Establishment and Characterization of a Novel Acute Promyelocytic Leukemia Cell Line (UF-1) With Retinoic Acid–Resistant Features

By Masahiro Kizaki, Hiromichi Matsushita, Nobuyuki Takayama, Akihiro Muto, Hironori Ueno, Norihiro Awaya, Yohko Kawai, Hiroya Asou, Nanao Kamada, and Yasuo Ikeda

All-trans retinoic acid (RA) induces complete remission in a high proportion of patients with acute promyelocytic leukemia (APL). Nevertheless, most of these patients develop RA resistance and relapse. The mechanisms of RA resistance by APL cells are still unclear. To understand the characteristics of human leukemia, human leukemic cell lines are useful tools for study. APL cells have a strikingly low proliferation potential in vitro; thus, only one APL cell line has been established. We developed a novel APL cell line (UF-1) from a patient clinically resistant to all-trans RA. Cell surface markers in the UF-1 cells were positive for CD7, CD13, CD33, and CD38. Cytogenetic analyses revealed additional abnormalities, 46XX, add(1)(q44), add(6)(q12), add(7)(q36), t(15;17) (q21;q21). Molecular analyses showed a PML/RARA fusion transcript. Sequence analysis of the RARα gene in RA-resistant HL-60 cells disclosed a point mutation in codon 411 (C to T substitution), whereas UF-1 cells showed the normal sequence. All-trans RA did not change morphological features of the cell, NBT reduction activity, or their expression of CD11b antigens as determined by FACS analysis except at 10⁻⁴ mol/L. RA also did not alter the growth curve of the cells as determined by the MTT assay. These findings suggest that the UF-1 cell is the first permanent cell line with spontaneous RA-resistant APL cells. This RA-resistant APL cell line may be a useful model for molecular studies on the block of leukemic cell differentiation and as a means to investigate the mechanisms of RA resistance.

© 1996 by The American Society of Hematology.

VITAMIN A and its biological active metabolites, including retinoic acid (RA), regulate the growth and differentiation of a wide variety of different cell types. RA induces terminal differentiation of HL-60 cells and acute promyelocytic leukemia (APL) cells in vitro. APL is characterized by a differentiation block at the promyelocytic stage, occurrence of disseminated intravascular coagulation (DIC), and by a reciprocal translocation affecting chromosomes 15 and 17 [t (15;17) (q21;q21)]. Recent clinical studies have shown that complete remission (CR) could be achieved in a high proportion of patients with APL through all-trans RA treatment, but most patients relapse and develop RA-resistant leukemia. Clinical resistance to RA poses a serious problem for differentiation-inducing therapy, and the mechanisms of RA-resistance by APL cells are still unclear.

To understand the characteristics of leukemic cells, human cell lines are useful tools. The APL cells have strikingly low proliferation potential in vitro; thus, only one APL cell line has been isolated and is designated NB4. This cell line was established on a bone marrow stromal cell layer before emergence of an autonomous cell line. The cell can be induced to differentiate to granulocytes by all-trans RA. Also, the NB4 cells were cultured for a long time with high concentrations of all-trans RA (10⁻⁶ mol/L), which resulted in an RA-resistant cell line named NB4-RA. We report here the establishment and characterization of a novel APL cell line (UF-1) from a patient who was clinically resistant to all-trans RA. We also describe their lack of cellular differentiation as compared with NB4 and HL-60 cells in response to all-trans RA treatment.

