Overexpression of the Major Vault Transporter Protein Lung-Resistance Protein Predicts Treatment Outcome in Acute Myeloid Leukemia

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The monoclonal antibody LRP56 recognizes a 110-kD major vault protein (lung-resistance protein [LRP]) overexpressed in several P-glycoprotein-negative (Pgp-), multidrug resistant tumor cell lines. To determine the frequency of LRP overexpression, its prognostic significance, and its relation to Pgp, we analyzed bone marrow specimens from 87 consecutive patients with acute leukemia. Diagnoses included de novo acute myeloid leukemia (AML; 21 patients), leukemia arising from an antecedent hematologic disorder or prior cytotoxic therapy (secondary AML; 27 patients), AML in relapse (29 patients), and blast phase of chronic myeloid leukemia (CML-BP; 10 patients). A granular cytoplasmic staining pattern was detected by immunocytochemistry in 32 (37%) cases, including 7 (33%) de novo AML, 13 (48%) secondary AML, 11 (38%) relapsed AML, and 1 of 10 CML-BP. Among 66 evaluable patients with AML, LRP overexpression was associated with an inferior response to induction chemotherapy (P = .0017). Remissions were achieved in 35% of LRP+ patients as compared with 68% of LRP- patients. Although Pgp adversely affected response in univariate analysis (P = .0414), only LRP had independent prognostic significance when compared in a logistic regression model (P = .0046). Differences in remission duration (P = .075) and overall survival (P = .058) approached significance only for LRP. Sequential specimens from remitting patients receiving treatment with the Pgp modulator cyclosporin-A showed emergence of the LRP phenotype despite a decrease or loss of Pgp at the time of treatment failure (P = .0304). Significant associations were observed between LRP and age greater than 55 years (P = .017), Pgp (P = .040), and prior treatment with mitoxantrone (P = .020) but not with CD34. These findings indicate that overexpression of the novel transporter protein LRP is an important predictor of treatment outcome in AML.

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Intrins ic or Acquired resistance to antineoplastics limits the effectiveness of conventional treatment in acute myeloid leukemia (AML). Multidrug resistance (MDR) resulting from overexpression of the MDR1 gene or its plasma membrane product, P-glycoprotein (Pgp), has been implicated as a cellular mechanism contributing to chemotherapy resistance in this disease.1 Pgp has been linked to a number of adverse prognostic variables including age, leukemia arising secondary to an antecedent hematologic disorder or cytotoxic therapy, cytogenetic pattern, and a CD34 surface phenotype.2,5 Leukemias expressing the MDR1 phenotype show lower intracellular drug accumulation and diminished in vitro and in vivo sensitivity to the anthracyclines.4,8,9 For this reason, agents capable of reversing Pgp-mediated drug resistance in vitro have entered clinical testing as modulators of MDR.10,11 The predictive value of Pgp as an independent marker for treatment failure has been questioned in some studies.2,3 The reported incidence of MDR1 overexpression in clinically resistant leukemia shows considerable variation, and its correlation with in vitro chemosensitivity has been inconsistent.11-16 In a phase I/II trial evaluating cyclosporin-A as a modulator of MDR, we found that in remitting patients MDR1 gene message decreased or was absent at the time of relapse, thereby implicating alternate mechanisms of chemotherapy resistance.11 Recent evidence indicates that mechanisms other than Pgp contribute to the MDR phenotype in human malignancies.17,18 The MDR-associated protein (MRP) is a member of the adenosine triphosphate-binding cassette (ABC) superfamily of membrane transporters that confers resistance to anthracyclines and other natural products in tumor cell lines.19,20 Investigations of the prognostic relevance of MRP in AML remain inconclusive, showing infrequent overexpression in poor-risk cohorts but relative increase in gene message after treatment failure.21,22 The monoclonal antibody (MoAb) LRP56 recognizes a 110-kD protein initially identified in the Pgp-, MDR lung cancer cell line SW1573/2R120 and termed lung-resistance protein (LRP).19,23 Overexpression of LRP is demonstrable in tumor cell lines of diverse histiogenic origin that lack Pgp or MRP overexpression, but that show an energy-dependent drug accumulation defect weakly sensitive to chemosensitizers such as verapamil and cyclosporin-A.19,24 Immunocytochemical staining with LRP56 is strongly reactive with a molecule closely associated with endomembrane structures. Recent investigations indicate that LRP is a highly conserved major vault protein implicated in nuclear-cytoplasmic transport.25,26 To determine the clinical relevance of this novel drug-resistance phenotype, we evaluated the frequency of antibody staining and the prognostic significance of LRP overexpression in clinical specimens from patients with AML.

