CDX2 gene expression and clinical significance in children with acute lymphoblastic leukemia

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Abstract: This research was designed to investigate CDX2 gene expression and clinical significance in children with acute lymphoblastic leukemia. Marrow samples were taken from 160 CDX Children with newly diagnosed ALL, . Multiplex RT-PCR was used to detect common ALL fusion gene, while regular RT-PCR was used to detect CDX2 gene. Comparative semi-quantitative method was used to analyze the relative expression levels of CDX2 in each group and their relationship with the treatment efficacy. Among those 160 patients, 10 were BCR / ABL-positive, 8 were E2A / PBX1 positive, 26 were TEL / AML1-positive, 8 were SIL / TAL1 positive, and 42 were MLL abnormalities (including translocations and repeat). 130 out of those 160 patients showed CDX2 positive (81.3%). The control group showed CDX2 negative. CDX2 expressions in B-ALL and T-ALL groups were 0.4675 ± 0.2372 and 0.4563 ± 0.2031, respectively. The expression difference between these two groups was not statistically significant (P> 0.05). There were significant differences (P <0.05) for each positive group CDX2 fusion gene expression levels, wherein the fusion gene negative group was the highest (0.4935 ± 0.2450). After the chemotherapy, 119 cases of 130 CDX2 gene-positive patients reached CR (91.7%), while all 30 cases of CDX2 gene-negative patients reached CR (100%). The treatment difference was not statistically significant between these two groups. However, the CDX2 expressions were statistically different between the group of 119 CDX2-positive patients who achieved CR after chemotherapy and the 11 CDX2-positive patients who didn't reach CR after chemotherapy. CONCLUSIONS: CDX2 expression was common among ALL children. CDX2 expression levels were highly associated with the chemotherapy efficacy and can be used as an indicator to monitor MRD.

KEYWORDS: lymphoblastic leukemia; CDX2 gene; gene expression

Caudal-related homeodomain transcription 2 (CDX2) is one member of the non-HOX family of homeobox gene (Homeobox gene, HOX). CDX2 is located in 13q12 ~ 13. It not only controls embryonic cell growth and development, but also plays an important role in regulating the differentiation and proliferation of adult tissues. The abnormal expression of CDX2 was reported to be involved in the occurrence, development, and prognosis of many tumors [1, 2, 3, 4].

Acute lymphoblastic leukemia is the most common hematological malignancy among children. Some patients showed fusion gene abnormalities. We used Multiplex RT-PCR to screen children with these
anomalies and to measure CDX2 gene expressions in myeloid leukemia cells in children in different anomaly groups. We further explored CDX2 gene expression in child with acute lymphoblastic leukemia and its clinical value.

**Materials and Methods**

1.1 Clinical data

Marrow samples were collected from 160 child patients diagnosed in our department during the period between March 2010 and October 2012. All patients underwent marrow cell morphology diagnosis and the phenotypes were measured by flow cytometry. The diagnosis and treatment of all children followed the typical lymphoblastic leukemia treatment procedures [7]. Among the 160 patients, 95 were male and 65 were female, with age ranging from four months and 29 days to 14 years with a median age of 5 years. Samples from 16 healthy individuals were also taken as normal control. 16 cases of peripheral blood of healthy volunteers as normal controls. 10 of them were men and 6 were women. The controls were 17-61 years old with a median age of 31 years.

1.2 Main reagents

TRIzol total RNA extracts and TaqDNA polymerase were purchased from TAKARA. Lymphocyte separation medium was purchased from Tianjin TBD company. RT kits were purchased from Promega.

1.3 PCR primers

CDX2(NM_001265.4) primers were designed and synthesized by Shanghai Biotech Ltd.

CDX2 forward primer:

5’—GAAAACCAGGACGAAAGAAA—3’

CDX2 reverse primer:

5’—CTGCTGCAACTTCTTCTGTTG—3’

The leukemia fusion gene primers for Multiplex RT-PCR were synthesized according to the references. [8]

Method

1.4.1 Total RNA extraction

Fresh bone marrow from patients or peripheral blood from healthy controls were collected with EDTA added as anti-coagulator. The marrow or blood samples were added into lymphocyte separation medium to isolate the mononuclear cells. TAKARA RNAiso plusTRI zol reagent was used to extract the total RNA, with the extracts quantified with UV spectrophotometer (with the minimum requirement of OD260 / OD280 ≥ 1.7).

