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Abstract

A new myeloid cell line, MTO-94, was established from the bone marrow of a patient with myelodysplastic syndrome (MDS). MTO-94 cells matured in culture medium without the addition of growth factors, and yielded neutrophils with pseudo-Pelger Huët anomaly or hypersegmentation until 6 months. Ten months after the start of cell cultivation, MTO-94 consisted of myeloblasts. Surface phenotypes were as follows: CD7 90.3%, CD13 99.6%, CD33 75.6%, HLA-DR 96.3% and CD34 0.9%. The karyotype was 46, XY, i(17q). The proliferation of MTO-94 cells was enhanced by rhlL-3, G-CSF, rhGM-CSF and rhSCF but not by rhlL-6 and erythropoietin. MTO-94 cells with i(17q) might be useful in the study of biological aspects of not only MDS, but also hematological malignancies with i(17q) as the sole chromosomal anomaly.

KEYWORDS: isochromosome 17q, myeloid cell line, myelodysplastic syndrome

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Establishment of a New Myeloid Cell Line with i(17q) as the Sole Chromosomal Anomaly from the Bone Marrow of a Patient with Myelodysplastic Syndrome

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A new myeloid cell line, MTO-94, was established from the bone marrow of a patient with myelodysplastic syndrome (MDS). MTO-94 cells matured in culture medium without the addition of growth factors, and yielded neutrophils with pseudo-Pelger Huët anomaly or hypersegmentation until 6 months. Ten months after the start of cell cultivation, MTO-94 consisted of myeloblasts. Surface phenotypes were as follows: CD7 90.3%, CD13 99.6%, CD33 75.6%, HLA-DR 96.3% and CD34 0.9%. The karyotype was 46, XY, i(17q). The proliferation of MTO-94 cells was enhanced by rhIL-3, G-CSF, rhGM-CSF and rhSCF but not by rhIL-6 and erythropoietin. MTO-94 cells with i(17q) might be useful in the study of biological aspects of not only MDS, but also hematological malignancies with i(17q) as the sole chromosomal anomaly.

Key words: isochromosome 17q, myeloid cell line, myelodysplastic syndrome

Myelodysplastic syndromes (MDS) are hematological disorders with the potential of progressing to acute leukemia (1). MDS presumably results from the transformation of pluripotent stem cells. Its pathogenesis, including the mechanism of leukemic transformation, has been carefully studied in respect to hematopoietic stem cells, hematopoietic growth factors and oncogenes (2–6). Recent papers concerning MDS with an isochromosome 17q [i(17q)] suggest that MDS with i(17q) as the sole chromosomal anomaly might constitute a distinct MDS with characteristics somewhere between MDS and chronic myeloproliferative disorders (CMPD) (7, 8). In the past, we reported a case of refractory anemia with excess of blasts (RAEB) which developed into overt leukemia with CMPD-like findings such as leukothrombocytosis and hepatosplenomegaly (9). i(17q) was observed during the disease's course in this patient. In the present paper, some biological aspects of a cell line, designated MTO-94, which was newly established from bone marrow cells of this patient, are described.

Materials and Methods

Patient profiles. A 72-year-old man was referred to our hospital because of refractory anemia on June 9, 1992. Examination of his blood showed a red blood cell (RBC) count of $1.83 \times 10^6/\mu l$, hemoglobin (Hb) of 8.6 g/dl, a platelet (Plt) count of $2.8 \times 10^4/\mu l$, and a white blood cell (WBC) count of $5.6 \times 10^3/\mu l$ with 1% myeloblasts. A few erythroblasts and megakaryocytes were found. Giant thrombocytes, neutrophils with hypersegmentation or pseudo-Pelger Huët anomaly, were also found. Bone marrow smears revealed 15.0% myeloblasts and 13.2% erythroblasts. The megakaryocyte count was elevated to $540/\mu l$. Multinucleated erythroblasts, neutrophils with pseudo-Pelger Huët anomaly and micromegakaryocytes, were observed. Cytogenetic analysis by Giemsa-banding showed 46, XY, i(17q) in 15 of 20 cells examined. On the basis of hematological findings, the patient was diagnosed as having RAEB. On January 7, 1993, examination of his blood showed a WBC count of $42.3 \times 10^3/\mu l$ with 32.5% blasts; a diagnosis of acute leukemia was made. On March 11, bone marrow smears revealed 33.4% myeloblasts and the results of marker

