Prognostic Significance and Mechanism of CUEDC1 Gene in Acute Leukemia

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Abstract
Objective: To study the prognostic significance of the expression of acute myeloid leukemia (AML) gene, acute lymphoblastic leukemia (ALL) cells and U937, THP-1, HEL, Jurkat and MOLT-4 cells in patients with acute leukemia. Methods: The expression levels of AML and ALL splicing isomers in bone marrow cells of patients at different stages were detected by real-time quantitative reverse transcription-polymerase chain reaction. Results: The expression of CUEDC1 in AML, ALL and U937, THP-1, HEL, Jurkat and MOLT-4 cell lines were detected by RT-PCR, real-time quantitative PCR and Western blot. The low expression (MOLT-4) and high expression (THP-1) cell lines of CUEDC1 were screened. Conclusion: the expression of AML, ALL and MOLT-4 genes in AL patients has important prognostic significance.

Key words: CUEDC1 Gene; Acute Leukemia; Gene Expression; Prognosis.

Significado Pronóstico y Mecanismo del Gen CUEDC1 en la Leucemia Aguda

Resumen
Objetivo: estudiar la significacion pronostica de la expresion del gen de leucemia mieloide aguda (LMA), la leucemia linfoblástica aguda (ALL) celulas U937, THP - 1 células Jurkat, Hel, y Molt - 4 en pacientes con leucemia aguda.Métodos: los niveles de expresion de AML y empalmar isómeros en las células de la médula ósea de los pacientes en diferentes etapas fueron detectados por reaccion en cadena de la polimerasa de transcriptasa inversa cuantitativa en tiempo real.Resultados: la expresión de CUEDC1 en la LMA, todos y U937, THP - 1, Hel, y líneas de celulas Jurkat Molt - 4 fueron detectados por RT - PCR, PCR cuantitativa a tiempo real y Western blot.La baja de expresión (Molt - 4) y alta expresión (THP-1) células celulares de CUEDC1 fueron examinados.Conclusión: la expresión de la LMA, todos y Molt - 4 genes en AL pacientes tiene importante significado pronostico.

Palabras clave: CUEDC1 Gene; Leucemia Aguda Expresión Génica; Pronóstico;
1. Introduction

Multidrug resistance (MDR) in patients with acute leukemia (AL) is the main cause of failure of chemotherapy [1]. In China, with the process of urbanization and increasing industrial pollution, the incidence of disease is increasing year by year, seriously endangering human health and life safety [2]. Acute myeloid leukemia (AML) is a malignant clonal disease of hematopoietic stem/progenitor cells, and is the most important type of leukemia [3]. AML accounted for 13290 of the 44270 newly diagnosed leukemia cases found in the United States in 2008 [4]. Although the incidence of acute myeloid leukemia (AML) accounts for only one fourth of leukemia, AML-M3 is the most common type [5]. Followed by AML-M2 (AML-M2b) with t (8; 21), all other types were visible, but the incidence was lower. But acute myeloid leukemia has a higher mortality rate than acute lymphoblastic leukemia (ALL) [6]. Children with acute myeloid leukemia (AML) other than M3 have a low remission rate (CR) and a long-term disease-free survival rate of less than 50% [7]. AML has always been one of the most intractable problems in leukemia chemotherapy. The human MDR gene family is mainly associated with acute myeloid phenomena in leukemia [8]. The 170 glycoprotein encoded by MDR1 gene can pump drugs into leukemia cells and induce drug resistance. Acquired gene abnormalities lead to excessive proliferation of leukemic stem cells, accompanied by arrest of differentiation and inhibition of apoptosis [9]. The accumulation of immature or primitive cells in the bone marrow eventually inhibits normal hematopoiesis and infiltrates other organs and tissues. And can spread through the blood throughout the body, leading to anemia, easy bleeding, infection and organ infiltration in patients with such phenomena [10].

