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Short Communication

Evaluation of RT-PCR to Detect Translocations in Children Diagnosed with Acute Lymphoblastic Leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most common subtype of childhood cancer. Chromosomal abnormality, specially the replacement of chromosomal material is one of the main reasons in generating leukemia, wherein the kind of translocation play a key role in managing the remedy. The goal of the present study was to develop a reliable, rapid, and cost effective method to detect translocations, which are the main sources of leukemia.

Methods: Twenty seven samples were collected from leukemia affected individuals that were referred to the Shafa Hospital in Ahwaz from summer 2007 to spring2008. Total RNA was extracted from one milliliter whole blood, and then reversely transcribed using reverse transcriptase. Finally, multiplex RT-PCR was performed for each sample.

Results: Cell lines (K562, Jurkat E 6.1) that are harboring known translocations were used as positive control, with additional internal control to prove false negative results. Translocations t (9; 22), t (12; 21), t (1; 19) and t (4; 11) were observed in patients that have been diagnosed with the ALL, respectively. No Translocation has been seen in individuals suffering lymphoma.

Conclusion: Multiplex RT-PCR assay is an effective, sensitive, accurate, and cost-effective diagnostic tool, which can improve the ability to accurately and rapidly risk-stratify patients that were diagnosed with acute lymphoblastic leukemia.

Keywords: *Acute Lymphoblastic Leukemia, Translocation, Multiplex-RT-PCR*

Introduction

Acute lymphoblast leukemia (ALL) is a specific type of blood cancer. Leukemia is a current childhood cancer including 75% of the ALL, 20% of the AML (Acute Myelocytic Leukemia) and the remaining parts involving the CML (Chronic Myelocytic Leukemia) and the CLL (Chronic Lymphoblast Leukemia) (1). The worldwide statistics demonstrate this type of cancer in about 27% of children up to 15 yr old (1). In addition, environmental and genetic factors are considered as two essential factors in the children's infection to illness (2). Among the environmental factors, we can refer to the essential changes of ionizing radiation (3), some viruses, chemical materials such as benzene, and genetic factor including essential alteration in DNA like chromosome translocations, deletions, and insertions (4). Many of the predominant genetic alterations in ALL and AML consist of chromosomal rearrangement, illegitimate recombination and the generation of in frame fusion genes. Meanwhile chromosomal rearrangement and especial chromosomal translocation play an important role in the formation of leukemia blasts. Currently, more than 50 chromosomal replacement have been detected in children's ALL, in which the four translocations t[12;21], t[1;19], t[4;11], t[9;22] have an essential role in prognosis and therapy (5). However, detection of translocations by conventional cytogenetic studies like karyotype and FISH (Fluorecent in situ Hybridisation) is very time and cost intensive.

Our aim was therefore to evaluate an accurate and reliable alternative method to detect common translocations in leukemia, especially in the childhood ALL

Materials and Methods

Biological samples

The samples were collected from Shafa Hospital (the only leukemia center in the Khuzestan Pro-

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vince) and chosen among the children referred to the center for the first time. Twenty seven samples were collected from individuals diagnosed with leukemia from summer 2007 to spring 2008, in which several parameters were concerned about such as be a new case without using anti cancer drugs and without receiving blood.

Flow Cytometry and morphological analysis

All the samples were diagnosed according to the type of leukemia by standard clinical and morphological properties, following by flow cytometry using specific antibodies against cell surface antigens. The panel of monoclonal antibodies used for flow cytometry estimation were consisted of CD3, CD7, CD5 (for T lymphocytes lineage) and CD19, CD22, CD20, CD10 (for B lymphocytes lineage); CD13, CD14, CD33 (for myeloid subsets).

RNA Extraction and cDNA Synthesis

Total RNA was extracted from leukemia cells by Trizol reagent according to the manufacturer's instructions (Fermentase). Five microgram of RNA was reversely transcribed using reverse transcriptase according to the manufacturer's instructions (Fermentase) in RT buffer containing 0.5 Mm d NTP mix, 1 Um Oligo-d T, 10U RNase inhibitor at 37 °C for 60 min.

Multiplex PCR analysis

To verify the integrity of the isolated RNA and the correct synthesis of the cDNA, an internal positive control has been co-amplified with the size of 690 bp performed with the primers E2A1 and E2A2 for the first round and E2A3 and E2A4 (Table 1) for the second round nested reaction (Fig. 1). The multiplex-PCR was carried out in two steps. The first PCR was carried out for each primer panel listed in the table 1 in a final volume of 25 µl with 10X PCR-Buffer, 200 uM d NTP, $MgCl_2$ 50 mM 5% DMSO (Sigma), 5 pmol of each primer pair and 0.5 ul of Taq DNA polymerase, and 1-2 ul cDNA as template. The initial PCR consists of: at 95 °C for 3 min, followed by 30 cycles of PCR amplification (annealing at 58 °C for 30 S, elongation at 72 °C for 30 S and denaturation at 95 °C for 30 S), and an extension step of 5 min at 72 °C. The second PCR consist of 1 ul aliquots from the first PCR reactions transferred to the second round mixtures that is identical to the first, but with second round primers. The primer sequences (Table 1) for both rounds of the PCR reactions were adopted as described previously (2).

Results

Standard morphologic and histopathologic examination and flow cytometric assay revealed that 21 patients suffer ALL and further six patients were diagnosed with lymphoma, respectively.

A multiplex RT-PCR was applied to detect four common chromosomal replacements including t[1; 19], t[12; 21], t[9; 22] and t[4; 11]. The synthesized cDNA was used as template for three parallel first round PCR reactions that have been made with the primers from the panel A, B, and C, respectively (Table 1). To enhance the amplification reaction, we used the internal second primers in a nested-PCR as has been shown in the Table 1.

The panel ABC has been co-amplified in all reactions as technical control to prove false negative reactions. With other words, the ABC panel confirmed the real absence of any translocation and produced always a PCR product from ubiquitously expressed transcription factor E2A-m RNA with the length of 690 base pairs (Fig. 1).

We additionally confirmed the detected translocations in patients resulted from nested-PCR by using the cell lines (K562, Jurkat 6.1, BL28) that harbor known translocations as mentioned in the Table 2. These positive controls were applied parallel to each RT-PCR, derived from patient's samples.

Finally, the translocation t(9; 22), known as Philadelphia chromosome, has been found in 10 patients suffering ALL (Fig. 1). The translocations t(12; 21) and t(1; 19) were the second frequent chromosome rearrangement, respectively (Table 2). The translocation t(4; 11) was detected in only one patient diagnosed with ALL. In three cases were two translocations detected in the same sample (Table 2). No translocation was observed in six patients having lymphoma as in three patients have been diagnosed with ALL, respectively.

Table 1: The properties of primers that were used in the multiplex PCR

Panel	1. Primer	Primer sequence (5'>3')	2. Primer	Primer sequence (5'>3')
A	PBX1	GCCACGCCTTCCGCTAAC	PBX1	CATGTTGTCCAGCCGCATCAG
	E2A1	TTCTCGTCCAGCCCTTCTACC	E2A3	CTACGACGGGGGTCTCCAC
	TEL	CACTCCGTGGATTTCAAACAGTC	TEL	CTCATCGGGAAGACCTGGCTTAC
	AML1A	AGCCGAGTAGTTTTCATCATTGC	AML1A	AGCACGGAGCAGAGGAAGTTG
B	BCR1	CGCTCTCCCTCGCAGAACT	BCR3	CACGTTCCTGATCTCCTCTGAC
	BCR2	GAGTCACTGCTGCTGCTTATGTC	BCR4	ACTGCCCGGTTGTCGTGTC
	ABL1	TTTTGGTTTGGGCTTCACAC	ABL2	ACACCATTCCCCATTGTGATTATC
C	MLL1	CCGCCTCAGCCACCTACTAC	MLL3	AGCAGATGGAGTCCACAGGATCAG
	MLL2	AGCACTCTCTCCAATGGCAATAGT	MLL3	GGACCGCCAAGAAAAGAAGT
	AF4-1	GAATTTGAGTGAGTTTTTGAAGATGTAT	AF4-2	GTTTTTGGTTTGGGTTACAGAACT
ABC	E2A1	TTCTCGTCCAGCCCTTCTACC	E2A3	CTACGACGGGGGTCTCCAC
	E2A2	TTTTCCTCTTCTCGCCGTTTCA	E2A4	AGGTTCCGCTCTCGCACTT