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analysis of blasts examined were compatible with myeloblasts. These were: CD5 10.8%, CD7 16.3%, CD13 63.9%, CD15 19.1%, CD33 76.8%, CD34 less than 5%, CD41 0% and HLA-DR 64.9% by the immunocytochemistry staining method. The patient died from pneumonia on August 9, 1994 in spite of oral administration of cytarabine ocfosfate and melphalan. Sequential analyses of chromosomes showed 46, XY, i(17q) in all of 20 cells examined (9).

Cell culture method. On July 27, 1994, a mononuclear cell fraction was obtained from the heparinized bone marrow aspirates by Ficoll-Conray fractionation (Pharmacia Biotech AB, Uppsala, Sweden) and cells (3.0×10^6 cells/ml) were cultured in suspension in 35 mm petri dishes (Falcon, Lincoln Park, NJ, USA) containing RPMI 1640 medium, 15% fetal calf serum (FCS; ICN Biomedicals Japan Co., Osaka, Japan), 10% heat-inactivated cord blood and antibiotics at 37°C with 5% CO_2 and 100% humidity. Cells were fed with fresh medium twice weekly.

Cell proliferation assay. Cultured cells were washed three times and cells (2×10^5 cells/ml) were re-cultured in a 96-well, flat-bottom culture plate (Falcon, Lincoln Park, NJ) containing 0.2 ml culture medium with or without growth factors, 100 U/ml recombinant human interleukin 3 (rhIL-3), 100 U/ml recombinant human IL-6 (rhIL-6), 50 ng/ml granulocyte-colony stimulating factor (G-CSF), 50 ng/ml recombinant human granulocyte-macrophage CSF (rhGM-CSF), 100 ng/ml recombinant human stem cell factor (rhSCF), and 20 IU/ml erythropoietin (EPO). The viable cells were quantified by colorimetric assay (10) with tetrazolium salt on an ELISA plate reader (Behring ELISA Processor II; Hoechst Japan Co., Tokyo, Japan) with a test wavelength of 570 nm and a reference wavelength of 650 nm. These assays detect living cells but do not detect dead cells, and the signal generated is dependent upon the degree of cell activity. The mean OD value at 570 nm obtained from wells containing culture medium alone was used as a background control. Growth factors used in this experiment were kindly provided by Kirin Brewery Co. Ltd., Tokyo, Japan.

Karyotype and surface phenotypes analyses. Cells were cultured in the culture medium as described above and harvested after 24 h. G-banding was performed according to the standard procedures after short-term trypsin treatment. Surface phenotypes of MTO-94 cells were analyzed with a flow cytometer using fluorescein-isothiocyanate (FITC)- or phycoerythrin (PE)-labeled monoclonal antibodies (MoAbs). The MoAbs used were as follows: CD2, CD3, CD7, CD13, CD14, CD19, CD20, CD33, CD41a, HLA-DR (all from Coulter Immunology, Hialeah, FL, USA) and CD34 (Dako Japan Co. Ltd., Kyoto, Japan). FITC- and PE-labeled mouse IgG and FITC-labeled mouse IgM (Coulter Immunology, Hialeah, FL) were used as negative controls.