Primary acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and secondary leukemia can all have MLL gene abnormalities [11]. Leukemia with MLL gene abnormalities is called MLL-related leukemia. In recent years, despite improvements in chemotherapy regimens, new drugs and the widespread use of stem cell transplantation in AML, the efficacy and prognosis have been significantly improved [12]. However, most AML (except acute promyelocytic leukemia) still relapses. AML is a serious threat to human life and health. The long-term survival rate is the lowest among all kinds of leukemia [13]. The 5 year survival rate of AML in 1970s was about 5.8%, up to 14.6% in 90s. At present, the 5-year overall survival rate under standard treatment is 20% - 35%, and most patients can not obtain long-term survival and cure [14]. With the development of chemotherapy, long-term disease-free survival rate has been improved, but drug resistance and relapse are still difficult problems in the treatment of childhood acute myeloid leukemia [15]. In recent years, it has also been reported that patients with overexpression of AML gene have poor overall survival in adult precursor B-cell ALL patients. Langer et al reported that BAALC overexpression was correlated with MDR1 gene overexpression. At present, only 55% - 60% of all acute leukemia patients have specific fusion gene expression, and the remaining part is normal karyotype [16]. Although some AML patients with normal karyotype were classified as moderate prognosis group, the heterogeneity of AML was very high, and 70-80% of AML patients had poor prognosis. Therefore, the research of non-specific genes related to leukemia development has become a research hotspot.

The expression of CUEDC1 in acute leukemia and leukemia cell lines was detected. The lentiviral vector overexpressing CUEDC1 and the lentiviral interference vector downregulating CUEDC1 were constructed. The stable overexpressing and low expressing CUEDC1 cell lines were obtained by infecting leukemia cell lines. To study the effects of up-regulation and down-regulation of CUEDC1 gene on proliferation and apoptosis of leukemia cells and cell cycle. To study the effect of up-regulation and down-regulation of CUEDC1 gene on gene expression profiles of leukemia cells, and to explore the possible pathways involved in the regulation of CUEDC1 signaling pathways.

2. Construction of THP-1 Cell Line Interfering with CUEDC1 Gene

2.1. ShRNA sequence design of CUEDC1 gene

According to the NCBI human CUEDC1 gene sequence (NM_001271875.1), three shRNA sequences interfering with the CUEDC1 gene were predicted and designed using the online software of thermo official website. The carrier was pHHi, the interference vector of the laboratory, and the restriction sites were Agel and BamHI. The sequences were as follows:

CUEDC1-shRNA1: ACCGGTCGAGGGGTGGCGGAAACGACTTTGCTCGAGCACAGCCATT TTTGGGATCC
CUEDC1-shRNA2: ACCGGTCGAGGGGTGGCGGAAACGACTTTGCTCGAGTAGGCTCTTGACCAGACAGCTTT TTTGGGATCC
CUEDC1-shRNA3: ACCGGTCGAGGGGTGGCGGAAACGACTTTGCTCGAGCACAGCCATT TTTGGGATCC
2.2. Construction of shRNA vector

The designed shRNA sequence was delivered to Anhui General Biology Company to synthesize and construct on pHHsi vector, and the sequence was verified to be correct. According to the characteristics of P Silencer 4.1-CMV Neo shRNA expression vector, the corresponding siRNA template with hairpin structure was designed. Each siRNA template consists of two complementary 55 BP DNA strands, which can be annealed to form DNA double strands with digestion sites. It is used to connect to the linearized vector p Silencer 4.1-CMV Neo processed by enzyme digestion. The P Silencer 4.1-CMV Neo shRNA vector was digested and digested by pHHsi. The mixed reactants were placed at 37 C, 4h, separated by 0.8% agarose gel electrophoresis, and the gel recovery kit was recovered and purified. According to Takara's instructions, the carrier and the insert were matched in a certain proportion, and the connection reaction was set up. The reaction lasted overnight at 16 C. The conjugates were transformed into DH5 strains. Kanamycin and leukoplakia blue were screened. White positive clones were selected, shaking bacteria and plasmids were extracted and identified by sequencing.

