RESEARCH ARTICLE

FHIT Gene Expression in Acute Lymphoblastic Leukemia and its Clinical Significance

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Abstract

Background: To investigate the expression of the fragile histidine triad (FHIT) gene in acute lymphoblastic leukemia and its clinical significance. Materials and Methods: The level of expressed FHIT mRNA in peripheral blood from 50 patients with acute lymphoblastic leukemia (ALL) and in 50 peripheral blood samples from healthy volunteers was measured via RT-PCR. Correlation analyses between FHIT gene expression and clinical characteristics (gender, age, white blood count, immunophenotype of acute lymphoblastic leukemia and percentage of blast cells) of the patients were performed. Results: The FHIT gene was expressed at 2.49±7.37 of ALL patients against 14.4±17.9 in the healthy volunteers. The difference in the expression levels between ALL patients and healthy volunteers was statistically significant. The rate of gene expression did not significantly vary with immunophenotype subtypes. Gene expression was also found to be correlated with increase of total leukocyte and decrease in platelets, but not with age, gender, immunophenotyping or percentage of blast cells. Conclusions: FHIT gene expression is low in acute lymphoblastic leukemia and could be a useful marker to monitor minimal residual disease. This gene is also a candidate target for the immunotherapy of acute lymphoblastic leukemia.

Keywords: FHIT gene - acute lymphoblastic leukemia - immunotherapy - tumor suppressor gene

Asian Pac J Cancer Prev, **16 (18)**, 8197-8201

Introduction

Acute lymphoblastic leukaemia (ALL) is a heterogeneous disease with distinct manifestations and prognostic and therapeutic implications (Mona et al., 2014). ALL in adults is a rare disease. The results of therapy remain unsatisfactory, and progress has been relatively slow (Marks, 2010). B-cell ALL (B-ALL) is a clonal malignant disease originated in a single cell and characterized by the accumulation of blast cells that are phenotypically reminiscent of normal stages of B-cell differentiation (Cobaleda C and Sanchez Garcia I, 2009) .Outcome of adult B-precursor ALL has considerably improved because of identification of clinical and genetic risk factors stratifying patients to different treatment groups (Gökbuget and Hoelzer, 2009). Commonly accepted risk factors in B-precursor ALL include age, performance status, white blood cell (WBC) count, lactate dehydrogenase concentration, the immunophenotype, response to induction therapy, level of minimal residual disease, cytogenetics and genetic aberrations (Gomes et al., 2014). Patients lacking clinical and molecular risk factors are considered standard risk (SR). Outcome for SR patients is still unsatisfactory (Gomes et al., 2014) indicating the clinical and biologic heterogeneity of these patients. Therefore, the identification of novel predictive molecular markers in adult B-precursor ALL may improve treatment stratification of this subgroup. The FHIT gene spans more than 1.6 Mb of genomic DNA and is composed of 10 exons. This gene, a member of the histidine triad gene family, encodes a diadenosine 5', 5'''-P1, P3-triphosphate hydrolase involved in purine metabolism. The FHIT gene located on chromosome 3p14.2 is a tumor suppressor gene that is deleted or inactivated in multiple human cancers. FHIT is believed to be a tumor suppressor gene, The FHIT gene at FRA3B is one of the earliest and most frequently altered genes in the majority of human cancers (Wang et al., 2014). The FHIT gene encodes a 1.1 kb mRNA which is expressed at low levels in most tissue types (ZHIWEI et al., 2015). Aberrant transcripts from this gene have been found in about half of all esophageal carcinomas, gastric carcinomas (Wang et al., 2014), and other carcinomas. Epigenetic changes contribute greatly to leukemia development (Wang et al., 2014). DNA methylation is a well studied mechanism in epigenetic. The hypermethylation of numerous genes has been detected in various types of tumors and hematological neoplasms (Wang et al., 2014). Previous studies have shown that DNA methylation is the most commonly detected alteration in ALL (Wang et al., 2014). Continuous gene expression monitoring during treatment could determine the effects of chemotherapy and whether leukemic cells are drug resistant until now, however, local investigations and data on FHIT gene are rare. Hence,

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this study investigated FHIT mRNA expression in acute lymphoblastic leukemia patients and its relationship with clinical data such as white blood cell count, bone marrow blast count and immunophenotype of leukemia. This study also presents theoretical values to monitor minimal residual disease (MRD) and the immunotherapy of leukemia.