MATERIALS AND METHODS

Patients. Specimens from 87 consecutive patients with AML (n = 77) or chronic myeloid leukemia in myeloid blast phase (CML-BP; n = 10) presenting between January 1992 and March 1994 were
evaluated. Sequential specimens obtained before treatment and at the time of treatment failure were compared in patients receiving treatment with the Pgp modulator, cyclosporin-A. Diagnostic categories of AML included de novo AML (21 patients), AML arising from an antecedent myelodysplastic syndrome or cytotoxic therapy for a primary malignancy (secondary AML; 27 patients), and AML in relapse (29 patients). Among relapsed patients, prior anthracycline treatment included daunorubicin (DNR) or idarubicin (17 patients) or mitoxantrone (11 patients). Median age was 53 years (range, 13 to 84 years), with a male predominance (54:33). Response to remission induction therapy was assessed in 66 evaluable patients with AML treated with one of four chemotherapy regimens. Treatment differed according to age, risk category, and prior therapy. Patients with de novo or late-relapse AML received conventional-dose cytarabine (100 mg/m²/d for 7 days) administered with DNR (45 mg/m²/d for 3 days) or idarubicin (12 mg/m²/d for 3 days; n = 24). Remaining patients with relapsed or secondary AML received either high-dose cytarabine (HiDAC; 3 g/m²/d for 5 days) followed by DNR and cyclosporin-A (Sandimmune; Sandoz, East Hanover, NJ) as premedication, or HiDAC plus DNR or mitoxantrone without cyclosporine (n = 6), or mitoxantrone (10 mg/m²/d for 5 days) and etoposide (100 mg/m²/d for 5 days; n = 3). Eleven patients were not evaluable for response, including 5 elderly patients with secondary AML who refused induction therapy and 6 patients who underwent bone marrow (BM) transplantation.

Response assessment. Response to treatment was assessed after a maximum of two courses of conventional-dose induction chemotherapy or after one course of an HiDAC-containing regimen. A partial response required normal peripheral blood counts with a BM aspirate containing greater than 5% but less than 25% blasts or a BM aspirate with less than 5% blasts in the presence of moderate thrombocytopenia (50,000 to 100,000/µL platelets) in a patient with secondary AML.

Cytogenetics. Cytogenetic analysis was performed on BM aspirates from patients with previously untreated AML by routine Giemsa-banding techniques after 24-hour culture and methotrexate synchronization, as previously described.27 Chromosome abnormalities were analyzed separately for relationship to LRP56 staining and according to prognostic groups using a modification of the Chicago classification in which t(8;21), abnormal 16, and t(15;17) were considered favorable and a normal karyotype of intermediate prognosis, whereas numerical or structural deletions of chromosomes 5 or 7, trisomy 8, complex patterns, and other chromosome abnormalities were considered unfavorable.28

Immunocytochemistry. Mononuclear cell fractions were isolated from heparinized BM aspirates by double Ficoll-Hypaque density gradient centrifugation. Cytopsins were prepared, and the blast reactivity with the MoAbs LRP56 (Dr R.J. Scheper, Free University Hospital, Amsterdam, the Netherlands) and the Pgp-specific antibodies JSB-1 (Sanbio bv, Uden, the Netherlands) and MRK16 (a gift of Dr T. Tsuruo, University of Tokyo, Tokyo, Japan) were determined by indirect immunoperoxidase using a previously described modified biotin-avidin technique.29 Sensitive parental (8226/s) and two MDR human myeloma cell lines selected with either mitoxantrone for non-Pgp MDR (MR20) or doxorubicin for classical MDR (Dox6) served as negative, LRP56+, or Pgp+ controls, respectively. Immunostained slides were scored without knowledge of clinical outcome, and specimens with reactivity in ≥20% of the blast fraction were considered positive.

Cytophorography. Surface-marker analysis was performed by flow cytometry using MoAbs directed against Pgp (MRK16), CD34 (Becton Dickinson, Mountain View, CA), and CD7 (Becton Dickinson), and myeloid-specific antigens.30 Cell suspensions were incubated with MoAbs for 30 minutes at 4°C, washed, and then incubated with fluorescein-isothiocyanate-conjugated goat antimouse Ig. Cells were washed and resuspended in buffer containing 0.5% paraformaldehyde fixative. Indirect immunofluorescence was analyzed by a FACScan flow cytometer (Becton Dickinson). A nonreactive murine anti-body of the same isotype served as control. Test samples were considered positive when labeling was detected in 20% or more of the gated cell population relative to that of the control.

