Quantification of the FLI1 and CXCR4 gene expressions in acute lymphoblastic leukemia (ALL) patients with t(12,21)

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ABSTRACT

The presence of the t(12,21) is associated with good response to therapy in acute lymphoblastic leukemia (ALL) but molecular background of this pathology is not clear. FLI1 gene plays important roles in normal regulation of myeloid hematopoiesis and leukemogenesis. The chemokine receptor CXCR4 gene may play a role in the homing of hematopoietic stem cells. Our aim was to investigate possible relationships between t(12,21) existence and expression changes of these two genes (FLI1, CXCR4) in ALL. Thirty-one ALL patients were investigated. Twenty-one of these patients were t(12,21) carriers. We used the Quantitative Real-Time RT-PCR. Obtained results were compared to normal bone marrow samples of five healthy subjects. Expression differences were not found significant in both groups. Our study was the first attempt to quantify these genes in t(12,21) patients. We conclude that Quantitative RT-PCR is a reliable method for the monitoring of these gene expressions and similar studies should expand to other translocations in haematology.

Key Words: Gene expression, FLI1 protein, CXCR4, RT-PCR.

ÖZET

t(12,21) pozitif akut lenfoblastik lösemide FLI1 ve CXCR4 gen ekspresyonu kantitasyonu

t(12,21) varlığı akut lenfoblastik lösemi (ALL)'de tedaviye iyi yanıtla ilişkilendirilmiştir fakat, bu patolojinin moleküler temeli net değildir. FLI1 geni miyeloid hemopoeziste ve lökomogenezisin normal düzenlenişinde önemli rol oynar. Kemokin reseptörü CXCR4 hematopoietik kök hücrelerin yön tayinlerinde önemli rol oynayabilir. Amacımız t(12,21) varlığıyla bu iki genin (FLI1, CXCR4) anlatım değişiklikleri arasındaki muhtemel ilişkileri ALL'de incelemekti. Otuzbir ALL hastası incelendi. Bu hastaların 21'i t(12,21) taşıyıcısıydı. Kantitatif Gerçek-Zamanlı PCR kullandık. Elde edilen sonuçlar beş sağlıklı kişinin normal kemik iliği örnekleriyle kıyaslandı. Anlatım farklılıkları her iki grupta da istatistiksel olarak anlamlı bulunmadı. Çalışmamız bu genlerin t(12,21) hastalarında kantifikasyonu için ilk girişimdir. Kantitatif Gerçek-Zamanlı PCR bu genlerin taranması için güvenilir bir yöntemdir ve benzer çalışmalar hematolojinin diğer translokasyonlarına doğru genişletilebilir.

Anahtar Kelimeler: Gen ekspresyonu, FLI1 protein, CXCR4, RT-PCR.

INTRODUCTION

The presence of the t(12,21) is associated with good response in ALL. These kind of gene fusions are well known incidents in leukemia while the other genetic changes are less known. Recurrent chromosomal translocations are of relevance in leukemogenesis because they influence the expression of some key regulatory genes, but identities of such genes are not completly clear. Among several newly identified genes, FLI1 and CXCR4 are attractive targets for researchers interested with leukemia. We found that FLI1 gene was down regulated while CXCR4 gene was up regulated during vitamin D dependent differentiation of leukemia cell line HL-60 cells in our previous studies using cDNA array technology[1]. Role of the FLI1 gene in leukemogenesis and hematopoiesis has been shown also in previous studies of other authors[2,3]. CXCR4 gene is the receptor of CXC chemokine SDF-1. It has been concluded that CXCR4 is expressed on CD34+ cells including more primitive, pluripotent progenitors, and may therefore play a role in the homing of hematopoietic stem cells[4,5].

In this study our aim was to investigate possible relationships between t(12,21) existence and expression changes of FLI1 and CXCR4 genes in 31 ALL patients. Twenty-one of these patients were t(12,21) carriers. We used Quantitative Real-Time RT-PCR (LightCycler, Roche Diagnostics GmbH, Germany). Obtained results were compared to normal bone marrow samples of the five healthy subjects. To our knowledge, our study was the first attempt to quantification of FLI1 and CXCR4 genes in t(12,21) patients by Quantitative Real-Time RT-PCR.

