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Acute myeloid leukemia cell lines MOLM-17 and MOLM-18 derived from patient with advanced myelodysplastic syndromes

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Abstract

The two acute myelomonocytic leukemia sister cell lines MOLM-17 and MOLM-18 and the Epstein–Barr-virus positive non-malignant B-lymphoblastoid cell lines (B-LCLs) B422 and B423 were established from the bone marrow sample of a 60-year-old Japanese male in the advanced leukemic phase of refractory anemia with excess of blasts, a subtype of myelodysplastic syndromes (MDS). MOLM-17/-18 are proliferatively responsive to the growth factors present in the culture supernatant of the 5637 cell line. The B-LCLs are constitutively growth factor-independent. MOLM-17 and B422 were established at eight months after the initial diagnosis, while MOLM-18 and B423 were derived from a sample one month later. Immunophenotyping of the first leukemia sample revealed a mixed lineage leukemia immunophenotype with positivity for terminal deoxynucleotidyl transferase (TdT), CD13 and CD19; the second sample revealed solely myeloid characteristics with positivity for CD13, CD41 and CD61, whereas TdT was negative. MOLM-17/-18 showed immunomarker profiles typical of the myelomonocytic lineage. The karyotype analysis of MOLM-17/-18 revealed various non-random numerical and structural abnormalities including del(5)(q?), −7, der(11)add(11)(p11.2)add(11)(q23), add(17)(p11.2), add(18)(p11.2), −20, −22 as common aberrations. Treatment with tumor necrosis factor-α induced pronounced cellular differentiation of both cell lines into macrophage-like cells. The overall profile of MOLM-17/-18 based on their extensive immunological, cytogenetic and functional characterization suggests that these cell lines together with the paired B-LCLs B422 and B423 may represent scientifically significant in vitro models which could facilitate investigations into the pathobiology of MDS.

Keywords: AML; Cell line; Differentiation; Leukemia; MDS; TNF-α

1. Introduction

Continuous hematopoietic cell lines have become indispensable model systems for leukemia-lymphoma research [1]. The cell lines derived from myelodysplastic syndromes (MDS) or overt leukemia after MDS are significantly rarer than de novo leukemia derived cell lines [2]. The pathogenesis of MDS is not fully elucidated and the knowledge regarding the mechanism of leukemic transformation in MDS is only limited. MDS are clonal hematologic disorders which are clinically and morphologically characterized by ineffective hematopoiesis. Myelodysplastic conditions are often described as “preleukemic” syndromes in which the malignant clone is already established, progressing ultimately to acute myeloid leukemia (AML); however, not all cases seem to terminate in overt AML. As a corollary MDS and AML have been described as a progression of neoplastic hematopoiesis

Abbreviations: B-Ags, B-cell-associated antigens; My-Ags, myeloid/monocytic-associated antigens; n.t., not tested; Plt-Ags, platelet/megakaryocyte-associated antigens; T/NK-Ags, T-cell/NK-cell-associated antigens; TdT, terminal deoxynucleotidyl transferase

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[3]. Non-random recurrent cytogenetic aberrations have been seen in cells of the myeloid and erythroid cell lineages from patients with MDS.

The classification of MDS by the French–American–British (FAB) cooperative group has been widely utilized [4]. The FAB classification evolved into the World Health Organization classification [5].

Here we describe the characteristic and unique features of the novel growth factor-dependent AML-derived sister cell lines MOLM-17 and MOLM-18, established from a patient with overt leukemia at the final stages in the progression of MDS. We have also investigated the cellular differentiation capacity of these cell lines in response to various cytokines and all-*trans*-retinoic acid (ATRA) treatment.

The new continuous growth factor-dependent leukemia sister cell lines MOLM-17 and MOLM-18 in direct comparison with the paired "normal" B-lymphoblastoid cell lines (B-LCLs) B422 and B423 could provide a useful in vitro model system for the study of pathogenetic events and specific genetic alterations, underlying the development of MDS and its progression to fulminant leukemia.