Results and Discussion

Three months after cultivation, cell growth became marked and stable. Culture cells were found to be Epstein-Barr nuclear antigen (EBNA)-negative by the *in situ* hybridization method (Dako Japan Co. Ltd., Kyoto, Japan). Until 6 months, this cell line, designated MTO-94, showed maturation of myeloid cells in culture medium without the addition of growth factors (Table 1), and yielded neutrophils with pseudo-Pelger Huët anomaly or hypersegmentation resembling the original cells (Figs. 1-a, 1-b). MTO-94 culture consisted of 34.5% myeloblasts, 16.5% promyelocytes, 12.0% myelocytes, 5.5% metamyelocytes, 5.0% band-form and 19.0% segmented neutrophils 4 months after cultivation. By cytochemical analysis, MTO-94 cells were positive for peroxidase, naphthol AS-D-chloroacetate esterase, alkaline-phosphatase, periodic acid-Schiff (PAS), acid phosphatase and Sudan black B staining. Surface phenotypes of MTO-94 cells were as follows: CD7 90.3%, CD13 99.6%, CD33 75.6%, HLA-DR 96.3% and CD34 0.9%. These findings suggest that MTO-94 is an early myeloid cell. The reduction value of nitroblue tetrazolium (NBT) by neutrophils was 44% (normal value by peripheral neutrophil: 60–85%), and some neutrophils showed phagocytic activity against Candida albicans (Figs. 2-a, 2-b). The karyotype of MTO-94 cells was 46, XY, i(17q), examined on 75th and 200th cultivation day (Fig. 3), which was the same as the karyotype for bone marrow cells. Ten months after the start of cell cultivation, MTO-94 consisted of immature cells with the morphological characteristics of myeloblasts.

Until now, cell lines of MDS origin have been established and have yielded some information concerning the pathogenesis of MDS (11, 12). From the bone marrow of a patient with refractory anemia with ringed sideroblasts (RARS), Tohyama *et al.* (13) established a myeloid cell line, MDS92. This cell line proliferated in the presence of IL-3 or GM-CSF, and transiently proliferated in the

Table I Classification of MTO-94 cells

	07/27/94[a]	08/30	09/30	10/28	11/25	12/26	01/27/95	05/26
Myeloblast	1.6%	5%	42%	30%	34.5%	66%	85.5%	100%
Promyelocyte	3.8		5	15	16.5	12	1.0	
Myelocyte	5.4	4	9	14	12.0	6	1.0	
Metamyelocyte	0.8	8	5	11	5.5	2	2.5	
N[b] · Band	1.2	4	2	6	5.0	4	1.0	
N[b] · Seg	10.6	14	23	11	19.0	10	6.5	
Eosinophil	1.0							
Basophil	1.2							
Lymphocyte	1.4							
Monocyte	9.2	34	12	12	5.0		2.5	
Reticulum cell	1.4	31	2	1	2.5			
Erythroblast	62.4							