2.3. Interference lentivirus packaging

ALL cells were cultured at 37 C and 5% CO2. The density of ALL cells was adjusted to 0.5 10^6/L. The cells were inoculated in a 15 cm cell culture dish for further culture. Jurkat cells, 10 UG U937 cells, 10 UG THP-1 cells and 10 UG HEL cells were mixed with CaCl2 (2.5 mol/L) solution and stored at room temperature for 20-30 minutes. CuEDC1 cells were co-transfected by calcium phosphate precipitation method. After 12 hours of culture, the culture medium containing the transfection mixture was discarded and replaced by complete medium for 72 hours. The supernatant of CUEDC1 cells was collected and centrifuged at 4 C for 7 min at 800 g. Cell fragments were discarded. The supernatant was filtered by 0.45 micron filter and centrifuged at 50 000 g for 90 min. The precipitates were recombinant lentiviral particles and stored at 80 C after loading. The titer of virus was detected by flow cytometry (FACS). CuEDC1 cells at logarithmic growth stage were inoculated with 1 *10^7 cells in a culture dish (inner diameter 150 mm) and cultured at 37 C for 24 hours in a 5% CO2 incubator. Lipofectamine 2000 liposome complex and two auxiliary plasmids DNA were added to the cells with 70% confluence degree. The virus supernatant was collected 48 hours later and centrifuged at 4 C and 4000g for 10 minutes. The supernatant was filtered by PVDF membrane and packed separately for the determination of virus titer and transfected cells.

2.4. Construction of interference stable strains

The expression level of CUEDC1 gene in three stable cell lines interfered by different CUEDC1 genes decreased to some extent. The most obvious decrease was in MOLT-4 cell line, and the expression level of CUEDC1 protein was about 10% of that in control group. CUEDC1 overexpressed cells and AML cells were inoculated in three 10 cm dishes with the number of 30,100,200 cells per dish. Fifteen percent FBS was cultured in complete medium and the screening pressure of G418 was removed. After 2 to 3 weeks of culture, the clone formation and fluorescence intensity were observed. Clone rings were used to digest 6 to 10 isolated red fluorescent clone clusters and inoculated into 24-well cell culture plates. 15% FBS was cultured in complete medium and changed regularly until the cells entered logarithmic growth phase. Cells with good homogeneity, stable fluorescence without attenuation or loss were observed and labeled under fluorescence microscope. After digestion, the cells were cultured, frozen and identified.

**Figure 1.** After lentivirus-mediated transfection, stable cell lines were obtained by purimycin screening, of which 1-3 was shRNA and NC was the control.
2.5. Detection of CUEDC1 knockout efficiency in stable cell lines

![Graph showing interference detection](image)

Figure 2. (A) PCR detection of interference effect qPCR, the results showed that sh1 and SH2 group had about 70% interference effect. (B) WB results showed that sh1 and SH2 groups had significant interference effects, of which sh1 interference efficiency reached 71%. The results of qPCR and WB showed that the cells of CUEDC1-shRNA1 and CUEDC1-shRNA2 had obvious interference effect. The two cells were cultured and frozen for further experiments.

3. Treatment and Method of AML Patients

3.1. Treatment of AML patients

The treatment of AML patients is based on the 2016 AML recommendations for hematology of the Chinese Medical Association. AML (non-M3) was treated with a combination of homoharringtonine or daunorubicin, cytarabine, etoposide (HAE or DAE) regimen. All-trans retinoic acid combined with anthracycline or arsenic (ATRA + anthracycline or ATRA + arsenic) was used to induce remission in M3 patients. Consolidation therapy for AML (non-M3 type) with induction chemotherapy reaching remission was followed by a course of treatment with the original regimen and DAE regimen for M3 patients. After radical remission, AML (non-M3) was treated with mid-and high-dose cytarabine combined with daunorubicin or etoposide (DA or EA) and mid-and high-dose cytarabine combined with high-dose tricuspid base (HA) for 6 courses. Consolidation therapy after complete remission of M3 with ATRA + anthracycline reaching complete remission was given anthracycline-based chemotherapy for 2 courses.