MATERIALS and METHODS

Our study is based on total RNA samples extracted from bone marrow aspirates of 31 ALL patients. Twenty-one of these patients were t(12,21) carriers. All translocations were detected for diagnostic purposes in Genetics Department of Institute for Experimental Medicine, Istanbul University by RT-PCR analysis. Spesific data of all patients were shown in Table 1. Patient control group was consisted of pooled bone marrow aspirates from 5 healthy volunteer donors. Cells from bone marrow aspirates were isolated by ficoll-hypaque centrifugation. Total RNA was extracted by using guanidium thiocyanate-phenol-chloroform extraction method as previously described[6]. RNA samples were treated with DNase I and Quantitative Real-Time PCR analysis were performed as we described previously[1,7]. Standard curves were obtained by using of serial dilutions of the $ß_2$-microglobulin gene. Sequences of gene-specific primers were CCGCTTCTACCCCAATGACT and GCGAAGAAAGCCAGGATGAG for CXCR4, CCACACTGGTGACACAGGAG, TCTTTGACACTCAATCGTGAGGA for FLI1, TGGGTTTCATCAATCCGACAT, CACGGCAGGCATACTCATCTT for $ß_2$-microglobulin genes. The obtained gene expression values were normalised using housekeeping gene $ß_2$-microglobulin levels. Melting curve analysis and gel electrophoresis of the products validated the reactions. SPSS Student's t-test were used for statistical analysis.

RESULTS

FLI1 and CXCR4 gene expressions were investigated in 31 ALL patients. Twenty-one of these patients were t(12,21) carriers. Obtained results were shown in Table 1. These results were compared to normal bone marrow samples of five healthy subjects. FLI1 gene ratios were 0.0600, 0.19000, 0.24000, 0.19000, 0.23000 and CXCR4 ratios were 1.7600, 1.15000, 1.10000, 0.76000, 1.30000 for normal bone marrow samples of five healthy subjects, 1 to 5 respectively. SPSS Student's t-test were used for statistical analysis. In t(12,21) patient samples FLI1 (p= 0.251) and CXCR4 (p= 0.190) expression differences were not found significant. These genes also were not found significant in non translocated patient samples, FLI1 (p= 0.759) and CXCR4 (p= 0.291). Amplification reactions demonstrating the gradual reducti-

Table 1. **Data of 31 ALL patients and validation of relative gene expression by Quantitative Real-Time RT- PCR

Patient	t(12,21)	(%) BCD	Sex	Age	WBC	FAB	Phenotype	Immunophenotype	FLI1 ratio	CXCR4 ratio
1	Positive	83	m	5	na	L1	Common preB	CD10, 19, HLADR	0.00700	1.64000
2	Positive	95	f	9	2600	L1	Common preB	CD10, 19, HLADR	0.21000	18.00000
3	Positive	54	m	7	8500	L1	Common preB	CD10, 19, HLADR	0.14000	16.00000
4	Positive	97	f	3.5	23.000	L1	Common B	CD 10, 19, 20, HLADR, CD7	0.04900	1.37000
5	Positive	98	f	5	6200	L2	Common preB	CD34, 10, 19, 22, 3, HLADR	0.06400	2.30000
6	Positive	94	f	4	3000	L1	Common preB	CD 10, 19, 20, 22, HLADR	0.01600	44.30000
7	Positive	87	f	9	22.000	L2	Common B-ALL	CD10, 19, 20, 22, HLADR	0.04000	12.90000
8	Positive	65	f	7	4100	L3	B-ALL	sIg	0.02400	1.11000
9	Positive	98	m	4	8500	L2	CALLA(+) B-ALL	CD3, 75, 7, 10, 19, 22, HLADR	0.00800	1.50000
10	Positive	86	f	3	15.200	L1	Common ALL	na	0.00500	123.00000
11	Positive	100	m	4.5	15.400	L2	CALLA(+) B-ALL	CD10, 19, 34, HLADR	0.01400	1.70000
12	Positive	100	f	3	25.000	L1	CALLA(+) B-ALL	CD10, HLADR	0.04500	20.00000
13	Positive	98	m	3.5	5000	L1	CALLA(+) preB	CD3, 5, 7, 13, 10, 19, 22, 34, 45	0.00010	1.07000
14	Positive	88	m	4	13.300	L1	Common ALL	CD10, 19, HLADR	0.57000	4.80000
15	Positive	89	f	3	1900	L2	CALLA(+) preB	CD10, 19, 34, 45, Ki67	0.00700	63.00000
16	Positive	97	m	4	8700	L1	CALLA(+) B-ALL	CD3, 10, 22, 19, 5, 7, 45, HLADR	0.41000	7.20000
17	Positive	96	m	10	50.000	L2	Common B-ALL	na	0.09000	1.90000
18	Positive	79	m	5	13.000	L2	PreB-ALL	na	0.00600	119.00000
19	Positive	na	na	na	na	na	na	na	0.29000	10.50000
20	Positive	na	na	na	na	na	na	na	0.06000	4.70000

