Correlation of Expression of Multidrug Resistance Gene 1 and Multidrug Resistance–Associated Protein with Chemo–therapy Efficacy in Malignant Lymphomas

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Abstract **Objective** To explore the correlation of chemotherapy efficacy in malignant lymphomas with the expression level of multidrug resistance gene 1 (mdr1) and multidrug resistance-associasted protein (MRP) mRNA. **Methods** Using the methods of semi-quantitative reverse transcriptase polymerase chain reaction(RT-PCR) to detect the expression levels of mdr1 gene and MRP gene in the lymph nodes of 23 patients with primary lymphoma (HD1, NHL22) and 23 recurrent(HD5, NHL18) lymphoma patients. **Results** The expression levels and positive rates of mdr1 gene in recurrent patients were higher than in patients with primary lymphoma(P<0.001). There was no difference between MRP gene expression level and positive rate in recurrent and primary patients(P>0.05) The chemotherapy effective rates in the patients with mdr1 gene positive expression were lower (33.33%) than in the patients with negative expression (85.71%)(P<0.001). There was no correlation between the expression of mdr1 and MRP gene (r=0.0723, P>0.05). **Conclusion** mdr1 gene expression level is dominant mechanism of clinical drug resistance of malignant lymphomas, wherase, MRP gene appears to play no role in that course. Mdr1 gene is relevant to chemotherapy efficacy, however, MRP gene expression level seems to have no impact on it.

Key Words Malignant lymphomas; Multidrug resistance gene(mdr1); Multidrug resistance--associsted protein gene(MRP)

The main reason of poor chemotherapy efficacy on malignant lymphomas(ML) is cancer cell's multiple drug resistance(MDR) to chemotherapy drug[1]. The typical MDR phenotype is mediated by I type mdr1 gene overexpression[2]. But in clinic, a number of patients resistant to drugs haven't mdr1 gene changes, it suggested that there are other mechanisms besides MDR, multiple resistance-associated protein(MRP) gene may be one of the reasons. So in this research, We examined the expression of mdr1 and MRP gene in ML and explored these factors' clinical significance and relevance to chemotherapy efficacy.

MATERIALS AND METHODS

Clinical materials

46 ML patients were selected in Shandong tumor hospital, including outpatients and inpatients from Janurary, 1997 to May, 1999. Among them, the primary and recurrent patients were 23 cases each, male and female patients were 38 and 8 respectively. The patients' ages were from 4 to 73 years old with average age of 36 years. In these cases, 40 non-Hodgking, 6 HD patients'. 8 human normal lymph nodes were normal control. The fresh samples from alive tissue detection were put into liquid nitrogen or stored in -80℃ refrigerator.

Methods

Drawing up chemotherapy regimen: These ML patients were verified by histopatholopy. The stage was determined by international criterion. HD patient' types were determined by pathologic diagnosis, NHL patients' maligmant degree were classified by international meeting. The patients' treatment effects were evaluated according to solid tumors' criterion. The patents' state remarks were above 85 scores according to karnofsky remark stander. As to chemotherapy protocol, the primary patients selected CHOP regimen; ProMA CE /CytaBOM regimen were used by recurrent patients. The standard dosages were used by the regimen. The deliberate detection results were adopted before and after the therapy. MDR type drugs, for example vinblastine, anthracycline or podophyllotoxin were included.

Mdr1 and MRP gene examined by RT–PCR:

Guanidine ifothiocyanate-phennol-chloroform one step method was used to extract total RNA from tissues, complimentary DNA synthesis was done according to the kit specification. β_2-microglobin(β_2−MG) was used to internal control of PCR reaction . The primers were

designed according to data [3]. The PCR products were electrophoresed by 120g/L polyacrylamide gel, electrophoresis strips were scanned by CS-9300 two waves chromatographic scanner. The ratios of mdr1/ β_2 -MG and MRP/β_2-MG equal to or exceeding 0.3 were judged positive. The expression mode were mdr1+ and MRP+ respectively.

The statistic analysis:

The results were treated by SAS (6.0) statistic software. Student t, x^2 test and correlation analysis were adopted.

RESULTS

PCR results of mdr1 and MRP gene in some samples

Two fluorescein strips were seen after PCR manifestation products were electrophoresed. PBR 322/MSPI was used to determine the size of strip. One was internal control β_2-MG at the position of 115bp, one was mdr1 at the 185bp position, the other was MRP at the 292bp position.These results were showed in figure 1.

The relations of mdr1 gene and MRP gene with clinical features, chemotherapy response, malignant degree and stage

Mdr1 gene expression levels had obvious differences among the primary patients and recurrent patients, complete recovery patients plus partial recovery patients and NC plus PD patients. As to MRP gene expressions, above phenomepheon were not seen. There were no correlations among mdr1 or MRP gene expression and ML patients' malignant degree and stage (P>0.05). These results were seen in Tab1 and Tab 2.

