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Expression of Bcl-2 and MDR$_1$ Genes in Children with Acute Leukemia

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Abstract: Objective To investigate the expression and significance of Bcl-2 and MDR$_1$ genes in children with acute leukemia. **Methods** The expressions of Bcl-2 and MDR$_1$ genes in 36 cases of acute leukemia and 10 cases of idiopathic thrombocytopenic purpura in the control group were examined by RT-PCR. **Results** The expressions of Bcl-2 in the incipient group and relapsed group were higher than that of the control group ($P < 0.05$). While in the incipient group and complete remission group, they were lower than that of the relapsed group ($P < 0.01$). The expression of MDR$_1$ gene in the relapsed group was higher than those of control group, incipient group and complete remission group ($P < 0.01$ or 0.05). There was no difference of MDR$_1$ expression between the incipient group and the control group or the complete remission group and the control group ($P > 0.05$). There was no close relationship between the levels of Bcl-2 or MDR$_1$ and clinical features such as gender, age, initial WBC count, the percent of carcinocytes, hepatomegaly, splenomegaly and lymphadenhypertrophy ($P > 0.05$). The relationship between Bcl-2 and MDR$_1$ genes was not significant ($r_s = 0.308$, $P > 0.05$). **Conclusions** Bcl-2 and MDR$_1$ genes were associated with drug resistance by different mechanisms. **[Chin J Contemp Pediatr, 2003, 5(4): 294 - 296]**

Key words: Leukemia; Bcl-2 gene; MDR$_1$ gene; Drug resistance

儿童急性白血病 Bcl-2 基因 MDR1 基因表达及意义

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[摘 要] 目的 研究 Bcl-2, MDR$_1$ 基因与儿童急性白血病耐药的关系及其临床意义。**方法** 采用逆转录多聚酶链式反应(RT-PCR)技术,对 36 例急性白血病患儿及 10 例血小板减少性紫癜患儿(对照)骨髓单个核细胞中 Bcl-2 和 MDR$_1$ 基因的表达进行检测。**结果** ①初治组、复发组 Bcl-2 基因表达明显高于对照组,差异均有显著性($P < 0.05$)。初治组、完全缓解组 Bcl-2 基因表达明显低于复发组,差异均有显著性($P < 0.01$)。复发组 MDR$_1$ 基因的表达明显高于对照组、初治组、完全缓解组($P < 0.01$ 或 0.05)。初治组与对照组间及完全缓解组与对照组间的 MDR$_1$ 表达差异无显著性($P > 0.05$)。②Bcl-2 和 MDR$_1$ 基因的表达与白血病临床特征如性别、年龄、初诊时白细胞数、骨髓中幼稚细胞百分数及肝、脾、淋巴结肿大程度均无显著相关性($P > 0.05$)。③Bcl-2 与 MDR$_1$ 基因之间无显著相关性($r_s = 0.308$, $P > 0.05$)。**结论** Bcl-2 和 MDR$_1$ 基因可能通过不同的机制导致白血病的耐药。**[中国当代儿科杂志,2003, 5(4): 294 - 296]**

[关 键 词] 白血病;Bcl-2 基因;MDR$_1$ 基因;耐药

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At present, combined chemotherapy is the dominant therapeutic method of leukemia. However multidrug resistance (MDR) is the key reason leading to therapy failure. The main mechanism of MDR is associated with MDR$_1$ gene and its coding protein P-gp[1]. Bcl-2, the apoptosis inhibiting gene, also plays a role in leukemia therapy and drug resistance[2]. We examined Bcl-2 and MDR$_1$ gene expression in 36 cases of children with acute leukemia by RT-PCR, and analysed the relationship amongst Bcl-2 gene, MDR$_1$ gene and MDR.

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Methods and subjects

Subjects

Thirty-six children with acute leukemia aged from 0.8-14 years old (20 boys and 16 girls) were enrolled in this study. According to the morphologic, immunologic and chromosomal features, 26 of them had acute lymphocytic leukemia (20 cases of L_1, 6 cases of L_2) and 10 had acute non-lymphocytic leukemia (ANLL) (4 cases of M_2, 3 cases of M_3, 2 cases of M_4, 1 case of M_5). They were assigned into 3 groups: incipient group, complete remission (CR) group and relapsed group. 10 cases of the control group were patients with idiopathic thrombocytopenic purpura (ITP).

