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SHP-1 AND *SHP-2* GENE EXPRESSIONS IN LEUKEMIA AND UNCLASSIFIED BLOOD DISORDERS

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ABSTRACT

Hematopoiesis and blood diseases include leukemia and unclassified blood disorders (UBDs). Leukemia, one of the leading causes of cancer death in the world, is divided into four major types including acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). The SHP gene group belongs to the protein tyrosine phosphatase (PTP) family, which plays an important role in preventing cancer growth. This group consists of two main genes, *SHP-1* and *SHP-2*. In this study, experiments were conducted to determine the expression of *SHP-1* and *SHP-2* genes in patients with leukemia and UBDs using real-time PCR. Results showed that while *SHP-1* gene expression was significantly decreased in all patient samples compared to control samples, *SHP-2* gene expression was significantly increased in patients with CML and other blood disorders but decreased in ALL patients compared to healthy subjects. These results are important for disease diagnosis and combination therapy based on *SHP-1* and *SHP-2* target genes.

Keywords: Blood diseases, blood disorder, disease diagnosis, gene expressions.

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INTRODUCTION

Hematopoiesis and blood diseases include many different types including leukemia, polycythemia vera, essential thrombocythemia, immune thrombocytopenic purpura, medullary dysplasia, myelofibrosis, hemophilia, anemia, bacterial sepsis, and thalassemia. However, there are other diseases related to blood diseases but not yet classified. Leukemia is a type of cancer found in hematopoietic organs and is caused by gene mutations in hematopoietic stem cells (HSCs) (Li et al., 2014; Liu et al., 2017b). The four main types of leukemia are acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Among these, AML is identified by abnormal proliferation of myeloid blasts (Keiffer et al., 2019); CML is characterized by the uncontrolled proliferation of myeloid cells at all stages of differentiation (Li et al., 2014); ALL is a disease related to arrested maturation of lymphoid blasts (Gauffin et al., 2009); and CLL is indentified by abnormal proliferation of CD5 positive lymphocytes (Yeung et al., 2019).

The SH2 domain-containing protein tyrosine phosphatase (*SHP*) gene group belongs to the protein tyrosine phosphatase (PTP) family, which plays an important role in preventing cancer growth (Wu et al., 2003). It consists of two main genes: *SHP-1* and *SHP-2*. The *SHP-1* gene regulates the growth and development of hematopoietic stem cells by inhibiting phosphorylation of many signaling related molecules and is involved in the regulation of antagonistic immune systems. A low expression level of this gene is due to the methylation of the promoter region of the DNA (Chim et al., 2004), which leads to the self-phosphorylation of several growth factors in cells (Haque et al., 1998). It imbalances the differentiation and growth of bone marrow cells and is associated with the etiology of the tumor (Amin et al., 2011; Liu et al., 2017b). *SHP-1* is considered a tumor suppressor gene in lymphoma, leukemia, and other types of blood cancers (Plutzky et al., 1992) and participates in inhibiting activation of JAKs/STATs and PI3K/AKT/mTOR signals in various cancers (Lorenz, 2009; Saraswati et al., 2019). Mice lacking *SHP-1* display abnormal growth of HSCs, resulting in hypersensitivity of erythrocyte precursors to erythropoietin as well as uncontrolled accumalation of myeloid stem cells (Dong et al., 1999).

SHP-2 regulates cellular physiological processes through phosphorylation of signaling molecules such as Ras-Erk, PI3K-Akt, Jak-Stat, NF-κB, and mTOR (Tonks, 2006; Yang et al., 2006). *SHP-2* gene mutation increases its protein expression and causes the phathogenesis of leukemia (Tartaglia et al., 2003; Loh et al., 2004). *SHP-2* is similar to *SHP-1* in terms of structure but their functions are different due to the disctint sequences in their C-terminal domain phosphatase (Poole et al., 2005). Activity of SHP-2 protein is triggered by growth factors such as epidermal growth factor (FGF), insulin, and insulin-like growth factor-1 (IGF-1) that increase proliferation, differentiation, and migration of HSCs (Chan et al., 2006). *SHP2*-deficient mice die during early infancy. Lacking of *SHP-2* in HSCs triggers activation of molecular signals such as ERK and AKT, leading to a progressive failure of renewing themselves, apoptotic death, and structural failure of the bone marrow (Chan et al., 2011).

The different expressions of *SHP-1* and *SHP-2* genes in cancer patients can be explained by various structures of C-terminal regions in their DNA sequence (Poole et al., 2005). However, compared to patients with reduced *SHP-2* gene expression, cancer patients with reduced *SHP-1* gene expression respond better to medications such as dexamethasone/thalidomide (Beldi-Ferchiou et al., 2017). Although there are many studies on mutations and expressions of the two genes *SHP-1* and *SHP-2* in hematopoiesis and blood diseases, the expression levels of these two genes in Vietnamese patients with leukemia and other blood disorsers have not been quantified. In this study, experiments were

conducted to isolate total RNA from blood samples of Vietnamese patients with leukemia and UBDs and the mRNA levels of *SHP-1* and *SHP-2* genes in these patients were determined. The results indicated that the expression levels of these genes were different from each other, which can help early diagnosis as well as further studies on combination therapy based on *SHP-1* and *SHP-2* target genes.

