EXPRESSION AND CLINICAL SIGNIFICANCE OF MULTIDRUG RESISTANCE GENE AND MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN GENE IN ACUTE LEUKEMIA

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ABSTACT

Objective: To evaluate the expression and clinical significance of multidrug resistance gene (mdr1) and multidrug resistance-associated protein (MRP) gene in acute leukemia. Methods: The expression of mdr1 and MRP assay in 55 patients with acute leukemia (AL) by reverse transcription polymerase chain reaction (RT-PCR). Results: The mdr1 and MRP gene expression levels in the relapsed AL and the blastic plastic phases of CML were significantly higher than those in the newly diagnostic AL and controls. The mdr1 and MRP gene expression levels in the clinical drug-resistant group were significantly higher than those in the non-drug-resistant group. The complete remission (CR) rate in patients with high mdr1 expression (14.3%) was significantly lower than that with low mdr1 expression (57.5%); similarly the CR rate in patients with high MRP level was also lower than that with low MRP level. Using both high expression of mdr1 and MRP gene as the indicator for evaluating multidrug resistance (MDR), the positive predictive value and accuracy increased in comparison with single gene high expression. Conclusion: Elevated level of mdr1 or MRP gene expression might be unfavorable prognostic factors for AL patient and may be used as an important index for predicting drug-resistance and relapse in AL patient. Measuring both mdr1 and MRP gene expression would increase accuracy and sensibility of evaluating MDR in acute leukemia.

Key words: Acute leukemia, Multidrug resistance gene, Multidrug resistance-associated protein gene, PCR.

Multidrug resistance (MDR) remains a major cause of failure in the chemotherapeutic treatment of acute leukemia (AL). Classical MDR phenotype is due to overexpression of membrane-bound glycoprotein (P170) encoded by multidrug resistance gene (mdr1). However, low P-glycoprotein levels were frequently found in clinically drug-resistant leukemia, and 1 or 2 indicated that overexpression of the mdr1 gene cannot be accounted for all cases of drug resistance in leukemia. Recently, multidrug resistance-associated protein (MRP) gene was found to be associated with drug-resistance in AL.[1] In order to evaluate the significance of the MRP and mdr1 expression in clinical drug resistance, the expression of mdr1 and MRP mRNA levels was investigated in 55 AL patients.

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MATERIALS AND METHODS

Patients and Controls

Fifty-five patients (41 males, 14 females) including 36 newly diagnosed patients (24 cases with AML, 12 ALL), 12 relapsed patients (7 AML, 5 ALL) and 7 CML patients in acute phase were investigated. Median age was 36 years (range:16–61 years). These patients were diagnosed by routine morphologic and cytochemical procedures. The classification was established according to French-American-British (FAB) criteria.

Ten healthy staff members in our hospital were investigated as normal controls.

Treatment Protocols

For remission induction, acute promyelocytic leukemia (M_3) patients were treated with all-trans retinoic acid (ATRA). Patients with AML and CML in acute phase were treated with daunorubicin (40–60 mg, i.v., days 1–7) or harringtoninum (4–6 mg, i.v., days 1–7) and cytosine arabinoside (150–200 mg, i.v., days 1–7). The chemotherapy protocols of ALL patients consisted of daunorubicin (40–60 mg, i.v., days 1–3, 15–17), vincristine (2 mg/week, i.v. four times), cyclophosphamide (600 mg/m^2, i.v., twice) and prednisolone (40 mg/m^2/d,

orally to 28d). Complete remission status was defined according to the criteria made at Suzhou leukemia symposium in 1987.

Total RNA Preparation

Bone marrow samples were aspired from the patients. Blood samples were prepared from the normal subjects. Mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation. Total RNA was prepared according to the modified method of Chomczynski et al..[2]

RT-PCR

Primer Sequences

The primer pairs selected for MRP, mdr1 and β_2-MG were previously described by Yang et al.[3] and Noonan et al..[4] The sequences are as follows: MRP gene, sense 5'-GGACCT GGA CTT CGT TCT CA-3', antisense 5'-CGT CCA GAC TTC TTC ATC CG-3', the predicted amplified fragment length is 292 bp; mdr1 gene, sense 5'-CCC ATC ATT GCA ATA GCA GG-3', antisense 5'-GTT CAA ACT TCT GCT CCT CA-3', the predicted length is 157 bp; β_2-MG gene, sense ACC CCC ACT GAA AAA GAT GA-3', antisense: ATC TTC AAA CCT CCA TGA TG-3', the predicted length is 114 bp.

cDNA Preparation

cDNA preparation was performed as described previously.[5] In brief, 20 μl RT reaction mixtures, containing 1 μg RNA, 5 mmol/L $MgCl_2$, 0.5 mmol/L of each dNTP, 20 U RNasin, 25 pmol each downstream primers for MRP or mdr1 and β_2-MG, 10 U AMV RTase, 4 μl 5×RT buffer, were incubated at 42°C for 1 h, heated to 95°C for 5 min.