1.4.2 cDNA Synthesis

25μl reverse transcription medium included 1ng total RNA, 500 ng random primers, 20 U RNA inhibitors, 5μl 5xRT buffer, 200 U MMLV reverse transcriptase (Promega products). The reaction was taken in the following conditions: 70 °C for 5 min, 37 °C for 60 min, and 70 °C for 10 min. The resulted mixture was stored at 4 °C for the following experiments.

1.4.3 PCR experiment

Into the 50 μl PCR mixture, the following reagents were added: 4 μl cDNA reaction solution, 5μl 10 x buffer, 2.0 μl of both upstream and downstream primers (6.25 μmol/L), 2.0 μl TaqDNA polymerase (1 u / μl), 1.0 μl dNTPs (10 mmol / L), and 34 μl sterile deionized water. CDX2 amplification conditions were as follows: 35 cycles of pre-denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 45 seconds, annealing at 58 °C for 50 seconds, and extension at 72 °C for 45 seconds. After 35 cycles, a final 7-minute extension at 72 °C was added. The amplified product was 221 bp longer. Multiplex amplification conditions were the same as reported previously [8].

1.4.4 Analysis of PCR products

The mixture of PCR products (5 μl for each product) was analyzed with 50-min electrophoresis (100 V) on a 2% agarose gel. The resulted gel was stained with ethidium bromide (EB) and scanned with Tannon2500 digital scanner. The CDX2 / β-actin ratio was calculated from the digital gel analysis.

1.5 Statistical analysis:

SPSS 17.0 statistical software was used for data analysis. The measurement data were presented as mean ± standard deviation (± x ± s). ANOVA was used for the multiple group comparison, while pairwise comparisons used q test. The intra-group comparison was presented as a percentage or ratio while the χ 2 test was used for the inter-group comparison. P <0.05 was considered statistically significant.

Results

2.1 Fusion gene expressions in children with acute lymphoblastic leukemia

Multiplex PCR analysis showed that among the160 ALL patients,
10 were BCR / ABL-positive, 8 were E2A / PBX1 positive, 26 were TEL / AML1-positive, 8 were SIL / TAL1 positive, and 42 were MLL abnormalities (including translocations and repeats). Electrophoresis results were shown in Figure 1.

2.2 CDX2 gene expressions in children with acute lymphoblastic leukemia

Among the 160 ALL patients, 130 showed CDX2 positive (81.3%), including 117 B-ALL patients (90%) and 13 T-ALL patients (10%). 16 normal controls were CDX2 negative. Electrophoresis results were shown in Figure 2. The CDX2 expression levels in B-ALL and T-ALL were 0.4675 ± 0.2372 and 0.4563 ± 0.2431, respectively. The difference on CDX2 expressions in these two groups was statistically significant (F = 130.428, P = 0.000), with the fusion gene negative group showing higher expression (0.5645 ± 0.2450).

2.2 The relationship between CDX2 gene expression and treatment efficacy on children with acute lymphoblastic leukemia

After remission induction therapy, 119 out of the 130 CDX2-positive patients reached CR (91.7%) while all 30 CDX2-negative patients reached CR (100%). However, this efficacy difference between these two groups was not statistical different (P > 0.05). For CDX2-positive patients, CDX2 expressions dropped dramatically for 119 patients who had positive responses to the chemotherapy and achieved CR (from 0.4575 ± 0.2812 before chemotherapy to 0 after chemotherapy (P < 0.01)). On the other hand, those 11 patients who didn’t respond to the chemotherapy only showed very small decline on the CDX2 expression (0.7275 ± 0.2011 before chemotherapy vs. 0.6472 ± 0.2504 after chemotherapy (P > 0.05). The response difference between these two subgroups was statistically significant.