Materials and Methods

Patients

Fifty ALL patients were enrolled between 2013 and 2015 admitted to Mansoura Oncology Center, Mansoura, Egypt. Specimens were selected from consecutive patients who had sufficient material available. They were 33 males (66%) and 17 females (34%) with mean age 38.40±13.44 years. Patients were diagnosed according to standard diagnostic methods including clinical, cytomorphological, cytochemical and immunophenotypic methods. In addition, 50 healthy subjects, of matched age 40.35±11.67 years) and sex (30 males (60%) and 20 females (40%); with normal laboratory findings; were selected as a control group. Informed consent was obtained from all patients.

Treatment protocol

ALL cases were treated according to our risk adapted chemotherapy protocol: The patients were stratified according to their prognostic factors into standard, high, and very high risk groups. The treatment plan included: Prephase for patients with high WBC and/or organomegaly. Induction phase I: Four drugs: Vincristine, Doxorubicin, L-Asparaginase and prednisone with intrathecal MTX. Patients that attained complete remission (CR) were subjected to cranial irradiation with 24 Gy and intrathecal MTX for four injections. Phase II induction with Cyclophosphamide and Cytarabine. Consolidation phase I: Vincristine, Doxorubicine and prednisone with Tripleintrathecal. Phase II consolidation: Cyclophosphamide, Cytarabine and Etoposide with triple intrathecal. Maintenance therapy: two years with 6 mercaptopurine and methotrexate. For patients with high and very high risks who were not planned to stem cell transplantation, one cycle of high dose Cytarabine and mitoxantrone (HAM regimen) was added between induction and consolidation. Bone marrow aspirate was done to evaluate response to chemotherapy (status post induction). Cases who died before treatment or who didn't receive treatment due to poor performance status or elderly cases kept on supportive treatment were excluded, as well as patients who received SCT in first CR.

Criteria of response and survival definitions

Complete remission (CR) was assessed after completion of induction chemotherapy. CR was defined as follows: granulocyte count of at least 1.5×10^9/L, platelet count of at least 100×10^9/L, no peripheral blood (PB) blasts, bone marrow (BM) cellularity of at least 20% with maturation of all cell lines and less than 5% blasts, and no extramedullary leukemia. Primary therapy failure (refractory disease) was defined as persistence of PB blasts or at least 25% blasts in BM after induction therapy. Relapse was defined as reappearance of PB blasts, more than 5% blasts in BM, or appearance of extramedullary manifestations after CR was achieved. OS was defined as the time from diagnosis to date of death. For patients achieving CR, DFS was the time from the date of first CR to an event (death in first CR or relapse).

Immunophenotypic analysis

Immunophenotypic analyses were performed by flow cytometry on fresh pretreatment BM and PB samples. A wide panel of monoclonal antibodies (Mo Abs) was used. Lymphoid markers included CD19, CD22 for B Lineage and CD1, CD2, CD3, CD4, CD5 and CD7, for exclusion of T-lineage and other markers used included HLADR,

CD10 and CD34. All the monoclonal antibodies were obtained from Coulter Hialeah, FL. A cell-surface antigen was considered positive when at least 20% of cells showed fluorescence intensity greater than the negative control.