PCR Amplification

50 μl of PCR mixtures (contain cDNA 4 μl, 1 mmol/L of each dNTP, 10×buffer 5 μl, 1.5 mmol/L $MgCl_2$, 50 pmol of each primers, 2.5 U of Taq DNA polymerase) were subjected to 29 cycles of denaturation (94°C, 40 sec), annealing (58°C, 60 sec) and extension (72°C, 70 sec).

Analysis of PCR Products

25 μl of PCR products were separated on a 8% PAGE gel. The PCR product bands were visualized by silver staining and photographed. The relative amounts of each PCR product for MRP or mdr1 were quantitated by densitometry on CS-930 type dual-wavelength TLC scanner (Shimadzu, Japan) and calculated relative to the amount of β_2-MG PCR product. The results were expressed as a ratio to β_2-MG. In order to illustrate the results conveniently, a cutpoint of 0.4 mdr1 expression level was chosen to discriminate the "high" or "low" mdr1 expression,[5] and a 1.0 cutpoint for MRP expression.[3]

Statistical Analysis

All data was analyzed using Chi square test, Student's t test and rank correlation analysis.

RESULTS

The mdr1 and MRP Expression

The expression of mdr1 and MRP gene at mRNA level was detectable by RT-PCR in 55 patients and in 10 controls. The expression positive ratios and median levels are shown in Table 1. The positive rate and median level of mdr1 expression of relapsed AL patients and CML patients at acute phase were significantly higher than that of patients at newly diagnosis and of controls ($P<0.01$). The positive rate of MRP expression revealed no difference in each group, but expression level of patients at relapse and at acute phase was significantly higher than that of patients newly diagnosed and controls ($P<0.05$).

Association of mdr1 and MRP Expression with Clinical Therapeutic Response

Table 2 shows the association of mdr1 or MRP expression with chemotherapeutic response. Forty patients were followed up in this study. The CR rate in patients with low mdr1 expression was significantly higher than that with high expression ($P<0.01$). The CR rate in patients with both mdr1 and MRP low expression was 61.9%, however, none of the six patients with both mdr1 and MRP high expression obtained CR.

Association of mdr1 and MRP Expression Levels with Drug-resistance

According to the clinical drug-resistance criteria,[3] forty AL patients, who had been followed up, were divided into two groups; drug-resistant group (27 patients) and non-drug-resistant group (13 patients). Table 3 demonstrated the expression levels of mdr1 and MRP in the two groups, and significant statistical difference was found in mdr1 and MRP expressions between two groups ($P<0.05$).

Evaluation of Value of mdr1 and MRP Expression as the MDR Predictor

In this study, high expression of mdr1 and MRP gene were used as an indicator for evaluating MDR in AL patients, the clinical drug-resistant criteria was to be the

gold standard. Then, the positive and negative predictive value and accuracy were calculated. As Table 4 demonstrates, using both high expression of mdr1 and MRP gene as the indicator for evaluating MDR, the positive predictive value and accuracy increased in comparison with single gene high expression.

Table 1. mdr1 and MRP expression in acute leukemia

	Numbers of patients	mdr1 expression		MRP expression	
		mdr1(%)	mdr1 level ($\bar{x}\pm s$)	MRP(%)	MRP level ($\bar{x}\pm s$)
Newly AL	36	14 (38.8)	0.408±0.186	24 (66.7)	0.465±0.253
Relapsed AL	19	15 (78.9)	0.735±0.249	12 (63.2)	1.157±0.449
Controls	10	2 (2.0)	0.155±0.089	6 (60)	0.085±0.089

Table 2. Association of mdr1 and MRP expression with chemotherapeutic response

	mdr1 level		MRP level		mdr1 and MRP level	
	low	high	low	high	low	high
No. of cases	26	14	29	11	21	6
CR cases	15	2	15	2	13	0
CR rate (%)	57.7	14.3	51.7	18.2	61.9	0