MATERIALS AND METHODS

Case history. The cell line described in this report was derived from a peripheral blood sample of a 30-year-old woman with APL. She was admitted to the Keio University Hospital on June 3, 1991 because of bleeding tendency. At that time, peripheral blood counts were as follows: white blood cells, 26.1 × 10⁹/L with 54% blast cells; red blood cell count, 2.84 × 10¹²/L; hemoglobin, 9.1 g/dL; hematocrit, 26.2%; and platelets 17 × 10⁹/L. The bone marrow smear revealed a hypercellular marrow infiltrated with 96% hypergranular promyelocytes. DIC syndrome was clinically very active. Cytogenetic studies showed the typical t(15;17) (q21;q21) translocation, and molecular analyses revealed a PML/RARA fusion transcript. She achieved a CR with combination chemotherapy consisting of daunorubicin, cytarabine, 6-MP, and prednisolone. A year later, she relapsed. A second short CR was achieved after another chemotherapy, but she relapsed again. On second relapse, all-trans RA (60 mg/day orally) was commenced, and subsequently the dose of all-trans RA was doubled; however, RA failed to induce differentiation of APL cells and she died of progressive disease on July 24, 1994.

Cell culture. On July 23, 1994, a sample of peripheral blood was obtained with the patient’s informed consent. Cells (1 × 10⁹/mL) were cultured in RPMI-1640 medium (GIBCO-BRL, Grand Island, NY) with 10% fetal bovine serum (FBS) (HyClone Laboratories, Inc, Logan, UT) in a 25-cm² flask at 37°C under 5% CO₂. No growth factors or feeder cells were used. The cells were fed either once or twice weekly by partial replacement of spent medium with fresh medium. After 4 to 6 weeks, continuous growth of the cells was observed and confirmed as a cell line (UF-1) after 12 weeks of culture.

The HL-60 and NB4 cells (the latter a generous gift of Dr Lanotte, Hôpital St Louis, Paris, France) were maintained in RPMI-1640 medium with 10% FBS. RA-resistant HL-60 cells (RS HL-60; a gift of Dr R.E. Gallagher, Montefiore Medical Center, Bronx, NY) were maintained in serum-free culture medium (Cosmmed 001; Cosmo Bio Co, Tokyo, Japan) containing all-trans RA (10⁻⁴ mol/L). Cell numbers and viability were assessed by the trypan blue dye exclusion test. The morphology of the cells was evaluated from...
ESTABLISHMENT OF A NOVEL APL CELL LINE

**Chemicals and hematopoietic growth factors.** All-trans RA was purchased from Sigma Chemical Co (St Louis, MO), and 9-cis RA was a generous gift of Dr H. Klaus (F. Hoffmann-La Roche, Basel, Switzerland). They were dissolved in 100% ethanol to a stock concentration of 1 mmol/L, stored at -20°C, and protected from light. Recombinant human granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), IL-6, and stem cell factor (SCF) were provided by Kirin Brewery Co, Ltd (Tokyo, Japan). Recombinant human macrophage colony-stimulating factor (M-CSF) was kindly provided by Green Cross Co (Osaka, Japan) and transforming growth factor beta (TGF-β) was a gift of Chugai Pharmaceutical Co (Tokyo, Japan).

**Surface marker analysis.** Cell surface antigens were detected by immunofluorescence staining with the monoclonal antibodies shown in Table 1. Monoclonal antibodies used in the study include: Leu1, Leu2a, Leu3a, Leu4, Leu9, Leu12, Leu15, Leu16, HLA-DR, LeuM3, CALLA, and HPCA-2 (Becton Dickinson, Mountain View, CA); WM47, WM54, and TUK4 (Dako Japan, Kyoto, Japan); and TP-80 (Nichirei, Tokyo, Japan). The cells were analyzed by flow cytometry (FACScan, Becton Dickinson).