Molecular analyses

Real-time quantitative polymerase chain reaction (RTQ-PCR) for FHIT gene: To assess molecular responses. From each patient and healthy subject 3 ml of PB or BM samples were collected in sterile EDTA vacutainers, total RNA was extracted from PB or BM blood cells. FHIT mRNA expression was normalized to the simultaneously analyzed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. The relative FHIT expression was determined using the comparative cycle threshold (CT) method. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene and FHIT were co amplified in the same tube using 1 μL cDNA, 1× master mix (IQ Mix; BioRad, Munich, Germany), GAPDH probe (VIC-5-CAAGCTTCCGTTCTCAGCC-3-TMRA) with GAPDH forward (5'-GAAGGTGAAGGTCGGAGTC-3') and reverse (5'-GAAGATGGTGATGGGATTTC-3') primers, and FHITprobe (5'-(FAM)-TGA TGA AGT GGC CGA TTT GTT- (TAMRA)-3') with FHIT forward (5'-TGTCGTTCAGATTTGGCCAAC-3') and reverse (5' - TCATAGATGCTGTCAT TCCTGT -3') primers. Reactions were performed using real-time PCR 7000 sequence detection system (Applied Biosystems, Foster City, USA). Positive and negative controls were included in all assays.FHIT and internal control transcript levels were quantified using real-time PCR analysis (TaqMan) on an ABI prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Specific PCR products were amplified and detected using dual-fluorescent non-extendable probes labeled with 6-carboxyfluorescein (FAM), reporter and 6-carboxytetramethylrhodamine (TAMRA), quencher at 5`-end and 3`-end, respectively. The relative mRNA expression of FHIT transcript was calculated using the comparative cycle threshold (Ct) method .

Statistical analysis

The statistical analysis of data was done by using excel program and SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 16. Qualitative data were presented as frequency and percentage. Chi square test was used to compare groups. Quantitative

Table 1. Clinical, Laboratory and Molecular Characteristics at Diagnosis According to FHIT Expression in ALL

	Total cases (n=50)	Low expression group (n=25)	High expression group (n=25)	P value
Age (years)	38.40±13.449	35.27 ±13.150	41.53 ±13.442	0.207
Sex				
Males	33 (66)	16 (64)	17 (68)	1
Females	17 (34)	9 (36)	8 (32)	
Fever	39 (78)	20 (80)	19 (76)	0.08
Fatigue	40 (80)	20 (80)	20 (80)	1
Weight loss	40 (80)	20 (80)	20 (80)	0.651
Pallor	37 (74)	20 (80)	17 (68)	0.682
Bleeding tendency	35 (70)	19 (76)	16 (64)	1
Splenomegaly	29 (58)	13 (52)	16 (64)	0.713
Hepatomegaly	27 (54)	17 (68)	10 (40)	0.143
Lymphadenopathy	29 (58)	10 (40)	9 (36)	0.00713**
Total leucocytic count (X109/L)	48.5(3.7-225)	56.000(5.1-225)	17.000(3.7-224)	0.0213*
Hemoglobin concentration (g/dL)	8.650(4.8-12.7)	9.000(4.8-12.7)	8.600(6.8-11)	0.406
Platelets (X109/L)	34.00(4-307)	29.00(15-307)	50.00(4-236)	0.0133*
Peripheral blasts (%)	45.50(16-89)	46.00(16)	45.00(21-54)	0.755
Bone marrow blasts (%)	56.00(23-90)	65.00(24-89)	55.00 (23-78)	0.755
LDH	931.50(234-4274)	1366 (234-3524)	797.00 (242-4274)	0.245
Immunophenotype subtypes			21(84)	1
B-ALL	42 (84)	20 (80)	4(16)	
T-ALL	8 (16)	5 (20)		

data were presented as mean and standard deviation. For comparison between two groups; student t-test and Mann-whitney test (for non-parametric data) were used. Kaplan–Meier test was used for survival analysis and the statistical significance of differences among curves was determined by Log-Rank test. Prediction of survival was done using multivariate analysis. For gene expression quantification, we used the comparative Ct method. First, gene expression levels for each sample were normalized to the expression level of the housekeeping gene encoding GAPDH within a given sample (ΔCt). Results were evaluated by using 2-ΔΔCT method as relative gene expression values. N.B: p is significant if ≤ 0.05 at confidence interval 95%.

Results

FHIT expression and relationship with clinical, laboratory and molecular features

ALL Patients were divided into 2 FHIT expression groups; high FHIT expression (above median) and low FHIT expression (below median). The clinicohematological features differed in patients with high FHIT and those with low FHIT mRNA expression levels. Patients expressing low FHIT gene had higher WBC (P =0.0213) and low platelets (p=0.0133) , Lymphadenopathy(0.00786) compared with patients with high FHIT expression group. There was no significant association between FHIT expression level and sex, hemoglobin concentration, peripheral or marrow blasts, or immunophenotypic subgroups of ALL. No other association was seen between FHIT expression and clinical features (Table 1).