a : bone marrow cells *b* : neutrophil

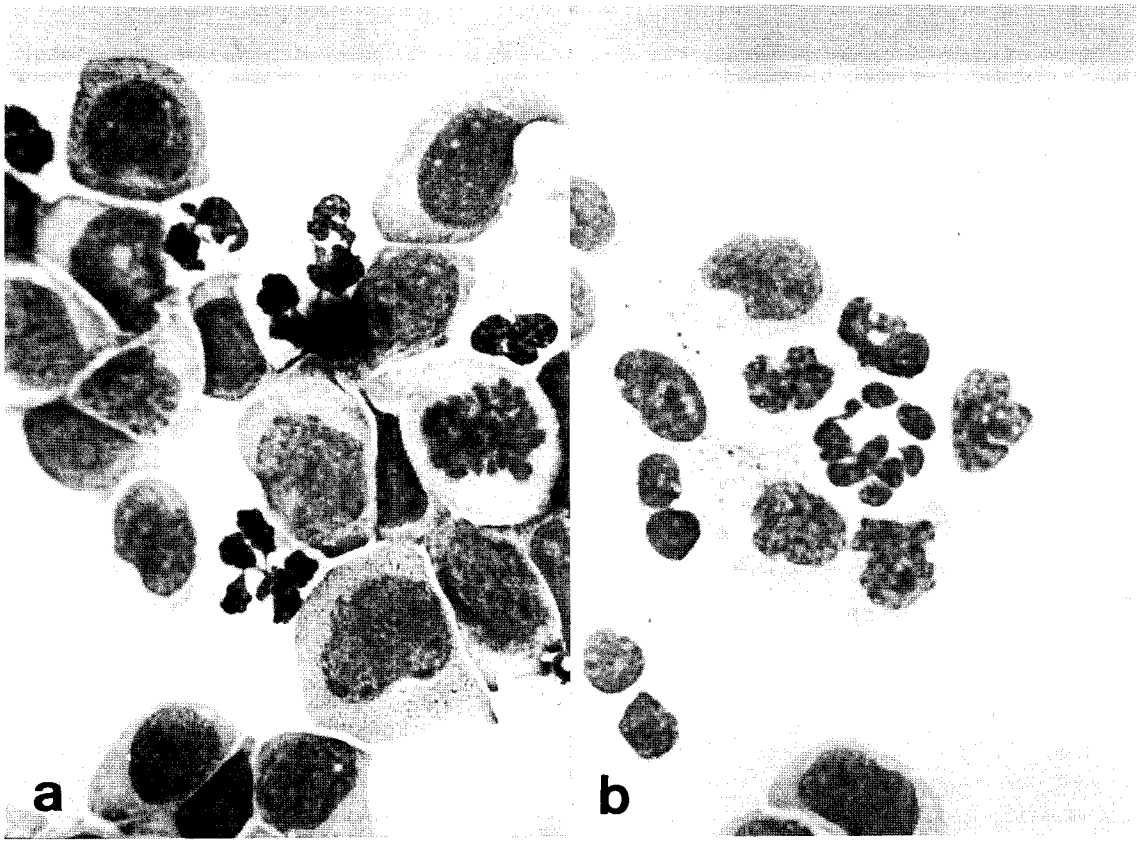

Fig. 1 May-Giemsa-stained cytospin preparation of MTO-94 cells. MTO-94 cells showed maturation of myeloid cells in culture medium without the addition of growth factors. Neutrophils with hypersegmented neutrophils (**a**, **b**) and pseudo-Pelger Huët anomaly (**b**) were visible.