**Cytogenetic studies.** Chromosomes were analyzed by the Giemsa banding technique as reported previously.15

**Assays for cellular differentiation.** UF-1, HL-60, and NB4 cells were suspended with either all-trans or 9-cis RA (10⁻⁶ to 10⁻⁴ mol/L) for 4 days. For analysis of cellular differentiation, cells were obtained and examined by morphology using Giemsa staining, nitroblue tetrazolium (NBT) reduction activity, and expression of cell surface antigens using two-color direct immunofluorescence staining technique. For NBT, cell suspension (2 × 10⁶/mL) was mixed with an equal volume of solution containing 1.25 mg/mL NBT (Sigma), 17 mg/mL bovine albumin, and 1 mg/mL 12-O-tetradecanoylphorbol 13-acetate (TPA) for 30 minutes at 37°C. After incubation, the medium was discarded, and formazan deposits were dissolved by adding 100 µL of dimethyl sulfoxide (Sigma) and measured at 580 nm by spectrophotometer. For analysis of cell surface antigens, cells were incubated for 60 minutes with human AB serum (Sigma) to block Fc receptors and then stained by using fluorescein isothiocyanate (FITC)-conjugated mouse antihuman CD14 and phycoerythrin (PE)-conjugated mouse antihuman CD11b antibodies (Becton Dickinson). Control studies were performed with a nonbinding control mouse IgG1 and IgG2 isotype antibodies (Becton Dickinson).

**Assays for cellular proliferation.** Cellular proliferation was measured by cell viability and nonradioactive cell proliferation assay system (MTT assay; Boehringer Mannheim, Indianapolis, IN). Cells (2 × 10⁴) were incubated with either RA or various cytokines for 4 days in 96-well plates (Flow Laboratories, Irvine, CA). Ten micromolars of MTT (5 µg/mL) was added to each well. The reaction was stopped after 4 hours of incubation by adding 100 µL of 0.04 N HCl in isopropanol, and the OD₅₇₀ was determined.

**Reverse transcription-polymerase chain reaction (RT-PCR) assay for PML/RARA fusion transcript.** The expression of the PML/RARA fusion transcript in UF-1 cells was examined by the RT-PCR method with primers flanking the fusion point. Total RNA was extracted from UF-1 and fresh APL cells as a positive control by the acid guanidinium thiocyanate-phenol-chloroform method. The RT reaction was carried out using 1 µg of total RNA, 100 pmol of random hexamer (Boehringer Mannheim), 10 U RNase inhibitor (Promega, Madison, WI), 200 U MMLV-RT (GIBCO-BRL), and deoxynucleotides (dNTPs, final concentration 0.5 mmol/L each: Pharmacia, Piscataway, NJ) in a total volume of 20 µL under conditions recommended by the suppliers. After cDNA synthesis,
Fig 2. Electron microscopic pictures of UF-1 cells cultured either in the presence or absence of all-trans RA (10^{-8} mol/L) for 4 days. (A) Control UF-1 cells, showing large primary granules positive for peroxidase reaction. The nuclear chromatin was primarily euchromatic, and prominent nucleoli were stained. Auer rods were distributed in the cytoplasm. (original magnification × 5,000). (B through D) UF-1 cells were treated with all-trans RA (10^{-6} mol/L) for 4 days. Most cells displayed immature features (B). Considerable nuclear segmentation and increased definition of heterochromatin areas were observed; nevertheless, these cells also had nucleoli (C) and Auer rods (D) that are features of an immature myeloid cells. (original magnification × 3,300).

40 cycles of PCR was sequentially carried out. The primers used in this study were as follows: 5'-primer, 5'-AGC TGC TGG AGG CTG ACG CGC GGT ACC-3' (PML exon3); 3'-primer, 5'-CAG AAC TGC TGC TCT GGG TCT CAA T-3' (RARα exon3). Each 100 μL of PCR solution contained 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 5 U Taq polymerase (Perkin-Elmer/Cetus, Norwalk, CT), 2 μL of cDNA (out of 20 μL total RT reaction), 100 pmol of each primer, and 200 mmol/L of each dNTP. PCR was performed using denaturing steps at 94°C for 2 minutes, annealing steps at 55°C, and extending steps at 72°C for 2 minutes, respectively. PCR products (10 μL) were electrophoresed on 2% NuSieve/1% SeaKem agarose (FMC, Rockland, MI) gels and transferred to nitrocellulose membrane (Hybond N⁺; Amersham Japan, Tokyo). Southern blotting of the amplified cDNA with hybridization to nonradioactive probe (ECL 3'-oligolabeling and detection system; Amersham) was performed.

