Expression of MDR1 Gene in Acute Leukemia Cells: Association With CD7+
Acute Myeloblastic Leukemia/Acute Lymphoblastic Leukemia

By Hiroshi Miwa, Kenkichi Kita, Kazuhiro Nishii, Norihisa Morita, Nobuyuki Takakura, Kohshi Ohishi, Nadim Mahmud, Shinichi Kageyama, Manabu Fukumoto, and Shigeru Shirakawa

MDR1 gene expression was examined in acute leukemia cells from 75 Japanese patients at diagnosis (50 with acute myeloblastic leukemia [AML]: 10 M1, 18 M2, 5 M3, 8 M4, 9 M5; 25 with acute lymphoblastic leukemia [ALL]: 13 B-precursor, 12 T-lineage). The results of MDR1 mRNA expression by reverse transcriptase polymerase chain reaction were confirmed by immunostaining using the anti-P-glycoprotein monoclonal antibody UIC2 and by a functional study using the rhodamine efflux test. Morphologically, AML M1 cases had the highest incidence of MDRI gene expression (6 of 10 patients). Phenotypically, CD7 and CD34 were the only surface markers that were significantly associated with MDRI gene expression (P < .01). In CD7+CD4-CD8- ALL, which is thought to originate from the lymphohematopoietic stem cell, expressed the MDRI gene with a high incidence (six of eight patients), whereas three surface CD3+ and one CD4+CD8+ T-cell ALL (T-ALL) did not have detectable MDR1 transcripts. Only two cases of 13 B-precursor ALL had MDR1 mRNA, of which had the Philadelphia (Ph) chromosome. No association was observed between MDR1 gene expression and CD34 positivity in ALL. Our results that MDR1 mRNA was frequently expressed in CD7+ AML and CD7+CD4-CD8- ALL, together with the previous reports indicating clinical similarities between these leukemias, provides a clue to clarify a relationship between CD7+ AML and CD7+CD4-CD8- ALL. In addition, MDR1 expression in CD7+ ALL/AML might be responsible for the poor response to conventional chemotherapies of these types of leukemia.

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Table 1. Nucleotide Primers Used for RT-PCR Study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense</th>
<th>Antisense</th>
<th>Ref*</th>
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<tr>
<td>MDR1</td>
<td>5'-CTGAAGAGGCTTGGGACGCA-3'</td>
<td>5'-CATAGAGGTCCTGACGACG-3'</td>
<td>761-780</td>
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<td>c-kit</td>
<td>5'-AAGGACTGATGAGGTTTTGTTTAC-3'</td>
<td>5'-CTGACCTCTAAATGGAAGTTC-3'</td>
<td>494-514</td>
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<td>G-CSFR</td>
<td>5'-ACCTGAGCAGGCACTTTGAGG-3'</td>
<td>5'-CTGGCTGTGGAGCTGAGTGG-3'</td>
<td>837-817</td>
</tr>
<tr>
<td>CD3e</td>
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<td>5'-CTGGCTGTGGAGCTGAGTGG-3'</td>
<td>1,790-1,810</td>
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<td>β-Actin</td>
<td>5'-CTGACGTTCATAATTGAAGTC-3'</td>
<td>5'-ACCTGGGAACAGCTGGAGTGG-3'</td>
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Abbreviation: G-CSFR, receptor for G-CSF.

References for primer sequences.

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MDR1+ ACUTE LEUKEMIA

Fig 2. Rhodamine 123 efflux test. (A) MDR1 mRNA-positive AML(M2), which is the same case in Fig 1A. (1) Negative control: leukemic cells were not stained with rhodamine. (2) After the staining, the cells were incubated in dye-free medium at 4°C. (3) Incubated at 37°C with verapamil, a potent P-gp inhibitor. (4) Incubated at 37°C without verapamil. The dye was effluxed from cells. (B) MDR1 mRNA-negative AML(M5) (same as in Fig 1B). Cells remained stained after the incubation at 37°C without verapamil indicating the absence of P-gp.