Methods

Bone marrow before therapy was collected in 10 ml tubes containing heparin as anti-coagulant. Mononuclear cells were separated by Ficoll density gradient centrifugation and cryopreserved at -70 ℃.

Total RNA was extracted by Trizol reagent, following the manufacturer's instructions. Their purity was examined by gel electrophoresis and measured by spectrophotometry. Bcl-2 PCR were carried for 28 cycles: 94 ℃/3 min, 55 ℃/30 s, 72 ℃/30 s, its amplified product was 233 bp. MDR_1 PCR were carried for 32 cycles: 94 ℃/3 min, 57 ℃/30 s, 72 ℃/30 s. β_2-microglobin (β_2-MG) PCR cycles were: 94 ℃/3 min, 57 ℃/30 s, 72 ℃/30 s for 32 cycles, its amplified product was 120 bp. The upstream primer of Bcl-2 was 5′-GGA TTG TGG CCT TCT TTG AG-3′ and the downstream was 5′-CCA AAC TGA GCA GAG TCT TC-3′. The upstream primer of MDR_1 was 5′-CCC ATC ATT GCA ATA GCA GG-3′ and the downstream was 5′-GTT CAA ATC TCT GCT CCT GA-3′. The upstream primer of β_2-MG was 5′-ACC CCC ACT GAA AAA GAT GA-3′ and the downstream was 5′-ATC TTC AAA CCT CCA TGA TG-3′.

The electrophoresis of the PCR products was carried on 2% agarose (100 v, 40 min). HL-60 cell was taken as positive control of Bcl-2, K562/ADR cell was taken as positive control of MDR_1, non-template PCR product was taken as negative control and β_2-MG as internal control.

The respective Bcl-2 mRNA expression level was determined by the ratio of electrophoresis scanning results of Bcl-2 and β_2-MG. The respective MDR_1 mRNA expression level was determined by the ratio of electrophoresis scanning results of MDR_1 and β_2-MG. It was analysed by EB-Kodak digital system.

Statistical analysis

Rank sum test was used to compare the difference of the results. Correlation analysis was used to analyze the relationship between Bcl-2, MDR_1 gene and clinical features, or the relationship between Bcl-2 gene and MDR_1 gene.

Results

The expressions of Bcl-2 in the incipient group and relapsed group were higher than that of the control group ($P < 0.05$). The expressions of Bcl-2 in the incipient group and CR group were lower than that of the relapsed group ($P < 0.01$), and the expressions of Bcl-2 in the incipient and relapsed group were higher than that of the control group ($P < 0.05$). MDR_1 expression in the relapsed group showed much higher than those of control group, incipient group and CR group ($P < 0.01$ or 0.05). The differences of MDR_1 between the incipient group and control group or the CR group and control group were not significant ($P > 0.05$). See Table 1.

Table 1 The expression of Bcl-2 and MDR_1 in 4 groups

Groups	n	Bcl-2 median	MDR_1 median
Control group	10	0	0.66
Incipient group	29	$0.76^{a,b}$	0.71^{d}
CR group	9	0^{b}	0^{b}
Relapsed group	7	0.98^{a}	0.98^{c}

Note: a vs control group $P < 0.05$; b vs relapsed group $P < 0.01$; c vs control group $P < 0.01$; d vs relapsed group $P < 0.05$

There was no close relationship between the levels of Bcl-2 or MDR_1 genes and clinical features such as gender, age, initial WBC count, the percent of carcinocytes, hepatomegaly, splenomegaly and lymphadenhypertrophy ($P > 0.05$). See Table 2.

There was no relationship between Bcl-2 and MDR_1 genes ($r_s = 0.308$, $P > 0.05$).

