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THE CLINICAL SIGNIFICANCE OF MULTIDRUG RESISTANCE GENE (mdr1) EXPRESSION IN ACUTE LEUKEMIA

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Objective: To study the clinical significance of multidrug resistance gene expression in acute leukemia. Methods: The relationships between drug resistance of leukemia cells and prognosis, multidrug resistance gene (mdr1) were examined in 85 patients with acute leukemia and 20 normal controls by reverse transcriptase polymerase chain reaction (RT-PCR). Results: The mdr1 positive rate in untreated group was 44.7%. The complete remission (CR) rate of mdr1 positive patients (23.9%) was significantly lower than that of mdr1 negative patients (88.5%) ($P<0.005$). The mdr1 expression level in relapsed-refractory group was higher than that of CR group. A gradually increased mdr1 mRNA level in CR patients indicated early relapse. Conclusion: The mdr1 positive rate in normal control and long-term survival patients was very low. The mdr1 expression was correlated with French-American-British Cooperative Group (FAB) classification. The mdr1 expression level was correlated with chemotherapeutic effect and prognosis. It is an unfavorable prognostic factor for patients with acute leukemia.

Key words: Leukemia, Drug resistance, Gene expression, Polymerase chain reaction.

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Recently, many studies have focused on the mechanism of multidrug resistance (MDR) of leukemia.[1] In this study, we examined the expression of mdr1 mRNA by reverse transcriptase polymerase chain reaction (RT-PCR) in patients with acute leukemia including newly diagnosed, complete remission, relapsed-refractory and long-term survival cases and evaluated the prognostic value of mdr1 in clinical drug resistance in acute leukemias.

MATERIALS AND METHODS

Clinical Samples

Eighty-five patients with acute leukemia were diagnosed according to the FAB criteria, including 47 newly diagnosed, 25 relapsed-refractory and 21 long-term survival cases. Median age was 37 years (14–60 years). 52 cases were males and 33 cases females. 17 patients were ALL (5 L_1 and 12 L_2), 68 patients were ANLL (1 M_0; 1 M_2; 9 M_2; 31 M_3; 7 M_4; 12 M_5; 3 M_6; 1 M_7); and 3 specific type of AML (2 acute mixed cells leukemias, 1 acute histiocytic leukemia). 20 volunteers served as normal controls.

Mononuclear Cells Isolation and Total Cellular RNA Extraction

Mononuclear cells from 5 ml of heparin-anticoagulated peripheral blood or 2 ml of bone marrow, were isolated by sedimentation on Ficoll Hapaque gradients in 2000 r/min for 16 minutes. Total RNA was prepared according to the method of Chomczynsk and Sacchi (Single-step method of RNA isolation by acid guanidine thiocyanate-phenol-chloroform extraction).

cDNA Synthesis and PCR Reaction

Primers for PCR

mdr1 and β_2 microglubulin primers for PCR were designed,[1] and synthesised in Molecular Biology Department of Hebei Medical University, as shown in Table 1.

Table 1. mdr1 and β_2m primers for PCR

Gene	Sequence	Products
mdr1		
Sense	CCCATCATTGCAATAGCAGG	
Antisense	GTTCAAACTTCTGCTCCTGA	157 bp
β_2m		
Sense	ACCCCCACTGAAAAAGATGA	
Antisense	ATCTTCAAACCTCCATGATG	115 bp

cDNA Synthesis:

Reverse transcriptase reaction volume was 15.5 μl, including lug total cellular RNA, antisense primer, 20 U RNasin, 1.6 U AMV reverse transcriptase, 200 μmol/L each dNTP, 3 μl 5×RT buffer, and incubated at 42℃ for 30 min.

PCR Reaction

PCR reaction volume was 40 μl, including 10 μl cDNA mixture, 150 pmol of each primers, 200 μmol/L each dNTP, 4 μl 10×PCR buffer, Taq DNA polymerase 1.25 U. Amplification was performed for 30 cycles, each cycle consisted of denaturation at 94℃ for 40 s; annealing of the primers at 55℃ for 40 s; and elongation at 72℃ for 60 s.

PCR Products Analysis

10 μl PCR amplification products were electrophoresed in 2% agarose gel containing ethidium bromide (EB) at 75 V for 30 min. The products were then visualized on an UV transilluminator and photographed. The bands were quantitative by Shimadzu Dual-Wavelength TLC Scanner CS-930 and the mdr1 mRNA expression level was expressed by mdr1/β_2m.