3.2. Real time quantitative reverse transcription polymerase chain reaction

Real time quantitative reverse transcription polymerase chain reaction (RT-PCR) was used. CDNA was used as template to amplify PCR. The RT-PCR reaction system of AML and ALL splicing isomers was as follows: mixture 12.5 μL, primers 0.5 μL, 10 mmol/L probe 0.3 μL, C DNA 4 μL, and double-steamed water 25 μL. The reaction conditions are as follows: thermal start-up at 95 C for 10 minutes, denaturation at 95 C for 15 seconds, annealing elongation at 60 C for 1 minute, 45 cycles, and fluorescence signal collection at 60 C for elongation. Each specimen was examined by complex tube and the mean value was taken as the expression level of ALL.

3.3. Result

The expression of CUEDC1 in AML, ALL, U937, THP-1, HEL, Jurkat and MOLT-4 cells was detected by RT-PCR, real-time quantitative PCR and Western blot. CUEDC1 low expression (MOLT-4) and high expression (THP-1) cell lines were screened.

![Graph showing RQ vs Sample](image)

Figure 3. The relative expression of CUEDC1 mRNA (A) and CUEDC1 protein in acute leukemia cell lines (B)
4. Construction of Molt4 Cell Line over Expressing CUEDC1 Gene

4.1. Overexpression plasmid construction

Molt 4 plasmid and eukaryotic expression vector CUEDC1 were digested with Xho I and Al I and ligated with CUEDC1 ligase for 16 hours. The conjugates were then transformed into Molt 4 competent cells and inoculated into the LB solid medium containing kanamycin and cultured overnight at 37 C. A single colony was picked up and inoculated in a LB liquid medium containing kanamycin, which oscillates overnight at 37. The plasmid DNA was extracted by alkaline lysis, and the insertion of foreign gene was identified by enzyme digestion.

4.2. Over expression of lentivirus packaging

Lentiviruses were packaged with CUEDC1 cells, transfected with reagents, and inoculated into a 10 cm cell culture dish. The virus was packaged with three plasmid systems: a vector plasmid with transcription factors 10 UG and a package plasmid Molt4. The virus supernatant was collected 60 hours after packaging, filtered by 0.45 micron needle filter and frozen at - 80 C for use in infecting the target cells. Molt4 cells were inoculated into 6-well plate. When the cell density reached 30%-50%, the culture medium was removed. FIV-FLT3-ITD lentiviral supernatant was added to each hole, and 2 mL virus supernatant was added to each hole, and 8 ug/mL polyamine was added. After 6 hours, the virus solution was removed and replaced with fresh culture medium. After 48 hours of culture, the green fluorescence was observed under fluorescence microscope.

4.3. Construction of overexpressed stable strain

Six acute leukemia cell lines, which basically cover all types of leukemia, were selected. The six acute leukemia cell lines include Jurkat, NB4, HL-60, THP-1, HEL and K562. The cells were cultured in vitro (part of the cell library of Shanghai Cell Institute, Chinese Academy of Sciences).

![Figure 4](image1)

**Figure 4.** The stable overexpression cell line CUEDC1 was obtained. Puromycin concentration of 4 g/ml was selected according to the growth state of the cell and expanded culture. After cell expansion, the cells were collected to detect the expression of target protein by qPCR and WB.

![Figure 5](image2)

**Figure 5.** (A) PCR detection of overexpression qPCR, the results showed that over expression was about 2 times that of the original. (B) WB results showed over expression effect and protein expression increased by 2 times. The results of qPCR and WB showed that the cells in CUEDC1 overexpression group were obviously up-regulated, and the cells were cultured and frozen. Two cells were cultured and cryopreserved for subsequent experiments.
4.4. Cell proliferation

**Figure 6.** The effects of molt4 overexpression group and control group, THP-1 interference group and control group on cell proliferation were detected after stable cell lines were obtained.