Table 2 The relationship between Bcl-2 or MDR_1 and clinical features

Clinical features		number	Bcl-2	*r*	*P*	MDR_1	*r*	*P*
Gender	male	18	0.716	1.17	> 0.05	0.948	1.19	> 0.05
	female	11	0.832			0.968		
Age (years)	< 1 or > 10	9	0.64	0.07	> 0.05	0.972	0.16	> 0.05
	1 - 10	20	0.806			0.990		
WBC	$\geq 50 \times 10^9$/L	3	0	- 0.16	> 0.05	0.968	0.09	> 0.05
	$< 50 \times 10^9$/L	26	0.777			0.958		
The ratio of carcinocytes in bone marrow		29	0.76	- 0.1	> 0.05	0.965	0.11	> 0.05
Liver enlargement	+	21	0.832	0.22	> 0.05	0.985	1.02	> 0.05
	-	8	0.758			1.007		
Spleen enlargement	+	20	0.682	1.44	> 0.05	0.952	1.38	> 0.05
	-	9	0.771			0.978		
Lymphnode enlargement	+	17	0.76	0.16	> 0.05	0.958	0.83	> 0.05
	-	12	0.763			0.967		

Discussion

Drug resistance is the main cause of failure in treating leukemia. MDR is resistant to a great diversity of anti-tumor drugs of various structure and function. The MDR_1 gene and its coding protein P170 are typical pathways. According to the past researches, high level expression of MDR_1 gene was the result of repeated chemotherapy. Our results showed there was no difference of MDR_1 gene expression between the incipient group and control group, whilst the expression of MDR_1 in the replased group was higher than that of the control group, and the difference between the CR group and incipient group was not obvious. These indicated that chemical drugs activated the promotor of MDR_1 gene and induced expression of MDR_1. On the other side, these results supported that a drug resistant clone might exist in the patients before chemotherapy, but the number was small and difficult to detect. After chemotherapy, sensitive cells were killed and the resistant cells survived, so a high level expression of MDR_1 was detected.

Bcl-2 is an important gene to modulate apoptosis. Bcl-2 gene expression is associated with leukemia occurence and drug resistance. Our study showed that the expression of Bcl-2 gene in the relapsed group was higher than that of the incipient group and CR group. This suggested that there was a close relationship between Bcl-2 expression level and the response to chemotherapy. The main mechanism of drug resistance mediated by Bcl-2 is inhibiting cell apoptosis and prolonging cell survival period. Once the drug effect disappeared, the survived tumor cells could self-repair and recover to their initial state. We found that there was no close relationship between the expression of Bcl-2 and MDR1 gene and clinical features such as gender, age, initial WBC count, the percent of carcinocytes, hepatomegaly, splenomegaly and lymphadenhypertrophy. This was consistent with Coustan-Smith and Zhou's reports[3,4]. We also found that the relationship between Bcl-2 and MDR_1 was not significant. This was consistent with some overseas studies[5]. It indicated that the mechanism of drug resistance mediated by MDR_1 or Bcl-2 was different. MDR_1 mainly took part in MDR, whilst Bcl-2 was involved in regenerated resistance. These discoveries provided new clues to solve the problem of the resistance to cytotoxic drugs in the treatment of leukemia.

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elevated before chemotherapy, it decreased gradually right after CR, remained normal during continuous CR but increased at relapse. These indicated that TNF-α was associated with the heavier tumor burden at diagnosis and relapse. So serum TNF-α level may be a useful indicator in determining the efficacy and for monitoring relapse.

We found that TNF-α level in CSF was elevated in children with acute leukemia. This might result from autocrine of TNF-α in the central nervous system or the damage of blood-brain barrier (carcinocytes infiltrating CNS). CSF TNF-α may also be produced by stimulated astrocytes or microglia[10]. Our results suggested that although the clinical and CSF routine examinations were normal, CNS of leukemic children had in fact been infiltrated by carcinocytes at diagnosis. This was consistent with the findings by autopsy that CNS invasion occurred in over 90 % patients with acute leukemia at diagnosis. The decrease of TNF-α after CR also supported this conjecture. CSF TNF-α level was higher in the children with CNSL than those without CNSL. This indicated heavier tumor burden in CNS of former.

We also found that CSF TNF-α level was closely associated with the white blood cell count of CSF. CSF TNF-α level decreased more slowly than the white blood cells did after intrathecal chemotherapy. This is of great significance in judging the therapy effect of CNSL and deciding the course of intrathecal therapy. It is more appropriate that the normalization of TNF-α level in CSF be used as indicator to end intrathecal therapy.

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