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Expression of the B-Cell Lymphoma/Leukemia 11A Gene in Malignant Hematological Cell Lines through Quantitative Reverse Transcription Polymerase Chain Reaction

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OBJECTIVE The B-cell lymphoma/leukemia 11A (BCL11A) gene is essential for normal lymphoid development and has been associated with hematological malignancies. In the current study, the relative expression level of BCL11A in malignant hematological cell lines was evaluated through real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR).

METHODS The relative expression level of BCL11A mRNA in malignant hematological cell lines was determined through qRT-PCR using SYBR Green I dye. Glyceraldehyde-3-phosphate dehydrogenase was used as the reference gene to confirm the relative expression level of BCL11A gene mRNA.

RESULTS The relative expression level of BCL11A mRNA in cell lines from B-cell malignancies was significantly higher compared with that from acute myeloid leukemia ($P < 0.05$). Different cell lines with malignant B-cells exhibited a wide range of BCL11A expressions ranging from 27.37 to 93.38.

CONCLUSION The overexpression of BCL11A gene mRNA in malignant B-cells might play a role in B-cell lymphoma/leukemia.

KEY WORDS: B-cell lymphoma/leukemia 11A (BCL11A), malignant B-cells, real-time quantitative reverse transcription-polymerase chain reaction.

Introduction

B-cell lymphoma/leukemia 11A (BCL11) gene family members, including BCL11A and BCL11B genes, are associated with lymphocyte proliferation, differentiation, and survival[1,2]. BCL11B has been recently shown as one of the necessary genes for T-cell differentiation and is associated with T-cell malignancies[2–4]. BCL11A gene was initially found in mice as an ecotropic retrovirus integration site (Evi9), also known as COUP-TP interacting protein 1[5]. BCL11A gene encodes a C_2H_2 zinc finger protein, which is a Krüppel transcription factor, and is closely related to B-cell proliferation and differentiation[1,6]. Alternative splicing within the BCL11A locus leads to the formation of four major protein isoforms: eXtra Long, Long, Short, and eXtra Short[7]. Abnormalities involving BCL11A have been detected in a variety of B-cell malignancies and certain subsets of acute myeloid leukemia in humans[8–11]. However, whether BCL11A is a real proto-oncogene has been controversial[1,9,11]. Previous results have shown that the expression level of BCL11A in B-cell acute lymphocyte leukemia patients significantly increased compared with T-cell acute lymphocyte leukemia and healthy control groups[12]. In the current study, the expression level of BCL11A gene in

malignant B-lymphocyte cell lines was analyzed through real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Materials and Methods

Cell lines

Human B-cell leukemia/lymphoma cell lines (Daudi, Raji, SUDHL6, EB1, OCI-LY-3, and DG-75) and acute myeloid leukemia cell lines (U937, KG-1, Nomo-1, Monomal, and P3HR1) were provided by Prof. Ailin Guo (Institute of Pathology, Cornell University). The cells were cultured at 37 °C in an atmosphere containing 5% CO_2 in RPMI-1640 medium supplemented with penicillin (100 U/mL), streptomycin (0.1 mg/mL), and 10% fetal calf serum.

RNA extraction and cDNA synthesis

RNA was extracted using the Trizol kit (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into the first-strand cDNA using random hexamer primers and the reverse transcriptase Superscript II Kit (Invitrogen, USA) according to the manufacturer's instructions. RNA purity and concentration was measured using a spectrophotometer. The integrity of the RNA was evaluated using 1% agarose gel. The quality of the synthesized cDNA was determined through qRT-PCR using the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

