the reactivity against antiplatelet monoclonal antibodies. Leukemia cells obtained from peripheral blood or bone marrow specimens before and after initiation of chemotherapy were analyzed for c-sis gene expression by the RNA-DNA dot blot hybridization. Although the level varied, the mRNA of the c-sis gene was detected in all megakaryoblastic leukemia cells obtained at different clinical stages. The c-sis mRNA level in cells obtained after relapse was higher than that obtained before the initiation of therapy. The 25S c-sis mRNA was detected in a megakaryoblastic leukemia cell line established from this patient. The role of the expression of the c-sis gene in acute megakaryoblastic leukemia cells is discussed.

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MATERIALS AND METHODS

Immunofluorescence assay. Leukemic cells were separated from heparinized peripheral blood by Ficoll-Hyphaque density centrifugation and used for analysis including the formation E, EA (IgG-Fc), and EAC rosettes and the presence of surface immunoglobulins (slg). The cell surface phenotype was analyzed with indirect immunofluorescence using monoclonal antibodies OKT3, OKT4, OKT6, OKT8, and OKT11 for T cell markers; B1 and B4 for B cell markers; J5 for common acute lymphocytic leukemia (ALL) antigen; 12 for IA-like antigen; and MY4, MY7, and MY9 for myelomonocytic antigens (monoclonal antibodies for T cell markers were purchased from Ortho Pharmaceutical Corp, Raritan, NJ, and the others were from Coulter Immunology, Hialeah, FL). Platelet-specific antigen was studied with the antiplatelet antibodies P2 (anti-GpIIb-IIIa, Immunotech, Marseilles, France) and KOR-P17.

Leukocyte isolation. Leukemic cells were obtained from bone marrow and peripheral blood specimens before chemotherapy and after relapse. Informed consent was obtained from the parents of the patients and adult volunteers. The cells were separated from erythrocytes by centrifugation on a Ficoll-Hyphaque discontinuous density gradient at 400 g for 20 minutes at room temperature. Platelets (less than 20,000/μL) in peripheral blood were carefully removed by washing cells repeatedly. Normal lymphocytes were separated from peripheral blood of an adult volunteer in the same way. The cells were immediately frozen and stored in liquid nitrogen until analysis.

Preparation of RNA and hybridization. The quick blot system developed by Bresser et al was slightly modified and used for mRNA immobilization on nitrocellulose filter and dot blot hybridization. Preparation of poly(A)-RNA and Northern blotting analysis was done as described by Colamonei et al. The PstI 1.3-kb restriction fragment of simian sarcoma proviral DNA, which contains the v-sis region, was used for the dot blot and Northern blot hybridizations as a probe. A 3' untranslated fragment of a mouse cytoskeletal β-actin gene was used as a probe for the quantification of mRNA on the dotted filter. The hybridization was performed as described previously. Autoradiography was performed for three to seven days with an intensifying screen at -70°C.

RESULTS

Case report. A 10-month-old boy with Down's syndrome was referred to Chiba University Hospital in July 1985. Complete blood counts on admission were as follows: WBC, 1,400/μL with 62% lymphocytes, 13% neutrophils, and 25% blasts; hemoglobin, 7.6 g/dL; platelets, 20,000/μL. The bone marrow aspirates showed decreased cellularity and contained 12% blast cells, which were considered possible megakaryoblasts. Most of the blasts were 10 to 14 μm in diameter and had a narrow basophilic cytoplasm with projections (Fig 1). The blasts were negative for myeloperoxidase and alphaphyethyl butyrate esterase activity, but positive for alphaphyethyl acetate esterase and acid phosphatase activity. A
Fig 1. May-Grünewald-Giemsa preparations of blast cells from peripheral blood (A and B) and bone marrow (C and D) at time of diagnosis.

Trephine biopsy sample showed both hypocellular and hypercellular areas. Atypical megakaryocytes were surrounded by increased reticulin fibers. On the basis of the platelet peroxidase and antiplatelet monoclonal antibody examination the patient was diagnosed as having acute megakaryoblastic leukemia, and we started chemotherapy. He obtained a complete remission after 1 month but relapsed in the bone marrow in October 1985 and died in November 1985.