Table 1. **Data of 31 ALL patients and validation of relative gene expression by Quantitative Real-Time RT- PCR (continued from previous page)

Patient	t(12,21)	(%) BCD	Sex	Age	WBC	FAB	Phenotype	Immunophenotype	FLI1 ratio	CXCR4 ratio
21	Positive	na	na	na	na	na	na	na	0.16000	20.70000
22	Negative	100	f	1	140.000	L1	Common B-ALL	CD3, 10, 19, 20, HLADR	0.11000	27.00000
23	Negative	90	f	12	78.000	L3	CALLA(+) preB	CD10, 19, 22, 45, HLADR	2.34000	1.25000
24	Negative	78	f	2	37.000	L1	Common > B SALL	CD10, HLADR, CD2, CD7	0.00007	1.64000
25	Negative	63	f	2.5	10.600	L1	CALLA(+) B-ALL	na	0.01700	0.61000
26	Negative	56	m	12	10.500	L2	T-ALL	CD5, 7	0.11400	7.40000
27	Negative	70	m	12	30.000	L1	T-cell ALL	na	0.03600	7.70000
28	Negative	78	m	3	26.600	L1	Common ALL	CD10, 20, HLADR	0.00100	651.00000
29	Negative	94	m	5	254.000	L1	Common ALL	CD19, 20, HLADR	0.17000	733.00000
30	Negative	100	f	7	48.500	L1	CALLA(+) B-ALL	CD10, 19, 22, 45, HLADR	0.03600	4.52000
31	Negative	na	na	na	na	na	na	na	0.03000	26.00000

Abbreviations: WBC: White blood cells, BCD: Blast count at diagnosis, na: Data is not available.
Ratio: The measured expression of the FLI1 and CXCR4 genes in patients/The measured expression of the housekeeping $ß_2$-microglobulin gene in patients.

on in fluoresence as temperature increases. The rapid falls indicates the spesific products that melts at spesific temperatures as 86°C for CXCR4, 85.5°C for FLI1 and 81°C for $ß_2$-microglobulin. Gene specific amplifications were demonstrated with melting curve data (Figure 1).

DISCUSSION

Ets family members have been shown to play an important role in several signal transduction pathways[2]. Human FLI1 gene is an Ets family member and involved in malignancies. Up-regulation of FLI1 affects normal lymphoid cell function and apoptosis[8,9].

FLI1 gene may have an important role on tumorogenesis in fusion forms with other genes. There are other previous quantification studies of the FLI1 gene in literature but most of these studies belong to the quantification of the EWS-FLI1 fusion in Ewing's sarcoma family tumors. Some of them introduced the question of whether EWS-FLI1 gene fusion type might serve as a prognostic molecular indicator in this group of patients[10-12].

On the other hand, Levanon et al have found that, the FLI1 homologous sequence contains a breakpoint of the t(11;22) translocation associated with Ewing's tumors, and may have a similar function in RUNX1[13].

In the literature, it has been concluded that CXCR4 is expressed on CD34+ including more primitive, pluripotent progenitors, and may therefore play a role in the hematopoietic stem cells. CXCR4 expression is critical for the biological effects of SDF-1. The exact role of SDF is not very clear. It has been suggested that SDF-1 activates distinct signalling pathways that may mediate cell growth, migration and transcriptional activation and may contribute to leukemic marrow infiltration, by increased CXCR4 expression[4,5,14].