Real-time quantitative PCR with SYBR green I dye

The $2^{-\Delta Ct} \times 100\%$ method could be used to analyze the relative changes in gene expression from real-time quantitative PCR experiments[13–14]. Before using the method to detect BCL11A gene expression level, whether both BCL11A and GAPDH genes have high amplification efficiency should be determined first. Therefore, a validation experiment was conducted using serial dilutions of Daudi cDNA template covering five orders of magnitude, namely, 0.0001, 0.001, 0.01, 0.1, and 1. Standard curves were generated by plotting the Ct values against the logarithm starting quantity of the serially diluted cDNA template. The slope and correlation coefficient (R^2) were determined through linear regression analysis. Briefly, the primers were synthesized (Invitrogen, USA) as previously described[14]. The following primer sequences were used in the PCR reactions: BCL11A sense, 5′-AACCCCAGCACTTAAGCAAA-3′; BCL11A antisense, 5′′-GGAGGTCATGATCCCCTTCT-3′; GAPDH sense, 5′-ACCCAGAAGACTGTGGATGG -3′; GAPDH antisense, 5′-TTCAGCTCAGGGATGACCTT-3′. The total reaction volume is 20 μL. Reaction conditions started with the enzyme activation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 80 °C for 5 s. At the end of each run, a melting curve was performed starting at 65 °C up to 95 °C with an increase of 1 °C per 2 s to verify primer specificities. Each run was completed using melting curve analysis to confirm the specificity of the amplification and the absence of primer dimers. The qRT-PCR was repeated in at least three separate experiments. The PCR products were visualized and separated via 2% agarose gel electrophoresis.

Statistical analysis

Independent sample *t*-test analysis was used for the relative expression level of BCL11A gene mRNA in different samples, whereas the Mann–Whitney U-test was used for non-normally distributed data using the SPSS 13.0 statistical software. Differences were considered statistically significant at $P < 0.05$.

Results

PCR product analysis

The representative data of the accurate standard curve graphs and melting curves are shown in Fig. 1. The slope for the BCL11A reaction was −3.774, with an R^2 value of 0.984. The slope and R^2 values of the standard curves for the GAPDH reaction were −3.720 and 0.983, respectively. The amplification efficiency of the BCL11A gene was consistent with the high amplification efficiency of the GAPDH reference gene, thus allowing the relative quantification according to the $2^{-\Delta Ct} \times 100\%$ formula[13,14]. In all the samples tested, the specific peak of the GAPDH gene began at 88 °C, whereas that of the BCL11A gene began at 85°C (Fig. 1). Results of the 2% agarose gel electrophoresis revealed that the PCR products from the BCL11A gene and GAPDH reference gene were 125 and 114 bp, respectively (Fig. 1). This result also confirmed the specific amplification of the BCL11A gene.

The expression level of BCL11A in malignant B-cell lines

According to the relative quantitative real-time PCR formula $2^{-\Delta Ct} \times 100\%$, the relative expression level of BCL11A mRNA in cell lines from B-cell malignancies were significantly higher compared with those from acute myeloid leukemia ($P < 0.05$). The quantity of BCL11A in these B-cell lines varied from 27.37 to 93.38 (Fig. 2). The relative quantity of BCL11A in the acute myeloid leukemia cell lines ranged from 1.33 to 9.00.

Discussion

Many malignancies of mature B-cells are characterized by chromosomal translocations involving the immunoglobulin heavy chain locus on chromosome 14q32.3 and result in deregulated expression of the translocated oncogene. Although t(2;14)(p16.1;q32.3) is a rare event