Table 2: cell line[1] were used in this study as positive controls in the RT-PCR reactions

Cell line	Kind of translocation
BL28	t (12;21)(TEL/AML1)
K562	t (9;22)(BCR/ABL)
Jurkat6.1	t(1;19)(PBX1/E2A) & t(4;11)(MLL/AF4)

[1]From cell bank of Pasteur institute, Tehran, Iran

Table 3: Clinical diagnosis and multiplex-PCR data from ALL patients

Patient	clinical diagnosis	Multiplex RT-PCR finding
1	ALL	t (12;21)
2	ALL	t (12;21)
3	ALL	t (12;21)
4	ALL	t (12;21)
5	ALL	t (12;21)
6	ALL	t (12;21)
7	ALL	t (12;21), t (9;22)
8	ALL	t (12;21), t (9;22)
9	ALL	t (9;22)
10	ALL	t (9;22)
11	ALL	t (9;22)
12	ALL	t (9;22)
13	ALL	t (9;22)
14	ALL	t (9;22)
15	ALL	t (9;22)
16	ALL	t (9;22), t (4;11)
17	ALL	t (1;19)
18	ALL	t (1;19)
19	ALL	Negative
20	ALL	Negative
21	ALL	Negative
22	Lymphoma	Negative
23	Lymphoma	Negative
24	Lymphoma	Negative
25	Lymphoma	Negative
26	Lymphoma	Negative
27	Lymphoma	Negative

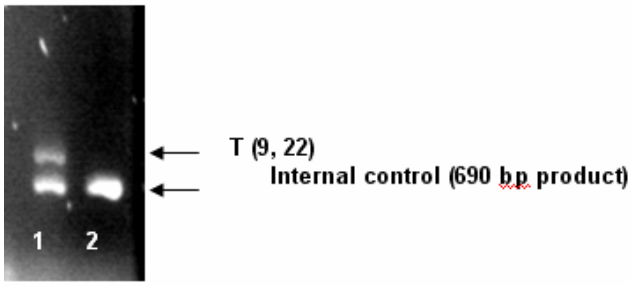

Fig. 1: To test the validation of the nested multiplex-RT-PCR, we first optimized the reactions with the cDNA derived from cell lines with known translocation; for instance the cell line K562 with expected translocation t (9; 22) demonstrates in the lane 1 the predicted fragment (upper band) resulting from the fused ABL-BCR mRNA in addition to the 690 bp product (lower band) that has been co-amplified from ubiquitous expressed E2A mRNA and was used as internal control. The sample in the lane 2 shows therefore a single band corresponding to the 690 bp. Thus, we can assume the absence of the Philadelphia chromosome in the mentioned patient

Discussion

The presence of chromosomal translocations in pediatric ALL affects not only the biology of leukemia cells but also plays an essential role in the prognosis of the disease. For instance, the translocation t[1; 19] causes a chimeric and fused transcript PBX1-E2A that is observed in a high frequency in infant's with consequently poor prognosis (6, 7), while the translocation t (12; 21) has appropriate good prognosis and a proper chemotherapy can increase 5 yr surveillance (8). Furthermore, the translocation t(4; 11) affects the fusion transcript of MLL-AF4 with moderate prognosis (9). The cyclically performance of high dosage to low dosage chemotherapy may increase the remedy rate largely (9). According to the recent reports, children who have the translocation t (9, 22) show dismal prognosis with at most 5 yr disease-free life in 25 to 30 percent of cases. While these patients undergo allogenic transplant, the remission rate will increase to 65% (10). Consequently, the diagnosis of these translocations is of great importance in determining the type of remedy (11). Nevertheless, molecular methods are very useful diagnosis tools to detection rapidly chromosome rearrangements because of their high accuracy and application.

In this study, we proved successfully the RT-PCR method for quick, easy, and low-cost diagnosis, which is applicable in some specific condition, such as applying simultaneous detection of four current chromosomal translocations as mentioned above. This method can be modified and extend to identify more translocations in leukemia patients.

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