Establishment of a Human Acute Myeloid Leukemia Cell Line (Kasumi-1) With 8;21 Chromosome Translocation

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A novel leukemic cell line with an 8;21 chromosome translocation, designated as Kasumi-1, was established from the peripheral blood of a 7-year-old boy suffering from acute myeloid leukemia (AML). The Kasumi-1 cells were positive for myeloperoxidase showing a morphology of myeloid maturation. The response in proliferation assay was observed in the culture with interleukin-3 (IL-3), IL-6, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage CSF (GM-CSF), but not with IL-1 or IL-5. Neither granulocytic nor eosinophilic maturation was observed in the liquid culture by the addition of dimethyl sulfoxide, G-CSF, or IL-5, respectively. In contrast, induction of macrophage-like cells was seen by the addition of phorbol ester. This is the first report of a human AML cell line with t(8;21) that has characteristics of myeloid and macrophage lineages. The cell line could be a useful tool for elucidating the pathophysiology of AML with t(8;21).

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Recombinant human interleukin-1β (IL-1β), IL-3, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and G-CSF were used as stimulating factors at the following concentrations: 100 U/mL of IL-1β, 10 ng/mL of IL-3, 5 ng/mL of IL-6, 10 ng/mL of GM-CSF, and 10 ng/mL of G-CSF. IL-1β and G-CSF were provided by Otsuka Pharmaceutical Co (Tokyo, Japan), and IL-3, IL-6, and GM-CSF were purchased from Genzyme Co (Boston, MA).

Induction of cellular differentiation. Cells were treated with the cell suspension and an equal volume of a growth-stimulating factor in 10% FBS-supplemented RPMI 1640 medium were cultured in 96 U-bottom microwell tissue culture plates (Nunc) at 37°C in a fully humidified incubator at 5% CO₂ for 48 hours. The cells suspended in RPMI 1640 medium without growth-stimulating factor, supplemented with 10% FBS were treated as a control. Then, 50 μL of 2 μCi/mL of ³H-thymidine (Amersham Japan, Tokyo) was added. After 6 hours of incubation, the cells were harvested on glass-fiber filters, and ³H-thymidine uptake was measured in a liquid scintillation counter.

The concentration of human recombinant IL-5 (Suntory Co, Osaka, Japan) was 1 ng/mL. The concentration of the other growth factors was the same as in the colony assay.

Induction of cellular differentiation. Cells were treated with...
Establishment of Kasumi-1 cell line. The Kasumi-1 cells have been continuously proliferating in a suspension culture for over 9 months with a doubling time of 40 to 45 hours.

Morphologic and cytochemical characteristics. The cells showed marked variations in both size and nuclear cytoplasmic (N/C) ratio. Granules were present in about 40% of the cells, but no Auer rods were seen. Some cells had vacuoles in the cytoplasm. The nuclei are often lobulated, showing a leptochromatic pattern. One to five nucleoli were found per cell. The basophilic cytoplasm contained fine azurophilic granules. Some cells had the characteristics of myelocytes, representing maturation of blast cells (Fig 1A).

The original leukemic cells and the Kasumi-1 cell line were positive for MPO (Fig 1B) and CAE. The Kasumi-1 cells were negative for NAP and NBE.

Surface marker analysis. Both the original leukemic cells and the Kasumi-1 cell line were positive for CD13 (OKM13) and HLA-DR (OKDR). However, the Kasumi-1 cell line was 37.1% positive for CD4 (OKT4A) and 50.1% for CD38 (OKT10) (Table 1). CD4 coexpressed with CD34, CD33, and CD13 in the Kasumi-1 cells was determined by double immunofluorescence. Further investigation at Mie University.
University showed that the Kasumi-1 cell line was positive for CD15 (LeuM1), CD34 (MY10), and CD71 (Nu-TERf).

Cytogenetic studies. Cytogenetic data are shown in Table 2. Chromosomal analysis of the leukemic cells in the second relapse showed t(8;21), der(9)t(9;?)q11;?), and a marker chromosome. The additional chromosomal abnormality found in the Kasumi-1 cell line was a minute marker chromosome designed as mar2 (Fig 2).

Southern blot hybridization. Southern blot hybridization showed no bands specific for Epstein-Barr virus and no rearrangement bands with IgH and TCRβ probes.

Proliferation studies by recombinant hematopoietic growth factors. To assess the effects of cytokines on Kasumi-1 cells, colony assay and 3H-thymidine uptake were studied. In the colony assay, increase in the number of colony formation was observed in the culture with G-CSF, IL-3, or IL-6 in the order of the activity, respectively, but not with IL-1 or GM-CSF (Table 3).

The increase of 3H-thymidine uptake was observed in the culture with G-CSF, GM-CSF, IL-3, or IL-6 in the order of the activity, respectively, but not with IL-1β or IL-5 (Table 4).