RESULTS

MDR1 mRNA expression in acute leukemia cells. We repeatedly examined the conditions of RT-PCR for detecting MDR1 mRNA in leukemia cells. By comparing the data of P-gp immunostaining and rhodamine 123 (Rh123) efflux test, we concluded that by using 30 cycles of PCR the most reliable results could be obtained when the primers listed in Table 1 are used. The cases having MDR1 mRNA specimens had no detectable P-gp and Rh123 efflux (Figs 1 and 2). The P-gp positivity in leukemic cells was also confirmed by immunocytochemical staining (data not shown). On the other hand, the MDR1 mRNA+ specimens had no detectable P-gp and Rh123 efflux (Table 2). The size of the RT-PCR products was in agreement with that predicted by the nucleotide sequences of the PCR primers, and the authenticity of the RT-PCR was confirmed by the strong hybridization to the MDR1 cDNA probe (RT-PCR Southern blot, Fig 3). Eight of 25 ALL cases and 15 of 50 AML cases examined had MDR1 mRNA (Table 2).

Co, St. Louis, MO) was studied as previously described. Briefly, the leukemic cells were incubated with 150 ng/mL of Rh-123 for 10 minutes and, after two washes, allowed to efflux the dye in dye-free media for 3 hours at 37°C or at 4°C. Rh-123 efflux study was also performed at 37°C in the presence of 50 μmol/L verapamil, a potent inhibitor of P-gp. After efflux, cells were analyzed by flow cytometry.

Statistical analysis. Difference in MDR1 mRNA positivity in relation to phenotypical markers were evaluated by χ² statistics.
Fig 3. RT-PCR Southern blot of MDR1 mRNA. (A) RT-PCR products were electrophoresed in agarose gel, transferred, and hybridized to MDR1 cDNA. M: molecular weight standard, ϕ × 174/HaelII. 1: Placenta as a positive control; 2 through 8: CD7+CD4−CD8− ALL: 9, 10: CD7+ AML; 11: AML(M3). (B) The original gel of RT-PCR of MDR1. (C) RT-PCR of β-actin using the same RT product as for MDR1.

Cellular characteristics of MDR1 mRNA+ ALL cells. Only 2 of 13 pre-B ALL cases had detectable MDR1 mRNA demonstrating a lower frequency than that of pre-T-cell ALL and AML (Table 2). One of the two MDR1 mRNA+ pre-B ALL cases had Ph1 chromosome and myeloid associated antigens CD11b and CD33. Although 10 of the 13 cases had CD34, no association with MDR1 gene expression was observed.

In pre-T ALL, 6 of 12 cases had MDR1 mRNA. Phenotype, immunogenotype, and cytokine receptor gene expression in the CD7+ ALL cases are shown in Table 3. The common phenotype of the MDR1 mRNA+ cases was CD7+/sCD3+/CD4−CD8− except one CD4+ case. CD34 was also positive in all MDR1+ pre-T ALL cases. Although sCD3 was not detected, cCD3 or CD3ε mRNA were detected in five of the six MDR1 mRNA+ cases. TCRs (αβ/δ) were not expressed on leukemic cells of any MDR1 mRNA+ case. Simultaneous gene rearrangements of TCR-δ and IgH were observed in one of the five MDR1 mRNA+ cases examined. No immune gene rearrangements were observed in the other four MDR1 mRNA+ cases examined.

Transcripts for the c-kit and granulocyte colony-stimulating factor (G-CSF) receptor gene were observed in four cases and all of the MDR1 mRNA+ cases. No MDR1 mRNA was detected in three sCD3+ cases and one sCD3−CD4−CD8− case examined. Here, leukemic cells of three sCD3+ cases and one CD4−CD8− case did not have c-kit or G-CSF receptor mRNA.

Phenotypical characteristics of MDR1+ AML cells. In AML, MDR1 mRNA was detected in 15 of 50 cases (30%). With regard to the French-American-British (FAB) subtype, M1 cases showed the highest incidence of MDR1 mRNA expression (Table 2). MDR1 positivity in relation to various phenotypical markers is given in Table 4. CD34+ AML showed significantly higher MDR1 mRNA positivity (P < .01). HLA-DR+ AML tended to display a higher incidence of MDR1 gene expression, although the difference was not statistically significant. CD7 expression was closely associated with MDR1 gene expression (P < .01). Because staining procedure for UIC2 was not suitable for the detection of CD34 and CD7, the double staining could not be performed. However, coexpression of P-gp and these antigens was supported by marked overlapping of percentage of cells expressing each antigen. Indeed, most of the leukemic cells effluxed the dye (Fig 2A). Other surface markers, CD11b, CD13, CD14, CD15, CD19, and CD33, did not show any significant association with MDR1 mRNA positivity (Table 4).