MATERIALS AND METHODS

Patients and control subjects

Fresh peripheral blood samples were collected from 325 untreated patients diagnosed with 5 types of blood diseases: 34 AML, 191 CML, 20 ALL, 26 CLL, and 54 UBDs, at the National Hospital of Hematology and Blood Transfusion and the 103 Military Hospital, Hanoi, Vietnam. The control group comprised 20 healthy subjects. No individuals in the control population took any medication or suffered from any known acute or chronic disease. All patients and volunteers gave written consents to participate in the study. Experimental procedures were performed according to the Vietnamese law for the welfare of humans and were approved by the Ethical Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology.

Total RNA extraction and expression level analysis using RT-PCR

Total mRNA was isolated using the Qiashredder and RNeasy Mini Kit from Qiagen according to the manufacturer's instructions. For cDNA first-strand synthesis, 1 µg of total RNA in 12.5 µl DEPC-H2O was mixed with 1 µl of oligo-dT primer (500 µg/ml, Invitrogen) and heated for 2 min at 70 ^{o}C. To determine transcript levels of SHP-1 and SHP-2, quantitative real-time PCR with the LightCycler System (Roche Diagnostics) was applied. The following primers were used: SHP-1 primers: 5'-GCCCAGTTCATT GAAACCAC-3' (forward) and 5'-GAGGG AACCCTT GCTCTTCT-3' (reverse); SHP-2 primers: 5'-GAGAGCAATGACGGCAAGT CT-3' (forward) and 5'-CCTCCACCAACGT CGTATTTC-3' (reverse) and GAPDH primers: 5'-GGAGCGAGATCCCTCCAAA-3' (forward) and 5'-GGCTGTTGTCATA CTTCTC-3' (reverse). PCR reactions were performed in a final volume of 20 µl containing 2 µl cDNA, 2.4 µl MgCl2 (3 µM), 1 µl primer mix (0.5 µM of both primers), 2 µl cDNA Master SybrGreen I mix (Roche Molecular Biochemicals), and 12.6 µl DEPC-treated water. The target DNA was amplified through 40 cycles of 95 ºC for 10 s, 62 ºC for 10 s, and 72 ºC for 16 s, each with a temperature transition rate of 20 ºC/s, a secondary target temperature of 50 ºC and a step size of 0.5 ºC. Melting curve analysis was performed at 95 ºC, 0 s; 60 ºC, 10 s; and 95 ºC, 0 s to determine the melting temperature of primer dimers and the specific PCR products. The ratio between the respective gene and corresponding GAPDH was calculated per sample according to the ΔΔ cycle threshold method.

Statistic

Data are presented as means ± SEM, n represents the number of independent experiments. Differences were tested for statistical significance using Student's unpaired two-tailed t-test or ANOVA, as appropriate. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Expression level of *SHP-1* gene

Similar to previous studies (Li et al., 2014; Liu et al., 2017b), the mRNA expression of *SHP-1* gene was significantly reduced in all patient compared to control groups. Accordingly, the expression rates between *SHP-1* and *GAPDH* genes in ALL, AML, CLL, and CML patients were decreased to 4.12; 5.49; 2.56; and 5.4 , respectively. Compared with control group, *P*-values of differences for ALL, AML, CLL and CML groups were 0.03; 0.02; 0.02; 0.01 and 0.02, respectively (Fig. 1). Similar to blood cancer patients, the mRNA expression of the *SHP-1* gene in patients with UBDs was also significantly decreased compared to healthy individuals (Fig. 1).

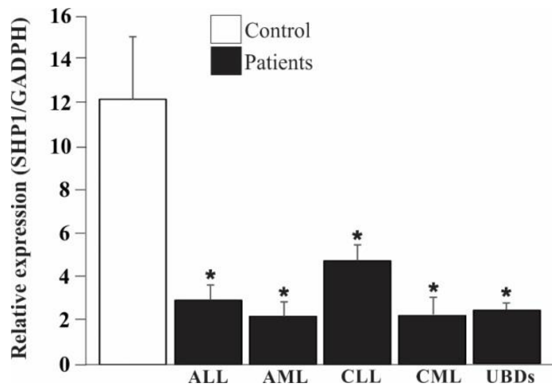

Figure 1. Relative expression of *SHP-1* gene on control and patient groups. * ($p < 0.05$) indicates a statistically significant difference between control and patient groups (ANOVA)

Numerous studies showed reduced expression of *SHP-1* gene in patients with leukemia (Table 1). SHP-1 is one of the important phosphatases to negatively regulate the phosphorylations of several proteins involved in differentiation, growth and development of bone marrow stem cells (Haque et al., 1998). Expression of this gene is decreased in patients with polycythemia vera (Wickrema et al., 1999) due to DNA methylation of its promoter region (Beldi-Ferchiou et al., 2017; Liu et al., 2017b). In this study, reduced expression of *SHP-1* gene in UBD patients is shown for the first time. This suggested that expression of *SHP-1* gene is negatively correlated to hematopoiesis and blood diseases.

