Evaluation of PPP2R5C gene expression in Iranian patients with B-Acute lymphoblastic leukemia and its association with clinical and laboratory findings

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Abstract

Introduction: PPP2R5C is one of the regulatory B subunits of protein phosphatase 2A (PP2A), which is a tumor suppressor. PPP2R5C plays a critical role in cell proliferation, differentiation, and transformation. Considering these vital functions, we investigate the gene expression in Iranian patients with B-Acute Lymphoblastic Leukemia (B-ALL) and its association with clinical and laboratory finding.

Materials and methods: In this case-control study, peripheral blood samples were collected from 60 B-ALL patients and 30 healthy controls. PPP2R5C expression levels were determined by Real-time PCR. After calculation of CT for target and control genes, we calculated ΔCT. Finally we compared the PPP2R5C expression levels in patients with control group.

Results: Significantly higher expression of PPP2R5C was found in the B-ALL patients (2.15±2. 50) compared with control group. There was no correlation between PPP2R5C expression and clinical and laboratory findings and FAB (French-American-British) subtype of patients.

Conclusion: we demonstrated PPP2R5C overexpression in B-ALL patients. Although there was no significant correlation between PPP2R5C expression, clinical and laboratory finding and also with FAB subtypes of patients.

Keywords: B-Acute Lymphoblastic Leukemia, PPP2R5C, PP2A, Gene expression

Introduction

Acute Lymphoblastic Leukemia (ALL) is a form of leukemia that is characterized by overproduction and subsequently bone marrow infiltration of lymphoblasts and other body organs involvement. Although it may be diagnosed at any age, it is the most common malignancy of infants and teenagers. In the current WHO

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classification, two subtypes, B-ALL and T-ALL, are recognized; B-ALL comprises the great majority of cases (1). In many cases the cause of leukemia is ambiguous but mutations, deletions, chromosomal defects, up or down regulation of cellular signaling pathways as well as dysregulation of Epigenetic events can be causative factors of leukemia (2-4). The opposing function of phosphatases and kinases is a more important mechanism involved in regulation of different signaling pathways, so aberrations in regulatory proteins of these mechanisms may be from significant causes of leukemia (5).

Protein phosphatase 2A (PP2A) is a Ser/Thr protein phosphatase with a heterotrimer structure. It has a catalytic subunit (C), a scaffold subunit (A) and several variable regulatory subunits (B). The regulatory subunits are four families which B56 family members consist of PPP2R5A, PPP2R5B, PPP2R5C, PPP2R5D, and PPP2R5E. Observations demonstrated that B56 family are more important in malignant transformation (6-10), by its inducing effect on dephosphorylation of P53. PPP2R5C plays a vital role in cell proliferation, differentiation, and transformation (11-13). With regard to the critical functions of PPP2R5C we investigated the gene expression of this protein in Iranian patients with B-Acute Lymphoblastic Leukemia and its association with clinical and laboratory findings.

Materials and methods

This study was performed in the Research laboratory of molecular hematopathology of Ghaem Hospital in Mashhad, Iran. 60 newly diagnosed and untreated patients with B-ALL and 30 individuals as control were enrolled into the study. All patients were categorized in FAB classification groups. At the beginning of study, informed consent was obtained from all groups. The clinical and laboratory parameter including lymphadenopathy, splenomegaly,hepatomegaly, demographic data, immunophenotyping results and other laboratory parameters, were retrieved from medical records of patients.

Peripheral blood was collected from all patients in K2-EDTA containing vials. Ficoll gradient centrifugation was used to isolate mononuclear cells. The RNA was extracted using RNX plus kit according to the manufacturer's instructions (Cinnagen, Tehran, Iran). Subsequently, cDNA synthesis was performed using primers of Random Hexamer. PPP2R5C gene expression analysis on patient's samples and control group was done using real time-PCR method. Gene expression levels, were applied by SYBRGreen (TAKARA) and relative expression was calculated from $2^{-\Delta\Delta CT}$ method. PPP2R5C gene expression normalized against the internal gene, beta-2-microglobolin (β2M), by specific primers depicted in table 1.

In order to ensure the specificity of the real-time PCR products, melting curves were plotted after each working run. For standard curve, 0.1, 0.01, and 0.001 dilutions were prepared from primers of PPP2R5C and β2M. Amplification curves were plotted following performing of the test. The results of PPP2R5C gene expression levels in patients were compared with that of the control group. Data were analyzed by SPSS, version19. The Kolmogorov–Smirnov test and Chi-Square independent sample t-test were used in case needed. P-value less than 0.05 were considered as significant.