**Allelic analysis by Fok I digestion.** PCR amplification was performed as described above, and PCR products (161 bp) were purified using a Geneclean kit (Bio 101, Vista, CA), and 150 ng of products was digested with 4 U of Fok I (Stratagene, La Jolla, CA) according to the suppliers condition at 37°C for 3 hours. The restriction products were then analyzed by gel electrophoresis and ethidium bromide staining.

**RESULTS**

**Establishment of UF-1 cell line.** We obtained leukemic cells from the peripheral blood of a patient with APL. The cells proliferated slowly for the first few weeks after the initiation of culture. The cells began to proliferate and continued to increase in numbers in liquid culture with RPMI-1640 supplemented 10% FBS; this occurred without the addition of hematopoietic growth factors. This new APL cell line (UF-1) was established and maintained in suspension culture for over 17 months and has a doubling time of 72 hours.

**Morphology.** The cells showed considerable heterogeneity in cell size and nuclear cytoplasmic ratio (N/C). The nuclei were large, round, and often lobulated, and a few nucleoli were found per cell. Numerous granules were present in all cells. Some cells contained large azulophilic granules that were compatible with hypergranulocytic promyelocytes (Fig 1A). NB4 cells were treated with 10^{-8} mol/
Table 1. Surface Antigen Characterization of Original Leukemia Cells, UF-1 Cells, and NB4 Cells

<table>
<thead>
<tr>
<th>CD</th>
<th>Monoclonal Antibody</th>
<th>Antibody Specificity</th>
<th>Original Leukemia Cells</th>
<th>UF-1 Control</th>
<th>RA*</th>
<th>NB4 Control</th>
<th>RA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Leu4</td>
<td>Mature T cells</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>CD4</td>
<td>Leu 3a</td>
<td>Helper T cells</td>
<td>1</td>
<td>&lt;1</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>CD5</td>
<td>Leu 1</td>
<td>Pan T cells/activated B cells</td>
<td>1</td>
<td>2</td>
<td>&lt;1</td>
<td>19</td>
<td>42</td>
</tr>
<tr>
<td>CD7</td>
<td>Leu 9</td>
<td>Pan T cells</td>
<td>1</td>
<td>&lt;1</td>
<td>34</td>
<td>40</td>
<td>66</td>
</tr>
<tr>
<td>CD8</td>
<td>Leu 2a</td>
<td>Suppressor T cells</td>
<td>2</td>
<td>5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>CD10</td>
<td>CALLA</td>
<td>Common acute lymphocytic leukemia</td>
<td>&lt;1</td>
<td>2</td>
<td>&lt;1</td>
<td>32</td>
<td>44</td>
</tr>
<tr>
<td>CD11b</td>
<td>Leu 15</td>
<td>Granulocytes/monocytes</td>
<td>ND†</td>
<td>1</td>
<td>17</td>
<td>20</td>
<td>96</td>
</tr>
<tr>
<td>CD19</td>
<td>Leu 12</td>
<td>Pan B cells</td>
<td>5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>CD20</td>
<td>Leu 16</td>
<td>Pan B cells</td>
<td>12</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>la (DR)</td>
<td>HLA-DR</td>
<td>Human la antigen</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>CD13</td>
<td>WM-47</td>
<td>Myeloid, hematopoietic cells</td>
<td>94</td>
<td>38</td>
<td>65</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>CD14</td>
<td>Leu M3</td>
<td>Monocytes</td>
<td>&lt;1</td>
<td>22</td>
<td>6</td>
<td>73</td>
<td>87</td>
</tr>
<tr>
<td>CD33</td>
<td>WM-54</td>
<td>Hematopoietic cells</td>
<td>98</td>
<td>91</td>
<td>51</td>
<td>98</td>
<td>37</td>
</tr>
<tr>
<td>CD34</td>
<td>HPCA-2</td>
<td>Hematopoietic cells</td>
<td>ND†</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>CD38</td>
<td>TUK4</td>
<td>Hematopoietic cells/activated marker</td>
<td>94</td>
<td>65</td>
<td>99</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>CD41</td>
<td>TP-80</td>
<td>Platelet GP IIb</td>
<td>&lt;1</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2</td>
</tr>
</tbody>
</table>

Cells were incubated for 30 minutes with the appropriately diluted monoclonal antibodies. After washing with phosphate-buffered saline, the cells were reincubated with fluorescent goat antimouse Ig for 30 minutes. After a second washing, the immunophenotypes of the cells were analyzed by flow cytometry.