Discussions

CDX2 gene regulates the differentiation and development of embryonic cells, and the differentiation and proliferation of adult tissues. It can only be in intestinal epithelial cells in healthy adults. It has been reported that CDX2 abnormal expression was usually related to gastrointestinal cancer occurrence, development, and prognosis [9-12]. Recently, some clinical reports showed that it showed high expression in adult leukemia cells [13-14], suggesting that CDX2 might induce leukemia.

Leukemia in children is mainly ALL, which is significantly different from adult leukemia in terms of leukemia type, CR ratio and cure rates. It’s very important to select the appropriate genetic markers in chemotherapy efficacy evaluation and MRD assessment.

This study showed that healthy adults didn’t have CDX2 expression in peripheral blood, while CDX2 expression was very common among children with ALL (81.3%). In addition, the CDX2 expressions in both T-ALL and B-ALL children were comparable, suggesting that CDX2 expression was closely related to the ALL onset and had the same effects on both T-ALL and B-ALL onsets. In addition, we carried out CDX2 comparative study on fusion gene-positive ALL children. The results indicated that each subgroup had a certain level of CDX2 expression and the difference among groups was statistically significant, with fusion-gene-negative group having the highest CDX2 expression. In addition, for CDX2-positive patients, the changes on CDX2 expressions before and after the chemotherapy were statistically significant for both CR and NR subgroups. Therefore, CDX2 expression levels could fairly reflect leukemia development and could be used to assess the effect of chemotherapy. This might be explained by that CDX2 is multifaceted in

![Figure 1: Multiplex PCR results for the fusion gene in ALL patients. PCR experiments were carried out separately for samples No1, 3, 4, and 6. PCR results confirmed sample No. 6 was BCR / ABL gene positive](image)

![Figure 2: Measurement results for CDX and β-actin gene. No1, 3, 5, and 7 were CDX samples from ALL patients, while No9 was normal control. No2, 4, 6, 8, 10 showed β-actin for each corresponding sample.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>n</th>
<th>P (%)</th>
<th>CDX2/β-actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR/ABL group</td>
<td>10</td>
<td>8</td>
<td>0.8</td>
<td>0.2736±0.0217</td>
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<tr>
<td>E2A/PBX1 group</td>
<td>8</td>
<td>6</td>
<td>0.75</td>
<td>0.2668±0.0212</td>
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<tr>
<td>MLL rearrangement group</td>
<td>42</td>
<td>36</td>
<td>0.86</td>
<td>0.5424±0.2235</td>
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<tr>
<td>TEL/AML1 group</td>
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<td>0.85</td>
<td>0.3575±0.1242</td>
</tr>
<tr>
<td>SIL/TAL1 group</td>
<td>8</td>
<td>6</td>
<td>0.75</td>
<td>0.3718±0.0372</td>
</tr>
<tr>
<td>Fusion gene negative group</td>
<td>66</td>
<td>52</td>
<td>0.79</td>
<td>0.5645±0.2450</td>
</tr>
</tbody>
</table>

Table 1: CDX2 expressions in 160 ALL patients
inducing leukemia. It not only contributes to the occurrence of leukemia by regulating the HOX gene expression but also is involved in the stem cell self-renewal and leukemic transformation [15-18]. With higher CDX2 expression levels, more hematopoietic genes and regulatory genes are affected, resulting in a decline in the effect of chemotherapy.

Individualized treatments have greatly improved the cure rate and long-term survival of child leukemia. An important basis for individualized treatment is minimal residual disease (MRD) detection. MRD is also the main cause of leukemia relapse. A variety of fusion gene-based MRD detection has been widely used in the clinical application, but the lack of biomarker is slowing the adoption of fusion gene-based MRD detection in child leukemia treatments. This study showed that in general CDX2 gene expression was high in child ALL and the CDX2 expression difference between the experimental group and the control group was significantly different. In addition, with leukemia in remission, CDX2 expression also turned from positive to negative. This dynamic change might be associated with the load reduction on leukemic cells in vivo, suggesting that the CDX2 gene might play an important role on MRD monitoring of ALL children after treatments.

In brief, we believe that for the fusion-gene-negative ALL children, CDX2 can be used as an indicator for MRD measurement. Monitoring CDX2 expression levels can help to adjust chemotherapy so that the best treatment effects can be achieved.

References: