The expression and clinical significance of CDX2 and WT1 gene in children acute lymphoblastic leukemia

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Abstract: The purpose of this study is to study and compare the expression and clinical significance of cdx2 gene and WT1 gene in acute lymphoblastic leukemia of children. The bone marrow of 160 Cases of early-on ALL children was collected to detect the cdx2 and WT1 gene expression in B-ALL, T-ALL and normal control group with RT-PCR method. The relative expression levels of the two genes and the relationship with efficacy were compared and analyzed with semi-quantitative method. The results showed that there were 130 cases (81.3%) of CDX2 positive and 81 cases (50.6%) of WT1 positive in the 160 ALL patients. The expression of the two genes was negative in normal control group. CDX2 expression levels in B-ALL and T-ALL were 0.4675 ± 0.2372 and 0.4563 ± 0.2031 respectively (P> 0.05); the WT1 expression levels were 0.5562 ± 0.4132 and 0.8065 ± 0.2678 (P <0.05). In 130 cases of CDX2 gene expression-positive children with ALL, WT1 gene expression was positive in 64 cases (49.2%); in the 30 cases of CDX2 expression negative, there were 17 patients (56.7%) with WT1 gene positive. The CDX2 gene and WT1 gene expression was no significantly correlated (P> 0.05). In the 130 cases of CDX2 gene-positive, 119 cases after chemotherapy reached CR (91.7%), and negative group of 30 patients all reached CR (100%); in the 81 cases of WT1 gene positive group of children, after chemotherapy 71 cases reached CR (88%), and 79 cases of negative group all reached CR (100%). The difference of the CR rates between the groups was not statistically significant (P> 0.05). The expression levels before and after chemotherapy between the 119 CDX2 positive cases that achieved CR after chemotherapy and 11 cases of children without achieving CR were compared, and the difference was statistically significant. The comparison of expression levels before and after chemotherapy of 71 WT1 positive cases that reached CR after chemotherapy and 10 children who did not achieve CR showed the difference was not statistically significant. Conclusion: in children with ALL, there is high expression of CDX2; the positive rate is higher than WT1 and there is no difference of expression in B-ALL and T-ALL. The expression level of CDX2-positive patients is related to the effect of chemotherapy. In children with ALL, the CDX2 is of more research value.

KEYWORDS: CDX 2 gene; leukemia; gene expression

Caudal-related homeobox transcription 2 (CDX2) is a member of non-HOX family in homeobox genes (HOX), located at 13q12 - 13. It not only controls the growth and development of embryonic cells, but also plays an important role in the regulation of the differentiation and proliferation of adult tissues. Its abnormal expression is believed to related with tumor occurrence, development and prognosis[1,2,3,4].

WT1 gene is the Wilm's tumor gene[5] separated from the chromosome 11p13 of Wilm's tumour cell and it is a zinc finger transcription factor which plays an important role on cell growth and differentiation. Most studies demonstrated its oncogene role in leukemia, and it is a transcriptional inhibitor or activator which regulates proliferation and / or differentiation.
of hematopoietic cells [8]. It is the most mature in present study and recognized by most scholars as ‘Pan leukemia genetic’ markers. Its expression is stable and it is related to the occurrence, development and prognosis of leukemia. Therefore it is the most common tumor-related genes in leukemia MRD detection.

Acute lymphocytic leukemia is the most common blood system tumors in children. We applied RT-PCR method to detect the CDX2 gene expression in children’s myeloid leukemia cells, and used WT1 gene as a control gene to explore CDX2 gene expression and its relationship with prognosis in children with acute lymphoblastic leukemia.

Materials and methods

1.1 Clinical data

160 Specimens were selected from our department received from March 2010 to November 2011. All patients underwent cell morphological examination of bone marrow and flow cytometry for immune phenotype. The diagnosis and treatment were in accordance with treatment recommendations for children acute lymphoblastic leukemia [7]. There were 95 cases of male, 65 females, aged 4 month and 29 days - 14, with a median age of 5. Peripheral blood of 16 patients with healthy physical examination was taken as normal control, 10 males, 6 females, aged 17-61, with a median age of 31.

1.2 Main reagents

TRIzol total RNA extract and TaqDNA polymerase were TAKARA products; lymphocyte separation medium was from Tianjin TBD. Reverse transcription kit was made by promega.