Cytogenetic studies. Chromosome analyses were performed on the blast cells of the bone marrow aspirate. Thirty-three metaphases were analyzed by the Giemsa banding method, of which ten cells showed 47,XY,+17p+, and nine cells showed 48,XY,+21,+17p+,+marker. The additional portion of the short arm of chromosome 17 seemed to be derived from chromosome 11. The remaining 14 cells showed 47,XY,+21, which had been observed in peripheral blood cells cultured for 72 hours with phytohemagglutinin.

Electron microscopic and immunochemical examination. Platelet peroxidase staining was done by using the method of Breton-Gorious et al. C-sis mRNA was detected in all megakaryoblastic cells obtained at different clinical stages, whereas normal lymphocytes and other types of leukemia cells did not express c-sis mRNA at a measurable basal level (Fig 3A). Validation of the differences in c-sis mRNA levels was provided by quantification of the β-actin mRNA on the same filter (Fig 3B). The amounts of c-sis mRNA in cells obtained from the bone marrow (45% blasts, lane 3) and peripheral blood (90% blasts, lane 4) after bone marrow relapse were at least fivefold more than those obtained before initiation of chemotherapy (90% blasts, lane 2). The cells obtained from another megakaryoblastic leukemia patient also expressed c-sis mRNA (lane 5). To obtain enough mRNA for further analysis we cultured the original blasts and established a megakaryoblastic cell line (CMK) that shared the chromosomal change, der(17)(11;17), with the original blasts (Sato et al, submitted). Figure 3C shows that the 25S c-sis transcript was expressed in the CMK cell line.

DISCUSSION

This report is the first demonstration of the c-sis gene expression in freshly isolated megakaryoblastic leukemia cells. The c-sis expression in fresh hematopoietic cells has
$\text{actin mRNA. The same filter was probed with mouse cytoskeletal fl-actin cDNA.}$

"...from peripheral blood [over 90% blasts]; lane 3. leukemic cells A CELLS, c-sis analysis of tal /9-actin under low stringent conditions. (C) Northern blot hybridization was carried out with the v-sis mRNA. mRNA was immobilized on nitrocellulose filters, and v-sis gene, v-sis mRNA. Lane 1, normal leukocytes; lane 2, leukemic cells before initiation of chemotherapy (obtained from peripheral blood [90% blasts]); lane 4. acute megakaryoblastic leukemia (5-month-old boy with Down's syndrome); lane 6. acute lymphocytic leukemia cell; lane 7. acute leukemic cells obtained from bone marrow (90% blasts) of another..."

Fig 3. Detection of c-sis mRNA. (A) Quick blot analysis of c-sis mRNA, mRNA was immobilized on nitrocellulose filters, and hybridization was carried out with the v-sis DNA probe as described in Materials and Methods. Lane 1, normal leukocytes; lane 2, leukemic cells before initiation of chemotherapy (obtained from peripheral blood [over 90% blasts]); lane 3, leukemic cells after relapse (obtained from bone marrow [45% blasts]); lane 4, leukemic cells obtained from peripheral blood (90% blasts); lane 5, leukemic cells obtained from bone marrow (90% blasts) of another acute megakaryoblastic leukemia (5-month-old boy with Down’s syndrome); lane 6, acute lymphocytic leukemia cell; lane 7, acute myeloblastic leukemia cells. (B) Quick blot analysis of cytoskeletal $\beta$-actin mRNA. The same filter was probed with mouse cytoskeletal $\beta$-actin under low stringent conditions. (C) Northern blot analysis of c-sis mRNA. Lane 1, a megakaryoblastic cell line, CMK; lane 2, normal peripheral lymphocytes.

**REFERENCES**


16. Den Ottolander GH, Te Velde J, Brederoo P, Geraedts JPM, ...


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S Sunami, A Fuse, B Simizu, M Eguchi, Y Hayashi, K Sugita, S Nakazawa, Y Okimoto, T Sato and H Nakajima