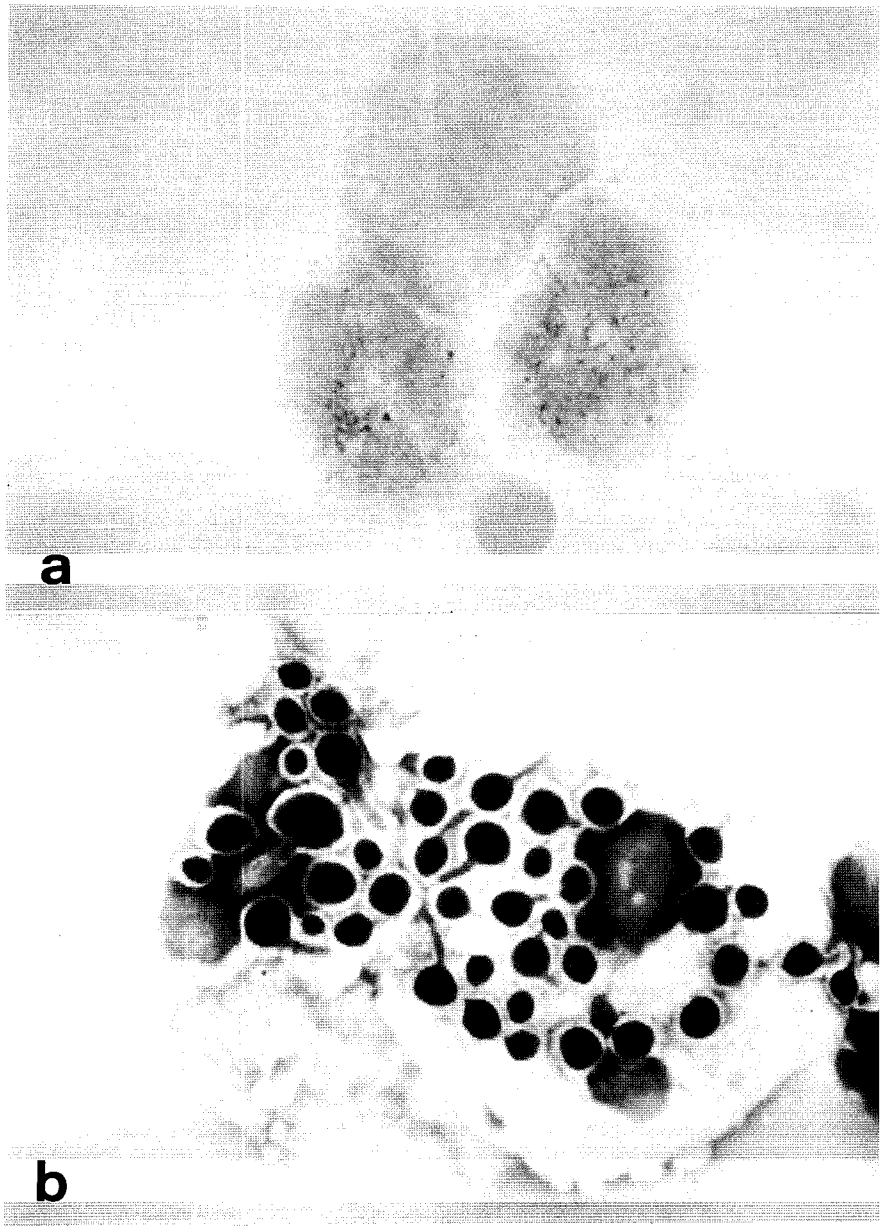

Fig. 2 NBT reduction and phagocytic activities of MTO-94 cells. (**a**): The reduction value of NBT by MTO-94 cells was 44% (normal value 60–85%). (**b**): Some MTO-94 cells showed phagocytic activity against Candida albicans.

presence of Steel factor with a tendency toward gradual maturation. MTO-94 cell line, presented here, is thought to be unique for the following reasons: Firstly, this cell line showed both proliferation and maturation in culture medium without the addition of growth factors until 6 months. Some of the neutrophils originating from MTO-94 showed pseudo-Pelger Huët anomaly or hypersegmentation, and MTO-94 cells also had the properties of neutrophils such as reduction of NBT and phagocytic activity against Candida albicans. The proliferation of MTO-94 cells was enhanced by rhIL-3, G-CSF, rhGM-CSF and rhSCF but not by rhIL-6 and EPO (Fig. 4). Effects of growth factors on differentiation, which could not be examined here because of a small quantity of cells, should be examined in the future. Secondly, the karyotype of MTO-94 was i(17q). A cell line with i(17q) as

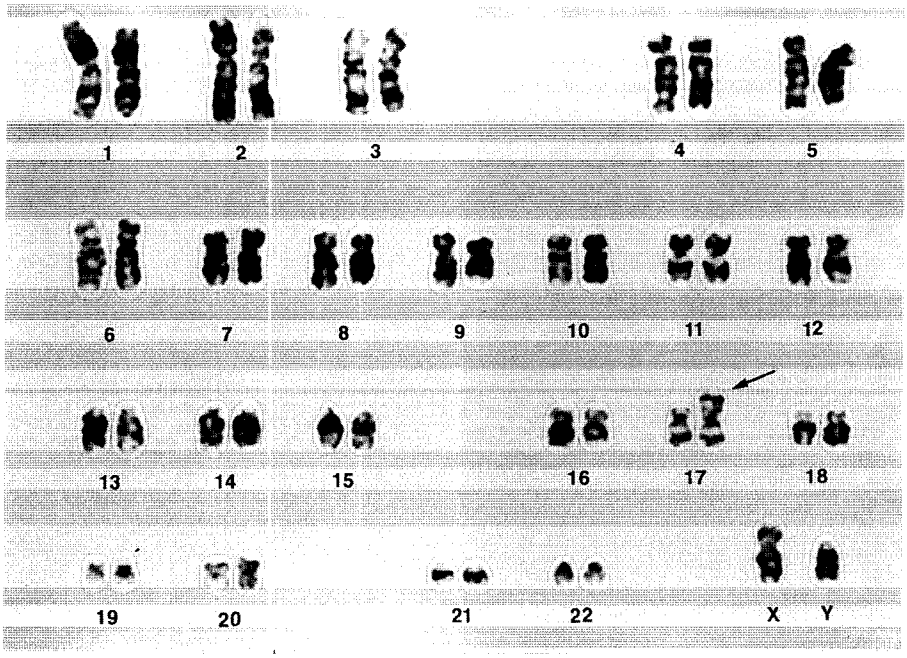

Fig. 3 Karyotype of MTO-94 cells. Karyotype analysis was performed using the conventional Giemsa-banding technique. The karyotype of MTO-94 cells was 46, XY, i(17q).