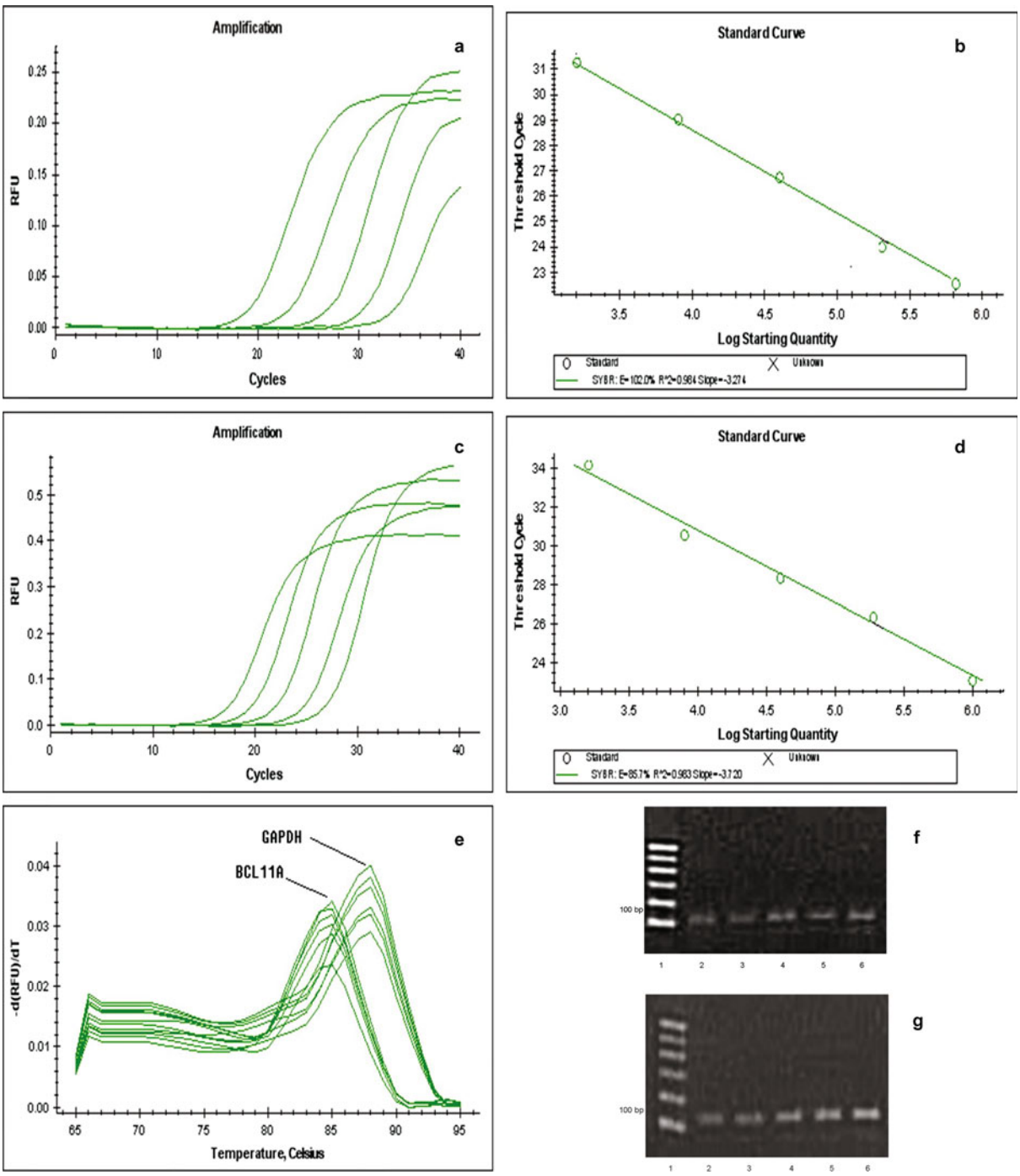

Fig.1. Accurate standard curves and melting curves, as well as agarose gel electrophoresis analysis of the BCL11A gene and GAPDH gene. Results shown are from representative experiments. The standard curves were generated by plotting the Ct versus the logarithm starting quantity of the serially diluted cDNA. (a and b) The slope and correlation coefficient (R^2) for the BCL11A gene are −3.774 and 0.984, respectively. (c and d) The slope and R^2 value for the GAPDH reaction are −3.720 and 0.983, respectively. (e) Melting curves of the BCL11A gene and GAPDH gene from a part of cell lines. The specific peak of the GAPDH reference gene begins at 88 ˚C, whereas that of the BCL11A gene begins at 85 ˚C. PCR products of the BCL11A gene (f) and GAPDH gene (g) via 2% agarose gel electrophoresis. The size of the PCR products of the BCL11A gene is 114 bp, whereas that for the reference gene GAPDH is 125 bp. Line 1, DNA ladder; Lines 2 to 6, PCR products from the malignant B-cell lines.