Table 3. The association of mdr1 and MRP expression levels with drug resistance

Group	No. of patients	mdr1 level ($\bar{x}\pm s$)	MRP level ($\bar{x}\pm s$)
Drug-resistant group	27	0.680±0.273	0.861±0.526
Non-drug-resistant group	13	0.351±0.133	0.184±0.108

Table 4. Evaluation of mdr1and MRP expression as the MDR predictor

	mdr1 high level	MRP high level	Both mdr1 and MRP high level
Positive predictive value (%)	85.7 (12/14)	81.8 (9/11)	100 (6/6)
Negative predictive value (%)	42.3 (11/26)	41.8 (12/29)	47.6 (10/21)
Accuracy (%)	57.5 (12/14)	52.5 (9/11)	59.3 (6/6)

The Correlation of mdr1 Expression with MRP Expression

The rank correlation analysis was performed on expression levels of mdr1 and MRP gene in 55 AL patients, and a correlation was found (r=0.33, r's=0.28, $P<0.01$).

DISCUSSION

Association of mdr1 Expression with Clinical Drug-resistance and Chemotherageutic Response

Although conflicting results have been published on the clinical significance of mdr1 expression in AL, most investigators confirmed that mdr1 was expressed in only 13–40% of AL patients newly diagnosed and in 80% of AL patients at relapse. A high level of mdr1 expression in newly diagnosed patients was often associated with low CR rate, short CR duration and high relapse rate, and served as a unfavorable prognostic factor.[6] In the present study the positive rate and median level of mdr1 expression in AL patients at relapse and CML patients at acute phase were significantly higher than that of patients newly diagnosed (78.9% vs 38.8%, 0.735±0.249 vs 0.408±0.186) ($P<0.01$). The mdr1 expression level in drug-resistant group (0.680±0.273) was significantly higher than that in non-drug-resistant group. The CR rate (14.3%) in patients with high mdr1 expression was significantly lower than that with low expression (57.7%) ($P<0.01$). Our results suggested that high expression of mdr1 gene was related to low CR rate, high recurrent rate and low response to chemotherapeutical regents, and which might serve as an indicator to evaluate the prognosis of acute leukemia.

However, our results also found that mdr1 expression was negative in 14 of 27 patients who revealed clinical drug-resistance. On the other hand, 47.6% of the patients with mdr1 negative expression demonstrated clinical drug-resistance. Therefore, MDR mediated by mdr1 expression was only one of many cooperative mechanisms of multidrug resistance.

Association of MRP Expression with Clinical Drug-resistance and Chemotherapeutic Response

MRP gene was cloned from an overexpressed cDNA in non-Pgp MDR small cell lung cancer cell line (SCLC) by Cole et al. in 1992,[7] and has been extensively studied. Gene transfection with a full-length MRP cDNA in laboratory has confirmed that MRP overexpression could result in MDR. Clinically, the characteristics and significance of MRP expression remain unclear and may be as mdr1, elevated MRP level might serve as a reverse indicator for evaluation of chemotherapeutic response and prognosis.[1] Our results found that MRP expression levels in AL patients at relapse and CML patients in acute phase were significantly higher than those in newly diagnosis (1.157±0.449 vs 0.415±0.253) ($P<0.05$). The CR rate of patients with high MRP expression was lower than that with low expression (18.2% vs 51.2%), even though the statistically was not difference significant. These suggest that elevated MRP expression was related to unfavorable prognosis of acute leukemia.

Evaluating the Value of mdr1 and MRP Expression as the MDR Predictive Indicators

One of the purposes of investigation of MDR is to predict the clinical drug resistance accurately in malignancies and to help in the chose of chemo-therapeutic regents. However, the result of evaluating MDR by a single indicator is not satisfactory. In the present study, according to the clinical drug resistant criteria, we used both mdr1 and MRP high expression to evaluate MDR. The results suggested that the positive predictive value and accuracy were increased in comparison with single indicator, and these were in agreement with the observation made by Yang et al..[3]

In conclusion, overexpression of mdr1 and MRP was related to drug resistant phenotype, high recurrent rate and low CR rate, and could serve as important unfavorable prognostic factors in AL. Measuring both mdr1 and MRP gene expression would increase accuracy and sensibility for evaluating MDR in acute leukemia.

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