* Cells were cultured with all-trans RA 10⁻⁶ mol/L for 4 days.
† Not done.

L all-trans RA for 4 days, which resulted in differentiation toward mature granulocytes (Fig 1C and D). In contrast, all-trans RA (10⁻⁷ mol/L), however, did not induce morphological differentiation of UF-1 cells toward either mature granulocytes or monocytes (Fig 1B). These results suggest that UF-1 is the first permanent APL cell line with spontaneous RA-resistance.

The electron microscopy revealed that the nuclear chromatin was primarily euchromatic, and prominent nucleoli were stained in UF-1 cells. Large numbers of peroxidase positive granules and Auer rods were detected in the cytoplasm (Fig 2A). Most cells continued to be immature with all-trans RA treatment (Fig 2B). Nevertheless, the nuclei of some all-trans RA-treated cells displayed considerable segmentation.

![Fig 3. Karyotype of the UF-1 cell line, representing 46,XX, add(1)(q44), add(6)(q12), add(7)(q36), t(15;17)(q21;q21).](image)
and increased heterochromatin areas; but they still have nucleoli and Auer rod characteristic of immature myeloid cells (Fig 2C and D).

Surface marker analysis. Cell surface markers of both the original leukemic cells and the UF-1 cells as well as NB4 cells were analyzed and summarized in Table 1. Also, both cell lines were treated with all-trans RA (10⁻⁶ mol/L) for 4 days and cell surface marker analysis was performed. It showed that NB4 cells expressed markers of granulocytic lineage (CD13 and CD33). All-trans RA increased the expression of CD11b antigen ninefold, suggesting the terminal differentiation of NB4 cells to mature granulocytes (Table 1). Both of the original leukemic cells and UF-1 cells were positive for CD33 and CD38. Interestingly, UF-1 cells were only 38% positive for CD13 and 91% positive for CD7, whereas the original cells were negative for CD7 antigen (Table 1).

Cytogenetic studies. Cytogenetic study of the bone marrow specimen at diagnosis and first hospital admission showed the typical t(15;17)(q21;q21). The additional chromosomal abnormalities found in the UF-1 cell line were add(1)(q44), add(6)(q12), and add(7)(q36) (Fig 3).

RT-PCR assay for PML/RARα fusion transcript. We examined for the expression of PML/RARα chimeric transcripts in UF-1 cells using the RT-PCR assay. As shown in Fig 4, the PML/RARα fusion transcript was detected in both UF-1 cells and fresh leukemic cells from a APL patient who had a typical t(15;17) (positive control). These results were also confirmed by Southern blotting of the RT-PCR products (Fig 4). The identical size of the PML/RARα transcripts in UF-1 and the freshly isolated APL cells suggested that no large deletion was present in the DNA sequences of the RA receptor of the UF-1 cells.

Proliferation studies by RA and recombinant hematopoietic growth factors. To assess the effects of RA and cytokines on UF-1 cells, the MTT assay (a non-radioactive cellular proliferation assay) was used. Neither all-trans RA (10⁻⁶ mol/L), 9-cis RA (10⁻⁶ mol/L), nor various cytokines (G-CSF, M-CSF, and IL-6) affected the absorbance of the MTT assay (Figs 5 and 6A). The UF-1 cells displayed proliferative responses to GM-CSF (10 ng/mL), IL-3 (100 ng/mL), and SCF (100 ng/mL). In contrast, TGF-β (1 ng/mL) markedly inhibited the proliferation of UF-1 cells (Fig 5).

Effect of RA on proliferation and differentiation of HL-60, NB4, and UF-1 cells. HL-60, NB4, and UF-1 cells were cultured for 4 days with various concentrations of all-trans RA (10⁻¹⁰ to 10⁻⁶ mol/L) (Fig 6A and B). All-trans RA inhibited the cellular proliferation of HL-60 and NB4 cells in a dose-dependent manner (Fig 6A). However, all-trans RA did not change the absorbance of MTT assay in UF-1 cells. Cell proliferation decreased by only 10% to 20% after the UF-1 cells were exposed to higher concentration of RA (10⁻⁶ mol/L) (Fig 6A).

Induction of differentiation of these three cell lines into mature granulocytes by all-trans RA was assessed by the NBT reduction activity and expression of CD11b and CD14 antigens using FACS analysis (Figs 6B, 6C, and 7). NBT reduction activity and expression of CD11b antigen in HL-60 and NB4 cells was increased by all-trans RA in a dose-dependent manner (10⁻¹⁰ to 10⁻⁶ mol/L) (Fig 6B and C). In
marked contrast, all-trans RA did not alter the NBT reduction activity and expression of CD11b antigen in UF-1 cells except at $10^{-9}$ mol/L (Figs 6B, 6C, and 7). Expression of CD14 antigen was unchanged after exposure of each cell line to RA. Therefore, these functional analyses were consistent with our morphological assessment showing that UF-1 cells were resistant to induction of cellular differentiation by all-trans RA.

**Sequencing and allelic analysis by Fok I digestion in RARα.** We cloned and sequenced the RT-PCR products from a ligand-binding domain of RARα in HL-60, RS HL-60, NB4, and UF-1 cells. As previously reported,19,20 RS HL-60 cells showed a C to T substitution in RARα codon 411 (Fig 8A). This point mutation introduces a termination codon TAG, truncating the 52 amino acids at the C-terminus of RARα. However, UF-1, HL-60, and NB4 cells had the normal sequence (Fig 8A). The C to T substitution in RARα from RS HL-60 cells alters the recognition sequence for the restriction endonuclease Fok I (Fig 8B). PCR products from RS HL-60 cells were completely resistant to Fok I digestion, whereas those from UF-1, HL-60, and NB4 cells could be digested to completion.

**DISCUSSION**

The UF-1 is a novel human cell line derived from a patient with APL with a 15;17 translocation; the cells are resistant...
to all-trans RA and have the morphology of typical promyelocytes. To our knowledge, this is the second human APL cell line and the first that has an unselected resistance to RA.

Several differences contrast NB4 from UF-1 cells. The NB4 cells were established from bone marrow cells of a patient with APL in relapse; the cells were initially cultured on a bone marrow stromal layer of cells. On the other hand, UF-1 cells were established from a patient who was clinically resistant to all-trans RA; these cells were initially cultured without any supporting hematopoietic growth factors or stromal cells. The NB4 cells have a myeloblastic morphology without the typical APL granules, whereas the UF-1 cells are hypergranulocytic promyelocytes that are consistent with APL (M3 by the FAB nomenclature).