1.3 Primers

CDX2 (NM_001265.4) primers were designed and synthesized by Shanghai Sangon:

CDX2 upstream primer:

5’-GAAAACCAGGACGAAAGACAAA-3’

CDX2 downstream primers:

5’-CTGCTGCAACTTCTCTTGTTG-3’

WT1 gene primers see ref. [8]

1.4 Methods

1.4.1 Total RNA extraction Fresh bone marrow of patients or peripheral blood of healthy participants collected with heparin were added to lymphocyte separation medium followed by conventional separation of mononuclear cells. TAKARA RNAiso plus TRI zol reagent was used for extraction of total cellular RNA. The UV spectrophotometer quantitative measurement was carried out and OD260/OD280 ≥ 1.7 was required.

1.4.2 cDNA synthesis RT system 25μl, including total RNA 1ng, 500 ng of random primers, RNase inhibitor 20 U, 5xRT buffer 5μl, MMLV reverse transcriptase (Promega Company) 200 U. The steps were 70°C for 5 min, 37 °C for 60 min, 70 °C for 10 min and 4 °C storage.

1.4.4 PCR reaction 50 μl PCR reaction system was added cDNA reaction solution 4μl, 10 x buffer 5μl, 2.0μl each of downstream primer and upstream primer (6.25 μmol / L), Taq DNA polymerase (1 u / μl) 2.0 μl, dNTPs (10 mmol / L) 1.0 μl, sterile deionized water 34 μl. The CDX2 amplification conditions: 5 minutes of 94°C predenaturation, 45 seconds of denaturation at 94 °C, 58 °C annealing for 50 seconds, 72°C extension for 45 seconds. After 35 cycles, 7 minutes of extension at 72°C, the length of amplification product was 221 bp. WT1 amplification conditions: 5 minutes of 94°C predenaturation, 45 seconds of denaturation at 94 °C, 60 °C annealing for 50 seconds, 72°C extension for 45 seconds. After 30 cycles, 7 minutes of extension at 72°C, the length of amplification product was 343bp. The length of amplification product of internal reference β-actin was 154 bp.

1.4.5 PCR product analysis 5 μl of the PCR products were mixed well and were subject to 2% agarose gel electrophoresis (100 V) for 50min. Ethidium bromide (EB) staining followed by scan analysis with Tannon2500 digital gel analyzer. CDX2/β-actin and WT1/β-actin ratio were obtained.

1.5 Statistical Methods:

SPSS 17.0 statistical software was used for χ2 test or fourfold table exact probability method for analysis.

Results

2.1 CDX2 and WT1 gene expression of children with acute lymphoblastic leukemia

In the 160 ALL patients, there were 130 cases of CDX2 positive with positive rate of 81.3% and 81 cases of WT1 positive, with positive rate of 50.6%. Expressions of the two genes in 16 normal controls were negative. Electrophoresis results is shown in Figure 1.

CDX2 expression levels in B-ALL and T-ALL were 0.4675 ± 0.2372 and 0.4563 ± 0.2031. There was no statistically signifi-
cant difference between the two (P > 0.05); the WT1 expression levels were 0.5562 ± 0.4132 and 0.8065 ± 0.2678 and comparison of the two showed statistically significant difference (P < 0.05).

2.2 The correlation between CDX2 gene and WT1 gene of children with acute lymphocytic leukemia

In the 130 cases of ALL children with CDX2 gene expression positive, there were 64 cases (49.2%) of WT1 gene expression positive; in the 30 cases with CDX2 expression negative, 17 patients (56.7%) was WT1 gene expression positive. CDX2 and WT1 gene expression was not significantly correlated (P > 0.05) (Table 1).

2.3 Comparison of relationship of CDX2 and WT1 gene with efficacy for children with acute lymphoblastic leukemia

Of the 130 cases of children with CDX2 gene expression positive, after remission induction therapy, 119 cases reached CR (91.7%). All 30 negative cases reached CR (100%), and comparison of CR rates between two groups showed no statistical difference (P > 0.05). In the 81 cases of children with WT1 gene expression positive, after remission induction treatment, 71 cases reached CR (88%). All 79 cases of negative group reached CR (100%), and the CR rate comparison between CDX2 gene and WT1 gene negative group showed the difference was not statistically significant (P > 0.05). The CR rate comparison between positive groups showed the difference was not statistically significant (P > 0.05).