2. Materials and methods

2.1. Patient

A 60-year-old Japanese male was referred to us on 1 August 2000, and he was diagnosed as having MDS, subtype RAEB in transformation. On 11 April 2001, approximately eight months after the initial diagnosis, the disease appeared to have progressed further to overt leukemia, at which time the cells used to establish the first cell line were obtained from the patient's bone marrow (BM) sample with informed consent. His hemoglobin concentration was 7.3 g/dl, red blood cell count was $234 \times 10^4/mm^3$, white blood cell count was $1100/mm^3$ with 88% atypical immature monocytoid cells and platelet count was $1 \times 10^4/mm^3$. After one month, on 11 May 2001, additional cells were obtained for further culture attempts. MOLM-17 and the non-malignant B-lymphoblastoid cell line (B-LCL) B422 were established from the first cryopreserved BM sample, and MOLM-18 and B-LCL B423 were established from the second BM sample, respectively.

The first BM aspirate showed characteristics of somewhat a "mixed" lineage leukemia immunophenotype with positivity for CD19 (71%) and TdT (56%) while expressing CD13 (71%). The second BM aspirate displayed only myeloid characteristics: CD13+ CD19− TDT−; several platelet-associated antigens were positive: CD41 (40%), CD61 (41%), CD110 (82%). Antigenic profiles of the first and second sample were summarized in Table 1. The karyotype of the peripheral blood cells showed: 45, XY, del(5)(q?), −7, der(11)(p11)add(11)(q23), add(17)(p11), add(18) (p11), −20, −22, +r1 × 2 in 2/44 metaphases analyzed. The FAB morphological classification of the terminal stage cells suggested AML M5a. The patient failed to achieve complete remission during chemotherapy including cytarabine, aclacinon, etoposide, vincristin and fildesin. Also, only a poor response was obtained upon administration of granulocyte-colony stimulating factor (G-CSF) and macrophage-CSF. The patient died on 28 July 2001.

2.2. Establishment of cell lines

Using leftover cells from the immunophenotyping sample, cells were washed twice with fresh RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan). Mononuclear cells were suspended in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS: Invitrogen, Tokyo, Japan) and antibiotics, 100 U/ml penicillin and 50 μg/ml streptomycin, with 20% culture supernatant of the 5637 cell line (conditioned medium: 5637 CM). The 5637 cell line produces several cytokines, including G-CSF, interleukin-3 (IL-3), IL-6, stem cell factor and others [6,7]. Cells were incubated in a 24-well culture plate (Corning, Corning, NY) at 37 °C in a humidified 5% CO_2 atmosphere. The culture was then fed once a week by replacing one half to one third of the volume of the culture contents with fresh complete medium plus 5637 CM during the subsequent 12 weeks. In the 13th week, a slow yet sustained proliferation of the cultured cells was noted which was then designated as MOLM-17. B422 was established from the same specimen in a separate culture plate. MOLM-18 and its "normal" sister cell line B423 were established from the second BM specimen by exactly the same culture technique. 5637 CM was continuously used as the source of growth factors for maintaining MOLM-17/-18. Complete RPMI 1640 medium without growth factors was used for maintaining the constant proliferation of the B-LCLs B422 and B423 carrying polyclonal immunoglobulin (Ig) expression and Epstein–Barr-virus (EBV) infection. The cell lines were found to be free of mycoplasma infection. We attempted to characterize the cells as detailed as possible, as outlined previously [8].

2.3. Morphological studies

Microscopic observation was performed on cytospin smears of the established cell lines after May–Grünwald–Giemsa staining. Cytochemical staining for myeloperoxidase (MPO) was also performed using cytospin smears and standard methodology.

2.4. Immunophenotyping

Immunophenotyping of fresh leukemic blasts and of the cell lines was performed using the antibodies listed in Table 1. These analyses were done between three to five times. The sources of these reagents and the methods used have been described in detail elsewhere [9–11].