Table 1. Differences in *SHP-1* gene expression in patients with blood cancers worldwide

Type of cancers	Research region	Number of patients	Age	Sex (male/female)	SHP-1 expression level	References
ALL	Vietnam	20	19–72	14/6	Decreased	This study
AML	Vietnam	34	17–86	17/17	Decreased	This study
CLL	Vietnam	26	32–83	12/14	Decreased	This study
CML	Vietnam	191	16–81	104/87	Decreased	This study
UBDs	Vietnam	54	19–72	19/35	Decreased	This study
Acute leukemia	China	62	14–70	30/32	Decreased	Liu et al., (2017b)
CML	China	94	16–72		Decreased	Li et al., (2014)
ALL	Sweden	31	1–5	17/14	Decreased	Gauffin et al., (2009)
Non-hodgkin lymphoma	China	33	~ 60	22/11	Decreased	Liu et al., (2017a)
Polycythemia vera	USA	13			Decreased	Wickrema et al., (1999)
Multiple myeloma	France	45	~ 51,7	27/18	Decreased	Beldi-Ferchiou et al., (2017)

Expression level of *SHP-2* gene

Real-time PCR results (Fig. 2) showed that the expression level of *SHP-2* gene was different between patient groups. In CML and UBD patients, the mRNA expression of *SHP-2* gene is significantly increased compared to the control group. Compared with control group, *P*-values of differences were 0.01 and 0.001 for CML and UBD groups, respectively. The expression level of this gene was unaltered in both AML and

CLL patients and even reduced in ALL patients compared with healthy subjects. Similar to this study, elevated *SHP-2* gene expression in CML patients has been identified in several previous studies (Tartaglia et al., 2003; Xu et al., 2005a), i.e. *SHP-2* gene expression could be used in combination with genetic and immunological parameters to diagnose and classify different types of leukemia and blood diseases.

Elevated expression of the *SHP-2* gene is due to a point mutation at the terminal end of the N-terminus that causes leukemia (Pandey et al., 2017). In contrast, in multiple myeloma patients, reduced expression of *SHP-2* gene is due to DNA methylation (Beldi-Ferchiou et al., 2017). *SHP-2* gene expression varied with countries and types of blood cancer (Table 2). However, higher expression of SHP-2 gene in UBD patients might be an important marker to distinguish from leukemia.

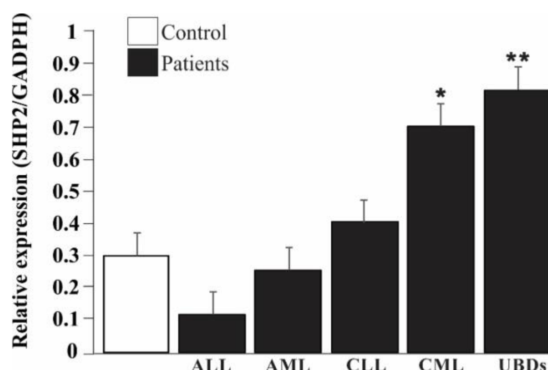

Figure 2. Relative expression of *SHP-2* gene in control and patient groups. * ($p < 0.05$) and ** ($p < 0.01$) indicate statistically significant differences between control and patient groups (ANOVA)

Table 2. Differences in expression of *SHP-2* gene in patients with blood cancers worldwide

Type of cancers	Research region	Number of patients	Age	Sex (male/female)	SHP-2 expression level	References
ALL	Vietnam	20	19–72	14/6	Decreased	This study
AML	Vietnam	34	17–86	17/17	Unaltered	This study
CLL	Vietnam	26	32–83	12/14	Unaltered	This study
CML	Vietnam	191	16–81	104/87	Increased	This study
UBDs	Vietnam	54	19–72	19/35	Increased	This study
Leukemia	China	52	16–69		Increased	Xu et al., (2005b)
Multiple myeloma	France	45	~ 51,7	27/18	Decreased	Beldi-Ferchiou et al., (2017)
Myelodysplastic syndromes	Europe	50			Increased	Tartaglia et al., (2003)
AML	Europe	26			Increased	Tartaglia et al., (2003)
Leukemia	Europe	62			Increased	Tartaglia et al., (2003)

CONCLUSION

Our results indicated different expressions of *SHP-1* and *SHP-2* genes in leukemia and UBDs, in which CML and UBDs were partially distinguished from other leukemia types based on the expression levels of these genes. The results are important for disease diagnosis and combination therapy based on SHP-1 and SHP-2 target genes.

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