The expression levels of the 119 cases of children with CDX2 positive who reached CR before chemotherapy was 0.5993 ± 0.3225, and after chemotherapy it was 0.3417 ± 0.1025 (P < 0.05). The expression level before chemotherapy of the 10 cases of children who did not reach CR was 0.5943 ± 0.3413, and 0.4863 ± 0.3355 (P > 0.05) after chemotherapy. The expression levels of CR group and non-achieving CR group before and after chemotherapy were compared, and the difference was not statistically significant.

Discussion

WT1 is currently the most widely used and recognized by most scholars as the ‘Pan leukemia’ genetic markers. Multiple studies have shown that WT1 not only has significantly increased expression levels in acute leukemia, especially in AML, but also is expressed in chronic leukemia. It has stable expression and is associated with the occurrence, development and prognosis of leukemia. It also reflects the changes in the load of leukemia. CDX2 gene regulates development and differentiation of embryonic cells as well as differentiation and proliferation of adult tissues. For normal adult it can be detected only in intestinal epithelial cells. Studies have shown that its abnormal expression is related to digestive tract tumor occurrence, development and prognosis [9-12]. Currently there are clinical reports that it is highly expressed in adult leukemia cells [13-14], which makes it attract much attention because of the possibility of becoming new leukemia gene.

Childhood leukemia is mainly ALL-based, and it is significantly different from adult leukemia in incidence type, CR rate and cure rates. It is critical of choosing suitable leukemia gene marker for assessment of chemotherapy effect and MRD detection. Bai Xiaoling’s and other studies have shown that WT1 is expressed in a variety of malignant hematopoietic diseases, with the highest expression rate in AL, suggesting WT1 can be used mainly as various AL tumor marker for monitoring MRD [15]. Boublikova L [16] found that in children ALL WT1 expression level displayed diversity and was significantly lower than in AML and in adult ALL. Expression levels of WT1 in bone marrow of some patients were lower than normal con-

Table 1: Correlation of CDX2 and WT1 expression in ALL Children

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tros but had a high recurrence rate. Therefore it was believed that WT1 is not suitable as children ALL MRD monitoring indicator.

This study shows that there is no CDX2 expression in healthy human peripheral blood. In childhood ALL CDX2 positive rate was higher (81.3%) than WT1 (50.6%), and CDX2 expression levels in B-ALL and T-ALL were 0.4675 ± .2372 and 0.4563 ± 0.2031, suggesting that CDX2 has a closer relationship with the incidence of ALL, and have the same causing effect of chemotherapy on B cells and T cells. Boublikova [16] and other studies have also pointed out that in childhood ALL, WT1-positive rate is much lower than the AML and adult ALL positive rate, indicating that the WT1 may be more relevant to these diseases. In this set of experiments, positive rate of WT1 gene in CDX2 positive group and negative group had no significant difference (P> 0.05), suggesting the expression of the CDX2 gene and WT1 gene in childhood ALL has no significant correlation and they play important roles on pathogenesis of leukemia through different pathways. CR rates after chemotherapy in positive group and negative group of the two genes were compared and there was no statistically significant difference, suggesting no significantly different functions of CDX2 and WT1 gene on overall assessment of chemotherapy. However it is worth noting that, compared with WT1, CDX2 expression levels of CR group after chemotherapy and NR group before and after chemotherapy showed significant difference in CDX2-positive patients. The CDX2 expression level can reflect the leukemic tumor burden to assess the effect of chemotherapy. This may be because the CDX2 causing effect on leukemia is multifaceted. It not only affects HOX gene expression and regulation to contribute to the occurrence of leukemia, but also is involved in stem cell self-renewal and leukemic transformation [17-20]. With the increase of CDX2 expression level, more hematopoietic genes and regulatory genes are affected, leading to a decline in the effect of chemotherapy.

In summary, we believe that CDX2 is more suitable for the detection of childhood ALL than WT1. High level of CDX2 expression indicates chemotherapy ineffective. For CDX2 positive patients, monitoring of CDX2 expression levels can provide better adjustment of chemotherapy program in order to achieve the best effect of chemotherapy.

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References