Induction of cellular differentiation. DMSO and G-CSF did not induce granulocytic maturation. IL-5 alone was not able to induce eosinophilic differentiation. In contrast, TPA induced the formation of macrophagelike cells that had the ability of adhesion to the wall of the culture bottle and showed about 10% positivity for NBE staining (Fig 1, C and D). After the culture with TPA, changes in the surface markers were observed; expression of CD4 completely disappeared (15.4% was positive before culture) and CD11b (Leu15) newly appeared in 11.4% of the Kasumi-1 cells. NAP activity was never positive after the culture with these inducers.

DISCUSSION

It has been well established that AML with t(8;21) has some specific clinical features, such as (1) generally younger patients, (2) a frequency of 10% to 40% of FAB M2 leukemias; (3) good response to chemotherapy with a high remission rate and a relatively long survival; (4) the presence of Auer rods in both the blast cells and mature granulocytes; (5) abnormal maturation of the leukemic cells, eg, scant or eccentric localization of the granules; (6) low activity of neutrophil alkaline phosphatase; (7) eosinophilia in the bone marrow in some cases; and (8) occurrence of solid tumor with myeloblastoma features outside the bone marrow cavity.

The Kasumi-1 cell line is a novel human cell line derived from a patient with AML with an 8;21 translocation, which is positive for MPO and has the morphology of myeloid maturation. In surface-marker analysis, the Kasumi-1 cells were positive for CD13, CD15, CD33, CD34, HLA-DR, CD38, CD71, and CD4. It has been reported that CD34 is expressed in pluripotent stem cells, CD33 in colony-forming unit granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM), and CD13 in colony-forming unit granulocyte/macrophage (CFU-GM). This evidence strongly suggests that the Kasumi-1 cell line has originated from an early myeloid stem cell. The Kasumi-1 cells could proliferate well in the presence of G-CSF, GM-CSF, IL-3,
or IL-6. The highest increases in colony formation and 
'H-thymidine uptake were observed by G-CSF. G-CSF acts
on lineage-restricted stem cells, mainly on the granulocyte
series.20 Our results indicate the Kasumi-1 cell line is
committed to the granulocytic series. On the other hand,
this cell line showed a response to IL-3 that acts on
multipotential stem cells.21

From these results, we can speculate that this cell line
keeps the characteristics of a multipotential stem cell with a
differentiation ability to granulocytic series.

Regarding responsiveness to IL-6, there are several
reports that IL-6 exerts proliferative effects on multipoten-
tial stem cells or blast cells of AML in combination with
IL-3 or GM-CSF.22,23 It still remains a possibility that IL-6
acts on the Kasumi-1 cells synergistically with some growth factor(s) present in FBS for proliferation ability. Synergistic
effects of these growth factors on the Kasumi-1 cells are
under investigation.

In the present study, IL-5 alone did not induce the
Kasumi-1 cells into eosinophils. This fact is not in concor-
dance with the experiment of Ema et al.24 Further studies
are necessary for the final conclusion of the differentia-
tion ability of the Kasumi-1 cells into eosinophils in the presence of IL-5 in combination with other growth factor(s).

The Kasumi-1 cells differentiate into macrophagelike
cells when cultured with TPA. It has been reported that
most of the myelocytic leukemic cells differentiated into
macrophagelike cells in vitro culture with TPA.24,25 This fact
indicates that myelocytic leukemia involves the stem cells
that can differentiate into both granulocytic and macroph-
agelike cells. The Kasumi-1 cell line also seems to be
originated from such a progenitor cell.

Of the 148 reported cases, two were diagnosed as M4
(FAB classification).26 Although those cases with M4 are
very rare, our study indicated that some AML cases with
t(8;21) could be diagnosed as M4 if leukemic cells different-
te into macrophages in vivo. It would be necessary to
identify the biologic factor(s) that is responsible for the
promotion of differentiation of leukemic cells of AML with
t(8;21) to macrophagelike cells.

Tumor formation (histologically myeloblastoma) outside the
bone marrow has been reported in AML with t(8;21).4
In the present study, 10% to 20% of the cells adhered to the
wall of the culture bottles and some cells gathered around
the adherent cells and formed aggregations. This phenome-
on might be associated with the tumor formation in AML
with t(8;21). In addition, surface marker CD4 disappeared
and CD11b newly appeared after the culture with TPA.
This phenomenon is in concordance with the evidence
observed in the U937 cell line.25 It is possible that CD4
positive cells might have been converted into CD11b
positive macrophagelike cells. If the tumor formation re-
lates to the macrophage differentiation, CD4 would be an
important marker to predict the formation of myeloblast-
oma in AML with t(8;21).

Kamada et al7 reported the lack of neutrophil alkaline
phosphatase recovery of AML with t(8;21) in vitro
culture, suggesting genetic alteration of alkaline phos-
phatase-related genes. Detailed research on NAP activity
will be conducted in the future.

The Kasumi-1 cell line has the monosomy of chromo-
some 13. The Rb (retinoblastoma) antioncogene is located
on 13q14. The possibility of structural rearrangement of
this antioncogene also should be studied in the Kasumi-1
cell line.

In conclusion, the previously described investigation
shows that the cell lines with specific chromosome abnor-
malities provide important material for the study of biologic
mechanisms involved in neoplasia. This cell line would be
a useful tool for biologic research of t(8;21).

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