Statistical analysis. Differences in response rates and the associations between LRP56 reactivity and other prognostic variables were determined by a two-sided Fisher's exact test. Comparison of the effect of LRP and Pgp overexpression on response to induction chemotherapy, together and separately, was evaluated by logistic regression analysis. A logistic regression model was also fit by testing the effect of LRP56, Pgp, AML diagnostic group (de novo, secondary, relapsed), and all two-way interaction terms on response (complete or partial response versus refractory failure). To assess changes in LRP phenotype or Pgp expression after cyclosporine chemomodulation, a McNemar's test was performed. Duration of complete remission and overall survival were estimated with the methods of Kaplan and Meier. The effect of LRP and Pgp overexpression on survival was tested by log-rank analysis. A proportional hazards regression model was fit to test the effect of LRP56, Pgp, AML diagnostic group (de novo, secondary, relapsed), and all two-way interaction terms on both overall and progression-free survival.

RESULTS

Immunocytochemical staining with LRP56 was localized to the cytoplasm in a granular pattern as shown in Fig 1. The results of immunocytochemical staining for LRP and Pgp in 87 patient specimens is summarized in Table 1. Thirty-two specimens showed immunoreactivity in 20% or more of the blast population. LRP overexpression was detected in all disease categories, ranging in frequency from 10% in patients with CML-BP to 48% in patients with secondary AML. Among 66 patients with AML evaluable for response to induction chemotherapy, 36 achieved a complete remission (n = 32) or partial (n = 4) remission. LRP overexpression was associated with response to induction therapy (P = .0036); 68% of LRP+ patients achieved a hematologic remission as compared with 35% of LRP- patients (Table 2). This difference in response resulted from a corresponding higher prevalence of resistant disease in the LRP+ cohort. Both Pgp and LRP overexpression had an adverse impact on response to induction therapy in univariate analyses (P = .0414 and P = .0017, respectively). When analyzed according to both LRP and Pgp staining pattern, LRP retained prognostic significance (P = .0046), whereas Pgp did not (P = .147). A similar effect was observed after adjusting for disease category (ie, de novo, secondary, or relapsed), with only LRP retaining prognostic significance (P = .0035 v P = .113 for LRP and Pgp, respectively). Although patients with both LRP and Pgp overexpression had the lowest remission rate (31%) and the highest prevalence of resistant disease (56%; see Table 3), a significant interaction between LRP and Pgp was not shown in the logistic regression model (P = .358).

The probability of remaining in complete remission and of overall survival were analyzed according to LRP and Pgp phenotype. Median remission duration was 1 month in LRP+ patients and 4 months in the LRP- cohort. Neither LRP (P = .0752) nor Pgp (P = .6464) significantly affected remission duration for the group as a whole. However, within defined disease categories, LRP had an adverse impact on progression-free survival for patients with secondary AML in log-rank analysis (P = .0561). Differences in overall survival...
approached significance for LRP \((P = .058)\) but not for Pgp \((P = .093; \text{see Fig 2})\).

Characteristics of treatment that might select for the LRP phenotype were analyzed in the relapsed patient cohort. Prior treatment with mitoxantrone was associated with a significantly higher probability of LRP56 reactivity \((64\%)\) as compared with that for patients receiving DNR or idarubicin \((18\%; P = .020)\). Sequential samples from 17 patients obtained before treatment with DNR and cyclosporin-A and at the time of disease progression were available for analysis (Table 4). Those patients experiencing an initial response to induction therapy showed acquisition of LRP at the time of relapse \((P = .0304)\), despite a loss or a decrease in frequency of Pgp expression. A significant change in LRP expression in patients resistant to cyclosporine-modulation was not observed \((P = .32)\).

Among clinical and biological prognostic variables analyzed for relationship to LRP, significant correlations were found with Pgp \((P = .040)\) and age greater than 55 years \((P = .017)\). No association was found with CD34, CD7, or specific chromosome abnormalities. Cytogenetic analysis was successful in 47 of the 48 patients with de novo or secondary AML, and 23 patients had an abnormal karyotype (Table 5). There was no difference in the prevalence of LRP overexpression among patients with a normal versus abnormal karyotype \((P = .556)\). Although LRP overexpression was detected in only 1 of 8 cases \((12\%)\) with a favorable cytogenetic abnormality as compared with 7 of 15 cases \((47\%)\) with an unfavorable karyotype, this difference was not statistically significant \((P = .176)\).