Results

We studied 60 patients diagnosed with B-ALL and 30 individuals as control group. The patients group included 30(50%) males, 30(50%) females and the control group was made of 15(50%) males and 15(50%) females. Mean ± SD age of patients and control group were 13±14 years (range: 1-65 years) and 16±7 years (range: 3-42 years), respectively. FAB classification of patients include 45(75%), ALL-L1 and 15(25%) ALL-L2

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respectively. WBC counts, platelet counts and hemoglobin concentration in patients and the correlation between FAB classification and gene expression level in patients are shown in table 2. There was no significant relationship between the expressions level of PPP2R5C and these parameters. The included clinical features were hepatomegaly (44%), splenomegaly (66%), lymphadenopathy (42%) and petechia-purpura (24%). There was no significant association between the expressions level 3of PPP2R5C and clinical presentations (Table 3).

We examined the genetic abnormalities such as t (12; 21), t (1; 19) and t (9; 22), there was no significant correlation between the expressions level of PPP2R5C and genetic abnormalities (Table 4). Significantly higher expression of PPP2R5C was found in the patients (2.15 ±2.50) compared with healthy controls. The number of patients that had upregulated expression of PPP2R5C was 70% (42 out of 60). There was no significant relationships between the expressions level of PPP2R5C and different FAB subtypes (P value > 0.05).

Table 1. Primer sequence for two genes (PPP2R5C and β2M).

Gene	Primer	Sequence (5' to 3')
PPP2R5C	Forward	GTAATAAAGCGGGCAG-CAGG
	Reverse	CAAAGTCAAAGAGACGCAACA
β2M	Forward	CAGCAAGGACTGGTCTTTCTAT
	Reverse	CAGCAAGGACTGGTCTTTCTAT

Table 2. Characteristics and laboratory findings of the patients with B-ALL.

Characteristics	Fab subtype		PPP2R5C Expression Level		
	L1	L2	Up Regulate	Down Regulate	P value
Number of Patients, (%)	45(75)	15(25)	42(70)	18(30)	
Male	22	8	20	11	
Female	23	7	22	7	
WBC count (10^9/L, median)	51.05	62.16	57.55	49.14	0.641
Platelet count (10^9/L, median)	79.86	11.84	73.07	55.31	0.192
Hb, g/dl (median)	6.5	7.2	6.42	7.08	0.383

Table 3. Differences in expression level of PPP2R5C in patients with different clinical presentations.

Clinical presentations	Lymphadenopathy		Splenomegaly		Hepatomegaly		Petechiae-Purpura	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
PPP2R5C level	3.06	1.50	2.10	2.25	2.52	1.93	3.47	1.74
P value	0.227		0.757		0.418		0.118	

Table 4. Differences in expression level of PPP2R5C in B- ALL patients with different genetic abnormalities.

Translocation	t(12;21)		t(9;22)		t(1;19)	
	Positive	Negative	Positive	Negative	Positive	Negative
Number	11	49	17	43	7	53
PPP2R5C level	2.476	2.069	2.547	1.958	2.683	2.073
P value	0.639		0.488		0.550	

Discussion

PPP2R5C is one of the regulatory subunits of protein phosphatase 2A (PP2A) which is a key cellular serine/threonine phosphatase affecting the phosphorylation site of many proteins (9). PP2A is one of the Ras-Raf signaling pathway regulators. The normal function of Akt is tightly modulated by phosphorylation events (14). The function of PP2A in Wnt/β-catenin signaling is similar to its role in MAPK pathway (15). In response to γ radiation PP2A dephosphorylates p53 hence affecting apoptosis in mammalian cells [16]. PP2A also plays a prominent role in

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controlling the accumulation of the protoncoprotein, c-Myc (17).

Recent studies have demonstrated decreased expression of PPP2R5C in B-CLL, so it was concluded to be a marker of progressive disease in B-CLL. Researchers have compared expression of PPP2R5C in stable and progressive B-CLL without a control group (18). Another study, demonstrated that overexpression of PPP2R5C gene in ALL patients compared with control group (19). Two different mutations in lung cancer and intestinal cancer can disrupt B56γ -P53 interaction (12, 20).

It has been showed that PPP2R5C suppression by RNAi can inhibit the proliferation of the Molt4 and Jourkat T cells (21). It also has been showed that the proliferation of the PPP2R5C–siRNA –treated CML cell line was decreased in K562 Cell line (22). In our study significantly higher expression of PPP2R5C was found in Iranian patients with the B-ALL. We studied more patients than previous studies to confirm overexpression of PPP2R5C and also examined the relation of the PPP2R5C expression with clinical and laboratory findings that there was no significant relationship. Additionally, we also examined expression level of PPP2R5C among FAB subtypes of B-ALL (L1, L2) that there was no significant difference in expression level between them. In regard with the importance of PP2A in cancer, as demonstrated in several studies, expression pattern of PPP2R5C might be a critical finding in ALL patients. Definitive results for proven the upregulation of PPP2R5C in B-ALL patients particularly relation of the expression level of PPP2R5C with clinical and laboratory findings could be demonstrated by larger number of samples studied.

Conclusion

The present study revealed PPP2R5C overexpression in B-ALL patients. Although there was no significant relation between PPP2R5C expression clinical and laboratory finding and also with FAB subtypes of patients.

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