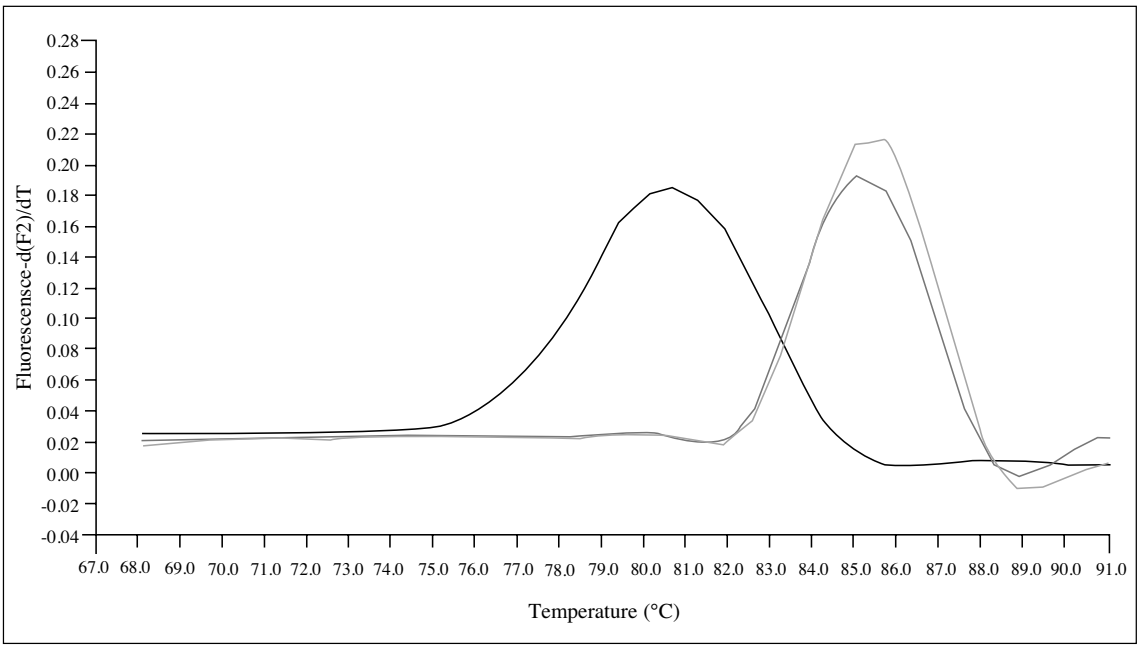

Figure 1. Melting curve analysis demonstrating the gradual reduction in fluoresence as temperature increases. The falls off at 86°C for CXCR4, 85.5°C for FLI1 and 81°C for ß$_2$-microglobulin indicates the spesific products that melts at this temperature.

There are many different methods for quantification of the gene expression including nested RT-PCR, northern blotting, cDNA array, RNAse protection assay. These technologies are valuable methods but have many problems as low sensitivitiy, high cost or long time requirements for the detection. Quantitative-Real-Time RT-PCR is most sensitive, rapid and reliable approach compared to conventional methods and cDNA array technology[15-19]. Normalization of the amplification product is the main problem of the Quantitative RT-PCR. Quantification errors are easily obtained by variation in the amount of starting material between different samples. The main approach for minimizing these errors is using a cellular RNA as an internal reference. The ideal gene used for normalisation should be expressed at a constant level in different tissues at all stages of development, and should not be affected by any type of diseases[19]. ß$_2$-microglobulin sequences have been defined very reliable for haematological experiments[20]. For this reason, we used ß$_2$-microglobulin as an internal controls for normalization our quantification results.

Based on our findings Quantitative Real-Time RT-PCR method should be considered as an approach to the gene statement analysis in haematology and here we propose an optimised strategy for FLI1 and CXCR4 gene detection. Larger studies are required to assess the impact of our approach in FLI1 and CXCR4 gene expression analysis in leukemogenesis. We conclude that Quantitative RT-PCR is a reliable method for the monitoring of these genes and similar studies should expand to other translocations in haematology.

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