The immunophenotype of APL is considered to be positive for CD13, CD33, and CD9 and negative for CD11b, CD14, CD15, CD34, and HLA-DR. The cell surface analysis of antigens showed that the NB4 cells were positive for myeloid markers (CD13 and CD33) and some T-cell markers (CD2). Expression of CD2 in AML has been described as a favorable prognostic factor. The UF-1 cells expressed myeloid markers, CD7 (91%) and only 38% positive for CD13, but not T-cell markers. The patient’s original leukemic cells were negative for CD7. AML expressing CD7 are considered to be an early hematopoietic cell origin. Kita et al reported that CD7-positive AML patients showed a poor prognosis and unfavorable responses to chemotherapy. Consistent with our patient’s original leukemic cells, CD7 was not expressed in 40 patients with APL. Why UF-1 cells, but not original leukemic cells, expressed CD7 and low level CD13 is unclear. Perhaps the CD7-positive and low CD13-positive leukemic cells represented a minor population of the original leukemia, but in vitro these more “immature” cells had a growth advantage during establishment and development of the RA-resistant leukemic cells. Furthermore, the CD7 may be an important molecule in the estab-
Establishment of this cell line, even in the absence of the addition of hematopoietic growth factors or stromal cells. Also, the UF-1 cells had an enhanced proliferation in the presence of GM-CSF, IL-3, and SCF but not G-CSF, M-CSF, IL-6, and TGF-β. The greatest stimulation was observed in the presence of GM-CSF or IL-3. As compared with G-CSF, M-CSF, IL-6, and TGF-β, IL-3, GM-CSF, and SCF act on very early hematopoietic stem cells, which supports the immaturity of the UF-1 cells.

All-trans RA induced terminal differentiation of HL-60 and NB4 cells to mature granulocytes. In contrast, all-trans RA did not change the morphological features, NBT reduction activity, expression of CD11b antigen, or growth rate of the UF-1 cells, showing that these cells are RA-resistant t(15;17) APL cells. The NB4-RA cell is a subline of NB4 cell, which is RA resistant after their continuous exposure to high concentrations of all-trans RA (10⁻⁶ mol/L). Therefore, UF-1 cell is the first permanent cell line with spontaneous RA-resistant APL cells.

Several mechanisms have been proposed to explain in vitro resistance to RA. One possible explanation is generation of a mutation in the nuclear RA receptor. As a result of this study, a point mutation in the RARα gene has been reported in several RA-resistant HL-60 sublines. Both subclones have the same point mutation, a C to T transition in codon 411 that is the ligand-binding domain of the RARα gene. This DNA alteration leads to a truncation of the protein at this site. These RA-resistant HL-60 cells developed under the artificial culture conditions of exposure to high concentrations of RA (≥10⁻⁶ mol/L).

Another possible mechanism of resistance to RA is either rapid drug metabolism or cytoplasmic sequestration by cytoplasmic RA binding protein (CRABP) that results in either lowering plasma or intracellular levels of active retinoids, respectively. Delva et al. reported that CRABP-II expression was increased at the time of relapse of APL compared with the levels before initiating all-trans RA treatment. Recently, we reported that acquired resistance to RA in samples from patients may be explained in part by altered intracellular drug metabolism including those concerning the induction of cytochrome P-450 enzymes and P-glycoprotein.
A third explanation may be that the proteins important in acquisition of RA resistance occur as a result of the additional chromosomal abnormalities (add(1)(q44), add(6)(q12), and add(7)(q36)) in this cell line. Further studies should examine the molecular basis of RA-resistance in UF-1 cells.

In conclusion, UF-1 cells may be a useful model for molecular studies of the defects in leukemic cell differentiation and a means to investigate the mechanisms of RA resistance in leukemic cells. It also will furnish a unique tool for designing and screening new therapeutic drugs to overcome RA resistance in patients with APL.

ACKNOWLEDGMENT

We appreciate the support and advice of Prof H. Phillip Koeffler (Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA) for his comments on the manuscript. We also thank Y. Enomoto (Department of Pathology, Keio University School of Medicine) for his excellent technical assistance of electron microscopy.

REFERENCES

11. Lanotte M, Martin-Thouvenin V, Najman S, Balerini P, Valent F, Berger R: NB4, a maturation inducible cell line with t(15;17) and a retinoic acid “resistant” t(15;17) acute promyelocytic leukemia cell line: Isolation, morphological, immunological, and molecular features. Leukemia 6:1281, 1992
topoietic stem cell factor from buffalo rat liver-conditioned medium.


Establishment and characterization of a novel acute promyelocytic leukemia cell line (UF-1) with retinoic acid-resistant features

M Kizaki, H Matsushita, N Takayama, A Muto, H Ueno, N Awaya, Y Kawai, H Asou, N Kamada and Y Ikeda