The results suggest that overexpression of CUEDC1 promotes cell proliferation, while downregulation inhibits cell proliferation.

4.5. Cell cycle

**Figure 7.** The effect of CUEDC1 overexpression on cell cycle of Molt-4 cells, (A) non-transfected cells, (B) blank vector control group, (C) overexpression group.

The results showed that there was no obvious phase change in cell cycle between normal and control cell groups, but the phase change in overexpression cell group was intense, S phase increased sharply, and most cells became aneuploid cells.
The results showed that there was no obvious phase change in cell cycle between normal cell group and control cell group, but S phase of interfering cell group decreased significantly, and some cells became aneuploid cells.

5. Cell Clone Formation

Table 1. Effects of overexpression and downregulation of CUEDC1 on the ability of AML cell clone formation

<table>
<thead>
<tr>
<th>Grouping</th>
<th>First experiment</th>
<th>The second experiment</th>
<th>The third experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colony number</td>
<td>Colony formation rate</td>
<td>Colony number</td>
</tr>
<tr>
<td>THP-1</td>
<td>7</td>
<td>17.5%</td>
<td>8.3</td>
</tr>
<tr>
<td>THP-1-NC</td>
<td>1.67</td>
<td>4.2%</td>
<td>1.67</td>
</tr>
<tr>
<td>THP-1-sh1</td>
<td>0.67</td>
<td>1.7%</td>
<td>0.67</td>
</tr>
<tr>
<td>Molt4</td>
<td>0</td>
<td>0.0%</td>
<td>0</td>
</tr>
<tr>
<td>Molt4-pCDH</td>
<td>6.3</td>
<td>15.8%</td>
<td>6.67</td>
</tr>
<tr>
<td>Molt4-CUEDC1</td>
<td>11.3</td>
<td>28.3%</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 8. Effect of down-regulation of CUEDC1 expression on cell cycle of THP-1 cells, (A) untransfected cells, (B) interfered control group, (C) interfered group

Figure 9. Effects of overexpression and downregulation of CUEDC1 on the ability of AML cell clone formation
The results showed that the cell clone formation rate of THP-1 interference group was significantly lower than that of normal group, and the cell clone formation rate of molt4 overexpression group was significantly higher than that of normal group.

5.1. Effect of stable over expression of CUEDC1 on proliferation of AML cells

The OD value of Lv-CUEDC1 overexpression group was higher than that of BC and Lv-Ctrl groups on day 1-2, but there was no significant difference between them. The difference increased gradually with time. On the 3rd to 5th day, the OD value of Lv-CUEDC1 overexpression group increased more significantly than that of BC and Lv-Ctrl group (P < 0.05, Fig. 10).

![Figure 10. Effects of CUEDC1 on cell proliferation of MOLT4 (A) and HEL (B) cells](image)

5.2 Effect of over expression of CUEDC1 on apoptosis of AML cells

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Early apoptosis</th>
<th>Advanced apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>2.18±0.36</td>
<td>0.83±0.34</td>
</tr>
<tr>
<td>Lv-Ctrl</td>
<td>2.20±0.45</td>
<td>0.88±0.64</td>
</tr>
<tr>
<td>Lv-CUEDC1</td>
<td>1.17±0.46 *</td>
<td>0.65±0.25</td>
</tr>
</tbody>
</table>

Note: *compared with group BC and Lv-Ctrl, P<0.05 has statistical significance.

![Figure 11. Effect of CUEDC1 on apoptosis of THP-1 cells](image)

Annexin V/7-AAD labeled flow cytometry showed that the percentage of apoptotic cells in early and late stage of Lv-Ctrl group had no significant change (3.08±1.09)% compared with that in BC group (3.01±0.7)%.