**DISCUSSION**

Clinical resistance to chemotherapy results from the interaction of numerous biological variables. In this study, we found that overexpression of the novel major vault transporter protein, LRP, has prognostic significance in AML. The gene encoding LRP was recently cloned and localized to chromosome 16p11.2 proximal to the MRP gene.\(^{26}\) Although LRP bears no significant homology to the adenosine triphosphate-binding cassette (ABC) membrane transporter superfamily, it shows striking identity to the major vault protein \((mVp\alpha)\). Vaults are highly conserved ribonucleoprotein complexes localized to nuclear membrane pores and cytoplasmic vesicles and are implicated in nuclear-cytoplasmic transport.\(^{34,35}\) Fluorescence microscopy studies support a similar function for LRP, showing rapid anthracycline redistribution from the nucleus to cytoplasmic vesicles in cell lines overexpressing the protein.\(^{36}\) Given that vaults

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**Table 1. Comparison of LRP and Pgp Overexpression**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients</th>
<th>LRP56 (%)</th>
<th>Pgp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>De novo</td>
<td>21</td>
<td>7 (33)</td>
<td>8 (38)</td>
</tr>
<tr>
<td>Secondary</td>
<td>27</td>
<td>13 (48)</td>
<td>17 (63)</td>
</tr>
<tr>
<td>Relapsed</td>
<td>29</td>
<td>11 (38)</td>
<td>11 (38)</td>
</tr>
<tr>
<td>CML-BP</td>
<td>10</td>
<td>1 (10)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>32 (37)</td>
<td>41 (47)</td>
</tr>
</tbody>
</table>

**Table 2. Remission Rate in Patients With AML According to LRP Phenotype**

<table>
<thead>
<tr>
<th>LRP Phenotype</th>
<th>No. of patients</th>
<th>Complete or partial remission</th>
<th>Resistant disease</th>
<th>Early deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP+</td>
<td>40</td>
<td>27 (68)</td>
<td>6 (15)</td>
<td>7 (18)</td>
</tr>
<tr>
<td>LRP-</td>
<td>26</td>
<td>9 (35)</td>
<td>14 (54)</td>
<td>3 (12)</td>
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</table>
**Table 3. Treatment Outcome According to MDR Phenotype**

<table>
<thead>
<tr>
<th>LRP+/Pgp Phenotype</th>
<th>No.</th>
<th>Complete or Partial Remission</th>
<th>Resistant Failure</th>
<th>Early Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP+Pgp+</td>
<td>25</td>
<td>19 (76)*</td>
<td>2 (8)</td>
<td>4</td>
</tr>
<tr>
<td>LRP+/Pgp-</td>
<td>15</td>
<td>8 (53)</td>
<td>4 (27)</td>
<td>3</td>
</tr>
<tr>
<td>LRP+Pgp-</td>
<td>10</td>
<td>4 (40)</td>
<td>5 (60)</td>
<td>1</td>
</tr>
<tr>
<td>LRP-Pgp+</td>
<td>16</td>
<td>5 (31)</td>
<td>19 (66)</td>
<td>2</td>
</tr>
</tbody>
</table>

Values shown in parentheses represent percentages.

are multi-unit protein structures, it is not surprising that LRP gene transfection alone is insufficient to confer MDR. Nevertheless, restoration of drug sensitivity in revertant cell lines supports a functional role for LRP in chemotherapy resistance. In addition, among 61 drug-unselected human tumor cell lines recently screened for expression of the MDR proteins, LRP, Pgp, and MRP, only LRP afforded the best predictive value for in vitro drug resistance.

In the present study, LRP overexpression was detected in 37% of patient specimens, including both previously treated and untreated patients (Table 1). Similar to that for Pgp, the highest frequency of detection was found in secondary AML (48%). However, LRP overexpression was uncommon in CML-BP (10%), indicating that other mechanisms, including Pgp, may contribute to chemotherapy resistance in this disease. Although the frequency of LRP detection in the relapsed patient cohort was identical to that for Pgp (38%), only prior treatment with mitoxantrone was associated with LRP overexpression. This finding is consistent with results of in vitro drug selection studies, indicating that mitoxantrone, rather than the anthracyclines, preferentially selects for the LRP phenotype.