RESULTS

mdr1 Gene Expression

The positive standard of mdr1 was determined according to visuable band of PCR amplification on an UV transilluminator.

mdr1 Expression in Normal Control

In normal control group, only 2 out of 20 cases expressed low level of mdr1 (0.05 and 0.09 respectively), the mdr1 positive rate was 10%.

The Relationship between mdr1 Expression Level and CR_1 Rate in Newly Diagnosed Group

The mdr1 positive rate in newly diagnosed group was 44.7% (21/47), the CR rate of mdr1 positive and mdr1 negative cases were 23.9% (5/21) and 88.5% (23/26) respectively, (P<0.005).

mdr1 Expression in Relapsed-refractory Patients

In relapsed-refractory group, 19 of 25 (76%) patients were mdr1 positive. The remaining 6 cases were consistently negative. In mdr1 positive samples, none obtained CR, but 3 relapsed patients were mdr1 negative, one obtained CR.

The Relationship between mdr1 Expression Level and Clinical Relapsed in CR Patients

In newly diagnosed group, 28 CR patients were followed up. 5 of whom showed mdr1 positive. The mdr1 positive rate and expression level in CR patients (17.9%, 0.24±0.07) were significantly lower than that of relapsed-refractory group (76%, 0.70±0.39) (P< 0.01). 2 CR patients expressed high mdr1 level and relapsed in 3 or 5 months after CR respectively. Meanwhile, the mdr1 mRNA expression level increased to 0.49 and 0.62 respectively.

The Relationship between mdr1 Expression and Leukemia Type

Regarding the subtype of AML, the mdr1 positive rate was the lowest in M_3 (12.9%) cases and the highest in M_5 cases (75%) ($P<0.001$), as shown in Table 2, 3.

Table 2. The mdr1 expression in ALL and AML

Group	n	mdr1+			*mdr1/β2m*	
		n	%	*P*	$\bar{x}\pm s$	*P*
ALL	17	13	76.5	<0.005	0.69±0.42	<0.05
AML	68	24	35.3		0.50±0.26	

Table 3. The mdr1 expression in AML sub-type

Group	n	mdr1+	
		n	%
M_0	1	1	100.0
M_1	1	1	100.0
M_2	9	3	33.3
M_3	31	4	12.9
M_4	7	2	28.6
M_5	12	9	75.0
M_6	3	1	33.3
M_7	1	1	100.0
AMLL	2	2	100.0
AHL	1	0	0.0
Total	68	24	35.3

DISCUSSION

Nooter et al.[2] reported that the mdr1 positive rate was 10%–70% in newly diagnosed ALL patients. The difference may be connected to examining methods or positive standards. Our results that the mdr1 positive rate was 44.7% in newly diagnosed group consisted with the observation by Campas et al.[3] We found that mdr1 expression level was closely correlated to the effect of chemotherapy. The first complete remission rate of mdr1 negative patients (88.5%) was significantly higher than that of mdr1 positive patients (23.9%) ($P<0.05$).

The mdr1 positive rate in relapsed-refractory group was significantly higher as compared with newly diagnosed group and CR group. In relapsed-refractory group, mdr1 was persistently negative in 6 cases. This indicates the existence of multidrug resistance mechanism other than mdr1 overexpression, such as overexpression of multidrug resistance-associated protein (MRP) gene, qualitative and quantitative abnormalities of topoisomerase II, or elevated activity of glutathione-s-transferase. So it is necessary to examine other multidrug resistant mechanisms.

There was some relationship between mdr1 expression level and clinical relapse. 5 out of 28 CR cases were mdr1 positive, 2 of whom expressed high mdr1 level and relapsed in a half year. Moreover, mdr1 expression level increased significantly at the time of relapse. Therefore, the increasing expression level of mdr1 can be regarded as a marker for early relapse.

In long-term survival patients, 3 out of 21 cases were mdr1 positive, one of which expressed high level and relapsed in 4 months after CR, accompanied by elevating level of mdr1 expression. This result indicated that mdr1 expression level could be regarded as a marker of clinical cure.

The mdr1 expression level is related to leukemia type. High relapse rate and low CR rate of adult ALL were associated with high mdr1 expression. The mdr1 expression level differs in AML subtype, M_3 cases expressed the lowest mdr1 level and M_5 cases expressed the highest. This result is in agreement with the observation by Poeta et al.[4] The mechanism of low mdr1 expression may be related to specific chromosome abnormalities and gene fusion in M_3. Some studies indicated that mdr2 mainly expressed in M_3 and mdr2 did not correlate to MDR.[5,6] Other studies indicated that mdr1 expression was correlated to CD_{34} expression, while M_3 cells are CD_{34} negative.[7–9]

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