The proportion of apoptotic cells in the early and late stage of Lv-CUEDC1 overexpression was lower than that in the control group. (Table 2, FIG. 11).
Table 3. Annexin V/7-AAD labeled flow cytometry was used to detect the apoptosis of CUEDC1 cells on Molt-4 cells (%; x±s; n=3)

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Early apoptosis</th>
<th>Advanced apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>5.49±0.62</td>
<td>2.94±0.46</td>
</tr>
<tr>
<td>Lv-Ctrl</td>
<td>5.52±0.43</td>
<td>2.99±0.38</td>
</tr>
<tr>
<td>Lv-CUEDC1</td>
<td>4.46±0.43 *</td>
<td>1.47±0.48 *</td>
</tr>
</tbody>
</table>

Note: *compared with group BC and Lv-Ctrl, P<0.05 has statistical significance.

Figure 12. Effect of CUEDC1 on apoptosis of Molt-4 cells

Annexin V/7-AAD labeled flow cytometry showed that the expression of CUEDC1 in Molt-4 cells was lower than that in BC group (8.43±1.08)%. The proportion of apoptotic cells in early and late stage of Lv-Ctrl group had no significant change (8.51±0.81)%. The percentage of apoptotic cells in the early and late stage of Lv-CUEDC1 overexpression (5.93±0.91)% was lower than that in the control group. (Table 3, Figure 12).

5.3. Drug resistance of over expressed CUEDC1 to AML cells

Table 4. The IC50 (half inhibitory concentration) values of high concentrations of three kinds of plasma were determined (ng/mL; x±s; n=3)

<table>
<thead>
<tr>
<th>Grouping</th>
<th>THP-1 (ng/mL)</th>
<th>MOLT-4 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>1.72±0.16</td>
<td>10.42±0.45</td>
</tr>
<tr>
<td>Lv-Ctrl</td>
<td>1.58±0.34</td>
<td>12.02±0.96</td>
</tr>
<tr>
<td>Lv-CUEDC1</td>
<td>4.17±0.63 ***</td>
<td>14.22±0.30 **</td>
</tr>
</tbody>
</table>

Compared with BC and Lv-Ctrl, ** refers to P<0.01, *** refers to P<0.001.

Figure 13. Inhibitory effect of high concentration of huperzine on proliferation of THP-1 cells (A) and MOLT-4 cells (B)

CCK-8 assay was used to detect the OD value of cells treated with different concentrations of homoharringtonine for 48 hours, and the inhibition rate and IC50 were calculated. Fig. 13 shows that the
The inhibitory rate of AML cell proliferation increases with the increase of homoharringtonine concentration, but the inhibition rate of Lv-CUEDC1 group is lower than that of BC group and Lv-Ctrl group at the same concentration. In THP-1 cells, the IC50 of Lv-CUEDC1 group was (4.17 ± 0.63) ng/mL, higher than that of BC group (1.72 ± 0.16) ng/mL and Lv-Ctrl group (1.58 ± 0.34) ng/mL (P < 0.001). In MOLT-4 cells, the IC50 of Lv-CUEDC1 group was (114.22 ± 0.30) ng/mL, higher than that of BC group (10.42 ± 0.45) ng/mL and Lv-Ctrl group (12.02 ± 0.96) ng/mL (P < 0.01) (Table 4).

Table 5. The IC50 (half inhibitory concentration) value of cytarabine in each group of cells (ng/mL, x±s,n=3)

<table>
<thead>
<tr>
<th>Grouping</th>
<th>THP-1 (ng/mL)</th>
<th>Molt-4 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>18.93±0.74</td>
<td>10.19±0.89</td>
</tr>
<tr>
<td>Lv-Ctrl</td>
<td>16.97±2.15</td>
<td>5.19±0.91</td>
</tr>
<tr>
<td>Lv-CUEDC1</td>
<td>27.35±1.20 **</td>
<td>35.41±3.59 ***</td>
</tr>
</tbody>
</table>

Compared with BC and Lv-Ctrl, * * refers to P<0.01, * * * refers to P<0.001.