Despite treatment and cohort heterogeneity, LRP was an important predictor of response to induction chemotherapy in patients with AML, independent of disease category. After adjusting for potential differences related to diagnostic group, LRP+ patients had a significantly lower remission rate and higher probability of resistant failure than did LRP- patients (P = .0035). Because Pgp expression was common in LRP+ specimens, stratification of outcome according to drug-resistance phenotype was analyzed to discern the relative contribution of each drug-resistance marker. As shown in Table 3, LRP overexpression was shown in the absence of Pgp, and each marker adversely influenced response to induction therapy. However, when adjusted for Pgp, only LRP retained prognostic significance (P = .0046). Although the prognostic impact of each marker appeared additive, a significant interaction was not shown in a logistic regression model. It is possible that the prognostic effect of Pgp was obscured by treatment with the Pgp modulator cyclosporin-A in some patients. If so, pretreatment screening of patient specimens for each of these drug-resistance markers may identify patients at greatest risk for treatment failure with conventional therapy, who might, therefore, be considered for alternate treatment strategies.

Previous investigations have shown that Pgp overexpression is principally restricted to CD34+ blast populations, a pattern that mirrors its regulation in normal hematopoiesis. Although we found no correlation between LRP and CD34, there was a trend favoring association with CD7. LRP overexpression was detected in 63% of CD7+ specimens as compared with 34% of specimens lacking a CD7 surface phenotype (P = .122). In view of recent reports that CD7 may have prognostic relevance in AML, LRP may contribute to the adverse biological behavior observed in this phenotypic cohort. This observation warrants further study in a larger group of patients.

LRP was associated with adverse prognostic variables including advanced age and Pgp, but no correlation was found with specific chromosome abnormalities (Table 5). The latter finding may relate to the comparatively small number of previously untreated patients analyzed. However, among karyotypically abnormal patients, LRP was detected in only 1 of 8 specimens harboring a favorable cytogenetic abnormality, as compared with 47% of cases with an unfavorable karyotype. We and others have reported a similar relationship between Pgp and cytogenetic pattern, indicating that leukemias harboring such favorable karyotypic abnormalities may have a more limited cytoprotective repertoire.
Treatment with a chemotherapy regimen using cyclosporin-A as a Pgp modulator selected for LRP overexpression despite a decrease or loss of Pgp expression (Table 4). This observation confirms that LRP may identify leukemic clones distinct from those harboring Pgp and is in agreement with results of in vitro selection studies. Myeloma cell lines selected for resistance to both doxorubicin and the Pgp modulator verapamil show LRP overexpression but lack Pgp (W.S. Dalton, unpublished data).

Our findings that LRP has independent prognostic relevance in AML indicates that this novel transport protein may contribute to chemotherapy resistance in this disease. These results require confirmation in a larger cohort of uniformly treated patients. Nevertheless, the relatively high prevalence of LRP overexpression observed in AML and its apparent selection by cyclosporin-A suggests that this drug-resistance phenotype may limit the effectiveness of Pgp modulators in clinical trials. The latter is under investigation in a Southwest Oncology Group trial in which participants are randomized to an anthracycline-containing induction regimen, with or without addition of cyclosporin-A. In a recent report from the Free University of Amsterdam, LRP overexpression conferred a lower response rate to cisplatin-based chemotherapy in patients with ovarian cancer, indicating that the prognostic value of LRP extends to other malignancies. Based on these findings and the results of the present study, development of strategies to overcome LRP-related MDR appears warranted.

Table 4. Change in Drug-Resistance Phenotype After Cyclosporine Chemomodulation

<table>
<thead>
<tr>
<th>Response Category</th>
<th>No. of Patients</th>
<th>PreTx</th>
<th>Progression</th>
<th>PreTx</th>
<th>Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant disease</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Relapse from complete remission</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>11 (65)</td>
<td>5 (29)</td>
<td>3 (18)</td>
<td>10 (59)*</td>
</tr>
</tbody>
</table>

Abbreviation: PreTx, pretreatment.
* P = .0304; represents comparison of LRP56 reactivity PreTx versus time of progression in relapsing patients.

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drug-resistant gene (mdr1) amplification are infrequently found in fresh acute leukemia cells: Sequential analysis of 15 cases at initial presentation and relapsed stage. Cancer 63:1534, 1989

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