Extramedullary tumor as presentation of leukemia: establishment of a new human GPIIb- and GPIIIa-positive leukemia cell line

Summary A 25-year-old man noted swelling of the right cervical lymph nodes in October 1983. Diagnosis of malignant lymphoma was made on the basis of pathological examination of biopsies. Despite both chemotherapy and irradiation treatment, blast cells appeared in the peripheral blood and bone marrow in April 1984. Immunophenotypic analysis demonstrated that the blasts in the patient’s peripheral blood expressed CD13, CD33, CD41a, and no markers for T or B lymphocytes, suggesting that he had been suffering from megakaryocytic sarcoma. We established a new cell line derived from the blasts in the peripheral blood, designated KH184. KH184 cells expressed glycoprotein (GP) Ib (CD42b) and GPIIb/IIIa (CD41a), while platelet peroxidase (PPO) activity was negative in an ultrastructural study. Both Northern blot and flow cytometric analysis of surface antigens and DNA content revealed that treatment with 12-O-tetradecanoylphorbol 13-acetate (TPA) did not induce the maturation of these cells. Various cytokines such as interleukin 3 (IL-3), interleukin 6 (IL-6), and leukemia inhibitory factor (LIF) had no effect in promoting the growth of KH184 cells.

KH184 cells expressing CD41a seem to possess unusual characteristics. KH184 cells, human GPIIlb- and GPIIIa-positive leukemia cells, which lack response to TPA-induced differentiation, provide a new and unique model for the characterization of factors that are implicated in the terminal differentiation of megakaryocytes, and should aid in studies of the mechanism underlying the occurrence of megakaryocytic sarcoma.

Key words Extramedullary tumor · Malignant lymphoma · Granulocytic sarcoma · GPIIb- and GPIIIa-positive leukemia cell line

Introduction

Granulocytic sarcoma is defined as a localized tumor mass consisting of immature cells of granulocytic lineage [9]. When granulocytic sarcoma occurs before the development of systemic leukemia with the involvement of bone marrow and peripheral blood, diagnosis is generally regarded as difficult. Since pathological findings in specimens obtained from granulocytic sarcoma are similar to those of malignant lymphoma, the condition is frequently misdiagnosed as malignant lymphoma. Although many studies using cytochemical staining with chloroacetate esterase (CAE) and histochemical demonstration of lysozyme [3, 8] have shown that granulocytic sarcoma cells have granulocytic characteristics, little is known about the exact nature of the cells presenting as granulocytic sarcoma. Recently, we established a new human leukemia cell line, designated KH184, from a leukemic patient who was first misdiagnosed as having malignant lymphoma and was later re-diagnosed as having megakaryocytic sarcoma. We investigated its megakaryocytic characteristics using genomic and immunological procedures. Further, we examined the responsiveness of the cells to 12-O-tetradecanoylphorbol 13-acetate (TPA) and cytokines.
Case report

A 25-year-old man was admitted to Kitano Hospital (Osaka, Japan) in October 1983, complaining of swelling of the right cervical lymph nodes. On admission, his peripheral blood count was: RBC, 5.04 x 10^6/μl; hemoglobin, 15.2 g/dl; platelets, 345 x 10^3/μl; and WBC, 6.9 x 10^3/μl with no blasts. The bone marrow specimen contained 67 x 10^3/μl nucleated cells with no abnormal blasts. Diagnosis of malignant lymphoma was made on the basis of pathological examination of biopsy specimens (Fig. 1A). CVP (cyclophosphamide, vincristine, and prednisolone) regimen following radiotherapy was begun; this resulted in complete remission. In February 1984, the patient was readmitted for relapse. Despite the fact that the patient received chemotherapy with VEPA (vincristine, cyclophosphamide, prednisolone, and adriamycin) as well as VEPAB (vincristine, cyclophosphamide, prednisolone, adriamycin, and bleomycin) and irradiation, no therapeutic response was obtained. In April 1984, blast cells appeared in the peripheral blood and bone marrow, revealing leukemic crisis. His peripheral blood count at this time was: RBC, 2.99 x 10^6/μl; hemoglobin, 8.4 g/dl; platelets, 66 x 10^3/μl; and WBC, 1.2 x 10^3/μl with 36% blasts. The hypocellular bone marrow after the intensive chemotherapy was infiltrated by malignant cells forming cell clumps (Fig. 1B). The cells were negative for myeloperoxidase (MPO) and periodic acid-Schiff (PAS). The patient died of disseminated intravascular coagulation (DIC) after high-dose methotrexate (MTX) therapy in July 1984. No postmortem findings were obtained. Fig. 2 shows the clinical course of this patient. Immunophenotypic analysis revealed that the mononuclear cells collected from the peripheral blood in April 1984, when leukemic crisis was suspected, expressed CD13, CD33, and CD41a, and did not express such markers for T lymphocytes as CD2, CD4, and CD7, or such markers for B lymphocytes as CD10, CD19, and CD20. Gene rearrangement analysis revealed no rearranged banding pattern but germline configuration for the TCR β, γ, and immunoglobulin JH genes. Furthermore, paraffin-embedded preparations from the pharyngeal lymph nodes on admission were negative for the monoclonal antibody, anti-human leukocyte common antigen (DAKO-LCA, DAKO Co., Carpenteria, CA) (CD45). The cells from the right pharyngeal lymph nodes, which had been frozen and kept for 7 years, also expressed CD41a. In accordance with these findings, the patient was diagnosed as having had megakaryocytic sarcoma.

Materials and methods

Cell culture

The mononuclear cells collected from the peripheral blood in April 1984 were suspended in RPMI 1640 (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 10% (vol/vol) fetal bovine serum (Hyclone Laboratories Inc., Logan, Utah), and cultured. The cell suspensions were serially diluted, and aliquots of the suspension were distributed in individual microwells. One of the clones, termed KH184, was used in our study. The cells were incubated at 37°C in a humidified 5% CO2 atmosphere in an incubator. In some experiments, TPA (Wako Pure Chemicals Industries Ltd., Osaka, Japan), recombinant interleukin 3 (IL-3; purchased from Genzyme Co., Boston, MA), recombinant interleukin 6 (IL-6; provided by Central Research Laboratories, Ajinomoto Co. Inc., Yokohama, Japan), recombinant granulocyte colony-stimulating factor (G-CSF; by Chugai Pharmaceutical Co. Ltd., Tokyo, Japan), recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF; Genzyme), recombinant macrophage-stimulating factor (M-CSF; Genzyme), leukemia inhibitory factor (LIF; Genzyme), erythropoietin (Epo; Genzyme), and stem cell factor (SCF; Genzyme) were added to the culture.

Morphological and cytochemical studies

Light microscopic examination was performed on May-Grünwald-Giemsa (MGG)-stained cytospin preparations. Cytochemical staining for MPO, acid phosphatase, α-naphthyl butyrate esterase, naphthol AS-D chloroacetate esterase, and PAS were performed by standard methods.

Chromosomal analysis

The karyotype of the cells was examined using a G-banding technique.

Ultrastructural studies

Simultaneous detection of platelet peroxidase (PPO) activity and GPIIb/IIIa by immunogold was performed as described in detail previously [5]. For sensitive detection of PPO activity, which is