Figure 14. Inhibitory effect of cytarabine on proliferation of U937 cells (A) and HEL cells (B)

The inhibitory rate of AML cell proliferation increased with the increase of cytarabine concentration, but the inhibitory rate of Lv-CUEDC1 group was lower than that of BC group and Lv-Ctrl group at the same concentration. In THP-1 cells, the IC50 of Lv-CUEDC1 group was (27.35 ± 1.20) ng/mL, higher than that of BC group (18.93 ± 0.74) ng/mL and Lv-Ctrl group (16.97 ± 2.15) ng/mL (P < 0.01). In Molt-4 cells, the IC50 of Lv-CUEDC1 group was (35.41 ± 3.59) ng/mL, higher than that of BC group (10.19 ± 0.89) ng/mL and Lv-Ctrl group (5.19 ± 0.91) ng/mL (P < 0.001) (Table 5).

5.4. What genes are involved in expression regulation and selection of CUEDC1

The second generation high-throughput sequencing (RNA-Seq) method was used to detect the levels of SH-CUEDC1, SH-control and empty leukocyte THP-1. CUEDC1 overexpression group, control group and empty leukocyte MOLT-4 group) gene expression profile changes, looking for interested candidate genes.

6. Conclusions

The protein products P glycoprotein and MOLT-4 of ALL gene are energy-dependent spillover pumps located on the cell membrane, which can pump many chemotherapeutic drugs into leukemia cells out of the cells, resulting in multidrug resistance (MDR). AML is a new MDR-related protein, which is a non-glycoprotein. It is different from P-glycoprotein and MOLT-4 gene in the mechanism of action, mainly through the nuclear target shielding mechanism to cause the MOLT-4 gene. The results of this study showed that the expression of the three genes were closely related to the clinical drug resistance of patients with AL, which was a very adverse prognostic factor. The prognosis of patients with refractory recurrence was significantly higher than that of patients with primary treatment, and the prognosis of patients with AL was very poor. It is suggested that the relapse and refractoriness of AL patients are not the result of single factor, but the result of drug resistance genes with different mechanisms.

Acute myeloid leukemia is a heterogeneous group of diseases, with the development of cytogenetics. Fusion genes such as BCR/ABL and PML/RARalpha have been widely used not only as diagnostic markers for different subtypes of AML, but also as important prognostic factors in clinical practice. However, 50% of AML with normal karyotype lacks effective prognostic marker genes. The role of ALL gene as a "pan" leukemia
marker in the prognosis of AML with normal karyotype has been paid more and more attention. In this study, we found that the expression level of ALL splice isoforms was significantly higher in the AML group than in the non leukemia group. These results suggest that the high expression of ALL splicing isomers in AML can be used as a marker gene of AML cells for clinical differential diagnosis or disease screening. In addition, the expression levels of AML and ALL splicing isomers in the first diagnosis group and relapse group were significantly higher than those in remission group and control group. The expression of ALL splicing isomers in children with AML at different remission stages decreased with the prolongation of remission time, and the ALL ratio in the initial diagnosis group, relapse group and drug resistance group was significantly higher than that in remission group. Therefore, we conclude that ALL splicing isomers may be involved in the occurrence of AML, and the main change is the alteration of ALL splicing isomers, which is consistent with the experimental results of AML-derived cell lines. It is further confirmed that monitoring ALL splice isoforms is of guiding significance for understanding the condition and prognosis.

The expression of CUEDC1 in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) cells and U937, THP-1, HEL, Jurkat, MOLT-4 cell lines in AL patients was detected by RT-PCR, real-time quantitative PCR and Western blot. It has important prognostic value for AL patients, and is worthy of clinical promotion.

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