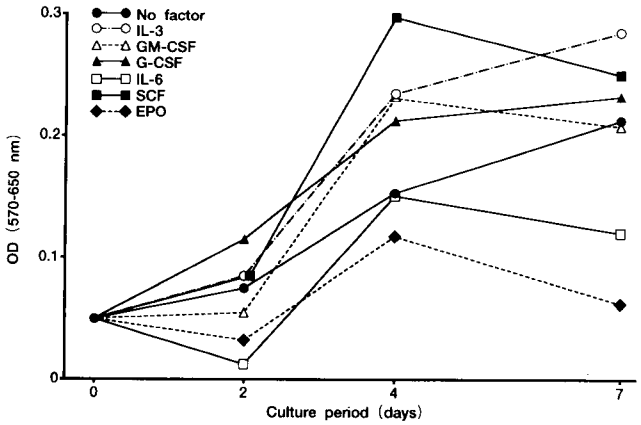

Fig. 4 Effects of growth factors on MTO-94 cells. Growth factors used were rhIL-3 (100U/ml), rhIL-6 (100U/ml), G-CSF (50ng/ml), rhGM-CSF (50ng/ml), rhSCF (100ng/ml), and EPO (20IU/ml). The proliferation of MTO-94 cells was enhanced by rhIL-3, G-CSF, rhGM-CSF and rhSCF but not by rhIL-6 and EPO. Results are expressed as the mean of triplicates.

the sole chromosomal anomaly is rarely reported. Caligaris-Cappio *et al.* (14) established a lymphoid cell line with i(17q) from a patient with B-chronic lymphocytic leukemia by infecting blood lymphocytes with the EB virus. MTO-94 was not infected with the EB virus. The isochromosome of 17q as the structural anomaly result not only in trisomy of 17q, but also in monosomy of 17p. The latter could well be the more significant event, because it leads to the loss of one allele of the p53 gene located at 17p13. p53 is considered to be a negative regulator of cell growth whose inactivation may be an important step in the pathogenesis or progression of various solid tumors and hematological malignancies including CML, especially in blast crisis (15, 16). Jonveaux *et al.* (17) found point mutations of p53 gene in 5 of 151 patients with MDS, and 3 of those 5 patients had anomalies of chromosome 17p. Fenaux *et al.* (18) also found the p53 mutation in 4 of 10 patients with acute myelocytic leukemia (AML) with 17p monosomy.

On the other hand, Schutte *et al.* (19) did not detect any structural abnormalities of the remaining p53 allele in 9 patients with blast crisis of CML and AML, who showed i(17q) as a cytogenetic anomaly. In the present case p53 gene mutations of bone marrow cells were not detected by single-strand conformation polymorphism (SSCP). MTO-94 cells were also negative for nuclear localization of the p53 protein by immunohistochemical staining (result not shown), but further analysis is required. Recent papers concerning MDS with i(17q) as

the sole chromosomal anomaly indicate that the diversities of MDS should be analyzed. Becher *et al.* (7) described that i(17q) seemed to identify a distinct subgroup of most MDS and, less frequently, myeloproliferative disorders that progress rapidly to acute non-lymphocytic leukemia. Sole *et al.* (8) also reported on a group of four MDS patients in which all four patients shared several characteristics such as male gender, advanced age, severe anemia, as well as a bone marrow with the following myeloproliferative characteristics: hypercellularity, prominent baso- and eosinophilia, and a marked increase of micromegakaryocytes. These findings suggest that MDS patients with i(17q) as the sole chromosomal anomaly may be classed as having a distinct MDS with characteristics somewhere between MDS and CMPD.

In conclusion, MTO-94 was established from the bone marrow of a patient with RAEB who developed into overt leukemia with CMPD-like findings such as leukocytosis, thrombocytosis and hepatosplenomegaly (9). MTO-94 with i(17q) might be useful in the study not only of pathogenesis, but also of diversities of MDS.

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