The correlation of mdr1 gene and MRP gene expression

There wes no correlation between mdr1 and MRP gene expression(R_S=0.072, P>0.05).

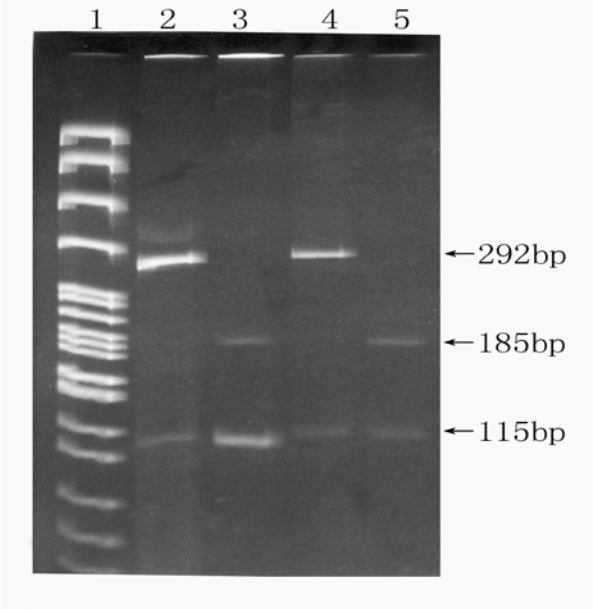

Fig.1 electrophoresis results of mdr1 and MRP gene expression in some patients analyzed by PCR
1 PBR322/MSP I DNA marker
2, 4 MRP gene expression in two patients
3, 5 mdr1 gene expression in two patients

Table 1 The relations of the expression of mdr1 or MRP gene with clinical features and chemotherapy response

group	n	mdrl			MRP		
		+	-	p value	+	-	p value
Primary	23	3	20	x^2=13.1	2	21	x^2=0
Recurrent	23	15	8	p=0.003	3	20	P=1
CR+PR	30	6	24	x^2=13.1	3	27	x^2=0.06
NC+PD	16	12	4	p=0.003	2	14	P=0.81

Table 2 The relations between the mdr1 or MRP gene and malignacy or stage

groups	n	Mdr1/β_2−MG ratio	MRP/β_2−MG ratio
low malignancy	11	0.50±0.63	0.27±0.31
Middle malignancy	14	0.45±0.42	0.23±0.24
high malignancy	21	0.54±0.45	0.27±0.30
Stage Ⅱ	13	0.33±0.56	0.21±0.25
Stage Ⅲ	20	0.51±0.42	0.25±0.32
Stage Ⅳ	13	0.67±0.45	0.32±0.26

DISCUSSION

MDR was the main mode of resistance to chemotherapy in ML patients. It was also the main factor inducing chemotherapy failure and influencing prognosis. Mdr1 gene overexpression made the patients producing resistance to many cellular toxicity drugs in ML. In recent years people found that MRP can also induce drug resistance, but its mechanism wasn't yet clear[4]. In the research of leukemia, it was thought that MDR was induced mostly by mdr1 gene overexpression, fewly by MRP gene overexpression[5].

There was statistic difference of mdr1 gene expression between primary and recurrent patients (P<0.001), the chemotherapy effective rate in patients with mdr1 gene positive expression was lower than in patients with mdr1 negative expression(P<0.001). These results were confident with the report of Liu Lixin[6], it suggested that the acquired drug resistance played an important role in ML partients. and mdr1 overexpression was the quotas of poor prognosis. There were no differences of MRP gene expression between primary and recurrent patients (P>0.05), it was the same as the results of Zhan[7]. Moreover, the chemotherapy effective rate in patients MRP gene positive expression was the same with that in MRP negative expression patients (P>0.05). These results showed that MRP was not the main mechanism of ML patients' drug resistance clinically. But because the research on MRP in ML patients was in early phase, more trials were needed to further verify its effect.

In this study, there were three patients whose MRP and mdr1 gene expression were positive concurrently. Correlation analysis showed that there was no correlation between mdr1 and MRP gene (P>0.05). Mdr1 and MRP gene expression can occur concurrently, they also can happen respectively. As to maliganant degree and stage, mdr1 and MRP gene both have no obvious correlation with that (P>0.05). We also found that there were 8.7 percent recurrent patients whose mdr1 and MRP were negative, but the chemotherapy effects were poor. These results suggestd that there were other mechanisms and ways besides MDR. Detecting many kinds of mechanisms concurrently can evaluate drug resistance more accurately in clinic.

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