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

By Hiroyo Asou, Satoshi Tashiro, Kazuko Hamamoto, Akira Otsuji, Kenkichi Kita, and Nanao Kamada

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 naphthol AS-D chloroacetate esterase (CAE), and neutrophil alkaline phosphatase (NAP) for morphologic studies and cytochemical reactions.

Surface marker analysis. Cell surface antigens were detected by flow cytometry (Ortho Cytron) at two laboratories (Hiroshima University and Mie University, Japan).

Southern blot hybridization. High molecular weight DNA was extracted from the Kasumi-1 cells. Ten micrograms of this DNA was digested with BamHI, HindIII, or EcoRI, and was subjected to the Southern blot hybridization technique with the Epstein-Barr virus Bioprobe (ENZO Diagnostic, Inc, New York, NY), IgJH, and T-cell receptor constant region (TCR-β) probes, as reported previously.

Colony assay. Colony assay was performed in methylcellulose semisolid culture with minor modifications, as previously described. The Kasumi-1 cell fraction was suspended in Iscove's modified Dulbecco's (IMDM) medium supplemented with 40% heat-inactivated fetal bovine serum and adjusted to the concentration of 3 x 10⁶ cells/mL. Fifty microliters of this cell suspension was gently suspended in an equal volume of IMDM supplemented with an optimal dose of single or combinatory growth-stimulating factors, 2% methylcellulose (Nakarai Chemical Co, Kyoto, Japan), and 2% deionized bovine serum albumin (BSA; Armour Pharmaceutical Co, Kankakee, IL) and cultured in a flat-bottom 96-well tissue culture plate (Nunc, Roskilde, Denmark) at 37°C in a fully humid incubator at 5% CO₂ for 7 days. An equal number of the Kasumi-1 cells in IMDM suspended with 20% FBS, 1% methylcellulose, and 1% BSA was treated as a control. The number of colonies consisting of more than 40 cells were counted under the microscope.

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inverted microscope (Olinpus Co, Tokyo, Japan). The whole cultures were performed in triplicate and the results were tabulated with mean standard error (see Table 3).

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).

Fig 1. (A) MG staining and (B) MPO staining of the Kasumi-1 cells (original magnification × 1,000). (C) MG staining (original magnification × 100) and (D) α-naphthyl butylate esterase staining (original magnification × 600) of the adherent cells of Kasumi-1 cells after the culture with TPA.

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.

3H-thymidine uptake. Cells were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated FBS and adjusted to a concentration of 5 × 10⁶ cells/mL. Fifty microliters of 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 lepto chromatic 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 Mic Univer-

Table 1. Reactivity of Original Leukemia Cells and the Kasumi-1 Cell Line With Monoclonal Antibodies

<table>
<thead>
<tr>
<th>CD</th>
<th>MoAb</th>
<th>Original Leukemia Cells</th>
<th>Kasumi-1 Cell Line</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Hiroshima</td>
<td>Mie</td>
</tr>
<tr>
<td>2</td>
<td>OKT11</td>
<td>7.1</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>OKT3</td>
<td>7.3</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>OKT4A</td>
<td>2.1</td>
<td>37.1</td>
</tr>
<tr>
<td>5</td>
<td>Leu1</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>7</td>
<td>OKT16</td>
<td>NT</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>OKT8</td>
<td>5.6</td>
<td>3.4</td>
</tr>
<tr>
<td>20</td>
<td>OKT20</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>21</td>
<td>OK7</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>11</td>
<td>OKB11b</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>14</td>
<td>OKM14</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>13</td>
<td>OKM13</td>
<td>74.8</td>
<td>98.9</td>
</tr>
<tr>
<td>15</td>
<td>LeuM1</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>33</td>
<td>MY9</td>
<td>NT</td>
<td>65.5</td>
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<td>34</td>
<td>MY10</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td>OKBCallA</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>19</td>
<td>OKB19</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>38</td>
<td>OKT10</td>
<td>NT</td>
<td>50.1</td>
</tr>
<tr>
<td>71</td>
<td>Nu-TERf</td>
<td>NT</td>
<td>53</td>
</tr>
<tr>
<td>22</td>
<td>OKDR</td>
<td>27.5</td>
<td>89.3</td>
</tr>
</tbody>
</table>

Abbreviations: MoAb, monoclonal antibody; NT, not tested.
*Tested by other antibodies: CD3 by Leu4, CD4 by Leu3, CD7 by TP40, CD8 by Leu2, CD20 by B1, CD14 by My4, CD13 by MCS2, CD10 by J5, CD19 by Leu12, CD38 by Leu17, HLA-DR by NU-la.

1.25% dimethyl sulfoxide (DMSO), 10^{-7} mol/L phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), 10 ng/mL of G-CSF, or 10 U/mL of mouse IL-5 (Genzyme). After 5 days of culture in RPMI 1640 medium with 20% FBS, DMSO, or TPA at the cell concentration of 2 \times 10^7/mL, cytopsin smears were prepared for morphologic observations and were analyzed for MG, MPO, NAP, NBE, and CAE stainings. Morphology of cells cultured with G-CSF or IL-5 was observed on the 7th and the 14th day of culture.

RESULTS

Table 2. Chromosome Analysis of Original Leukemia Cells and the Kasumi-1 Cell Line

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>No. of Metaphases Examined</th>
</tr>
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<tbody>
<tr>
<td>45,X,-Y,t(8;21)(q22;q22)</td>
<td>7</td>
</tr>
<tr>
<td>44,X,-Y, -16,t(8;21)(q22;q22)</td>
<td>3</td>
</tr>
<tr>
<td>43,X,-Y, -9, -13, -15, -16,t(8;21)(q22; q22), +der(9)(q9;2)(q11;?), +mar</td>
<td>3</td>
</tr>
<tr>
<td>46,XY</td>
<td>3</td>
</tr>
<tr>
<td>44,X,-Y, -9, -13, -15, -16,t(8;21)(q22; q22), +der(9)(q9;2)(q11;?), +mar1, +mar2</td>
<td>14</td>
</tr>
<tr>
<td>45,X,-Y, +4, -9, -13, -15, -16,t(8;21)(q22; q22), +der(9)(q9;2)(q11;?), +mar1, +mar2</td>
<td>7</td>
</tr>
<tr>
<td>46,X,-Y, +4, -9, -13, -15, -16,t(8;21)(q22; q22), +der(9)(q9;2)(q11;?), +mar1, +mar2, +mar3</td>
<td>4</td>
</tr>
</tbody>
</table>
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;7)(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 IgJH 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 ³H-thymidine uptake were observed by G-CSF. G-CSF acts on lineage-restricted stem cells, mainly on the granulocytic 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 multipotential 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 concordance with the experiment of Ema et al.¹⁴ Further studies are necessary for the final conclusion of the differentiation 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 This fact indicates that myelocytic leukemia involves the stem cells that can differentiate into both granulocytic and macrophagelike 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 differentiate 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).²⁷ 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 phenomenon 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.²⁸ It is possible that CD4 positive cells might have been converted into CD11b positive macrophagelike cells. If the tumor formation relates to the macrophage differentiation, CD4 would be an important marker to predict the formation of myeloblastoma in AML with t(8;21).

Kamada et al.⁷ reported the lack of neutrophil alkaline phosphatase recovery of AML with t(8;21) in vitro culture, suggesting genetic alteration of alkaline phosphatase-related genes. Detailed research on NAP activity will be conducted in the future.

The Kasumi-1 cell line has the monosomy of chromosome 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 abnormalities 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).

**REFERENCES**

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H Asou, S Tashiro, K Hamamoto, A Otsuji, K Kita and N Kamada