DISCUSSION

We describe MDR1 gene expression in acute leukemia in relation to the cellular characteristics. Our findings indicated that a close relationship between CD7 and MDR1 gene expression both in AML and ALL. In AML, MDR1 mRNA positivity was also associated with CD34 positivity. On the other hand, MDR1 gene expression was not associated with pre-B-cell phenotype in ALL. CD34 was not associated with MDR1 mRNA expression in pre-B ALL.

Difference in the sensitivity of the method for the detection of P-gp expression is a problem in examining clinical specimens.25 In this study, we carefully compared several

<table>
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<th>Table 2. MDR1 Gene Expression in AML and ALL Cells</th>
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<tr>
<td><strong>ALL</strong></td>
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<tr>
<td>CD19(+) CD10(−) ALL</td>
</tr>
<tr>
<td>CD20(−) c-ALL</td>
</tr>
<tr>
<td>CD20(+)/c-ALL</td>
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<tr>
<td>CD7(−) sCD3(−) ALL</td>
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</tr>
<tr>
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Abbreviation: c-ALL, common ALL.

* Number of positive samples/number of samples examined.
Table 3. Cellular Characteristics of CD7(+) sCD3(-) ALL Cells

<table>
<thead>
<tr>
<th>Genotype and Gene Expression</th>
<th>Immunogenotype</th>
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<tr>
<td>MDR1</td>
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Phenotype

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</tr>
</tbody>
</table>

Abbreviation: G-CSFR, receptor for G-CSF; (R), gene rearrangement.
* Number of positive samples/number of samples examined.
† Number of rearranged samples/number of samples examined.
‡ Both TCR-α and TCR-ζ.
§ Examined by antimyeloperoxidase antibody.

analytical methods and found that there was a good correlation among immunostaining (by UIC2) with 1% paraformaldehyde fixation, functional assay (Rh123 efflux test), and mRNA study by RT-PCR with 30 cycles. It is of interest that the Rh123 efflux test detects the functional P-gp/MDR1 gene expression of leukemic cells as well as that of the stem cells in normal bone marrow.12

The immaturity of CD7(+),sCD3(-),CD4(-),CD8(-) ALL, which is recognized to originate from lymphohematopoietic stem cells,22 is demonstrated phenotypically (frequent expression of myeloid antigens) and immunogenotypically (most of the cases had germline immune genes). In addition, mRNA for c-kit and G-CSF receptor is frequently expressed in these leukemic cells suggesting the multipotential features of CD7(+) ALL cells.22,23 Actually, CD7(+),CD4(-),CD8(-) ALL sometimes converts to AML in the clinical course, and these leukemic cells are capable of multilineage differentiation in vitro.24 Our finding that MDR1 gene is expressed in CD7(+),CD4(-),CD8(-) ALL cells is interesting together with the previous observation demonstrating MDR1 gene expression in hematopoietic stem cells.12

Eight of 13 CD7(+) AML cases had MDR1 mRNA, which was significantly higher than CD7(-) AML cases. CD7 is well known to be present on some immature myeloid cells,11-13 and it is recognized to be one of stem cell markers. We recently reported that clinical features of CD7(+) AML is similar to those of CD7(-)CD4(+)CD8(+) ALL.24 The frequent expression of P-gp supports an idea that there is a close relationship between CD7(+) AML and CD7(-)CD4(+)CD8(+) ALL. Further, because P-gp was not detected in pre-B ALL, P-gp might be expressed on a particular type of stem cell, which is probably with CD7.

It is well known that both CD7(+) AML and CD7(-)CD4(+)CD8(+) ALL show unfavorable outcome to conventional chemotherapy. So P-gp expression in CD7(+) AML/ALL may be related to the drug resistance of these leukemias. We should also note that some CD7(+) AML cases responded to the P-gp–related anticancer agents vincristine and doxorubicin (Adriamycin),24 which suggests the existence of a number of different mechanisms for drug resistance. Further investigations for drug resistance in vitro and in vivo are expected to answer this question and to help develop chemotherapy to improve the clinical outcome of this type of leukemia.

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