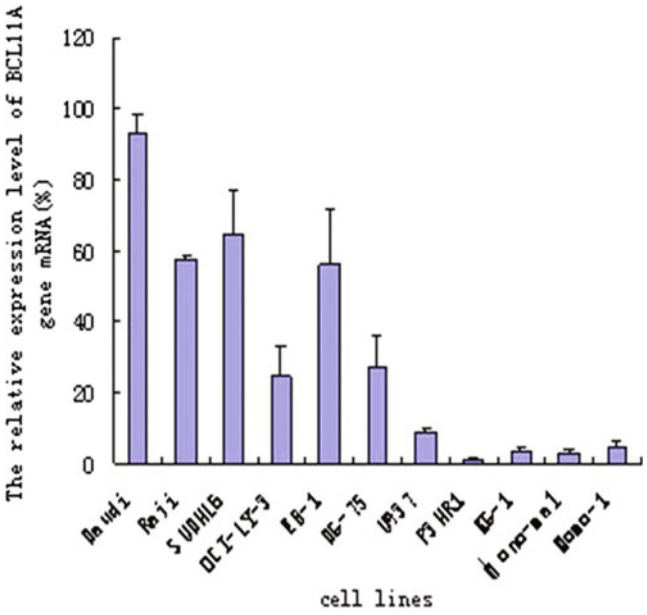

Fig.2. Relative expression level of BCL11A gene mRNA in different cell lines Results shown are the mean ± SD of triplicate experiments. The expression level of BCL11A gene was significantly different between malignant B-cell lines and acute myeloid leukemia cell lines ($P < 0.05$).

in B-cell malignancies, gains and amplifications of 2p16.1 (previously mapped as 2p13) have been reported in approximately 20% to 50% among the subtypes of diffuse large B-cell lymphoma (DLBCL) and in 50% of classical Hodgkin's lymphoma[10,15]. A previous study displayed an increased BCL11A gene copy number by fluorescence in situ hybridization in 15/20 (75%) primary mediastinal B-cell lymphoma (PMBL), suggesting its frequent genomic aberration in PMBL[9]. Although BCL11A gene expression has been detected and described in B-cell lymphomas, such as DLBCL and PMBL[9,16], little is known about the expression pattern of the BCL11A gene mRNA in B-lymphocyte malignancy.

In the present study, BCL11A mRNA expression was determined through real-time quantitative PCR using SYBR Green I dye. A specific melting peak was detected for the BCL11A gene in the different cell lines. Melting curve changes were not found in all cell lines. The high amplification efficiency of BCL11A was consistent with that of GAPDH. This result suggests that the $2^{-\Delta Ct} \times 100\%$ method can be used to quantify the relative expression level of BCL11A mRNA.

The expression level of BCL11A in the B-lymphocyte malignant cell lines was significantly different compared with those from acute myeloid leukemia cell lines. This result indicates that BCL11A may be associated with the development of B-lymphocyte malignancies. However, the BCL11A gene expression levels were quite different in these B-lymphocyte malignant cell lines, which may be related to the heterogeneity of B-lymphocyte malignancies including B-cell leukemia and B-cell lymphoma. Therefore, further studies using increased number of samples are needed to characterize the differences. Results of the current study are in accordance with Weniger's report, which detected BCL11A expression through immunohistochemistry and fluorescence using in situ hybridization in primary anaplastic B-cell lymphoma[9]. In conclusion, BCL11A mRNA expression level is increased in malignant B-cell lines. However, the tumorigenic function of BCL11A gene needs to be further studied in B-cell malignancies.

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Conflict of Interest Statement

No potential conflicts of interest were disclosed.

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