Outcome in ALL patients with respect to FHIT expression

Complete remission rate did differ significantly between high and low FHIT expression groups (p=0.0439), although a higher incidence of primary resistant disease (16% versus 28% respectively; p=0.0151). No influence on the relapse rate (P =1) was observed with respect to

Table 2. Clinical Outcome According to FHIT Expression in ALL

Response	ALL cases (n=50) No.	Low FHIT expression (n=25)	High FHIT expression (n=25)	p value
CR	29 (58)	15 (60)	18(72)	0.0439*
Refractory	14 (28)	7 (28)	4 (16)	0.0151*
Relapse	7 (14)	4 (16)	5 (20)	1
Total death	25 (50)	15 (60)	7 (28)	0.065

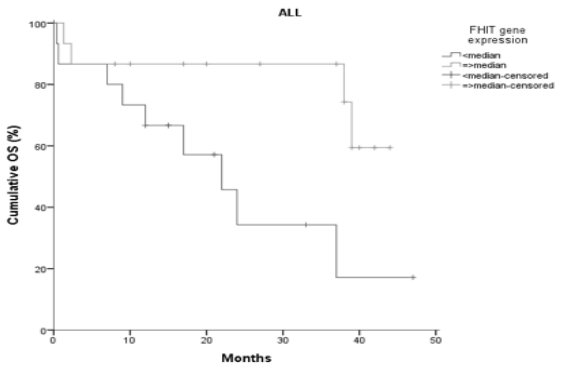

Figure 1. OS of ALL Patients According to FHIT Gene Expression

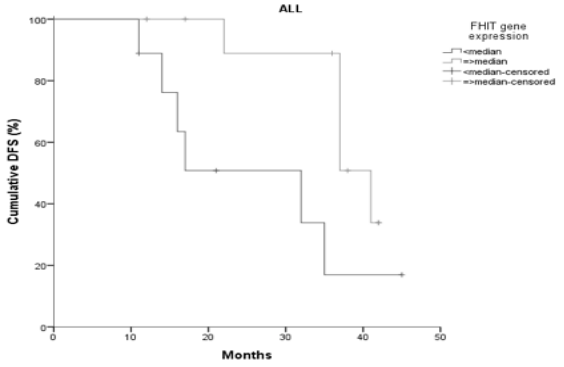

Figure 2. DFS of ALL Patients according to FHIT Gene Expression

Table 3. Multivariate Analysis for DFS and OS as Dependent Parameters Studied with Other Covariates in All Studied cases

Covariates	OS				DFS			
	P	HR	95% CI for HR		P	HR	95% CI for HR	
Age	0.194	0.963	0.921	1.006	0.931	1.002	0.967	1.037
BM blasts	0.574	1.095	0.976	1.093	0. 821	0.347	0.095	1.272
FHIT	0.025*	0.241	0.07	0.834	0.028*	0.22	0.057	0.846

FHIT expression. There were no significant differences regarding deaths in induction therapy (P =0.065) between the two FHIT expression groups. Total mortality rates during the entire period of the study were higher in high FHIT expression group when compared to low expression group, but did not reach significant level (P =0.063) (Table 2).

When applying FHIT expression, age, marrow blasts and immunophenotypes as covariates for prediction of resistant disease, FHIT remained a predictive factor for primary therapy resistance (P = 0.025; HR= 0.241; 95% confidence interval [CI], (0.070- 0.834) (Table 3).

Overall survival (OS) was significantly shorter in patients with Low FHIT expression compared with those with high FHIT expression (23.21versus 36.88 months; 4-year cumulative OS %, 57.1%, 86.7% respectively; p=0.017). In addition, low FHIT expression group showed no statically significantly shorter DFS than those with high expression (32.311versus 40.571 months; 71.1 % versus 100 % respectively; p=0.065) (Figure 1). FHIT expression was independently predictive for OS in multivariate analysis (p=0.028, HR=0.220, 95%CI=0.057-0.846).

Discussion

Acute lymphoblastic leukemia (ALL) remains one of the most challenging adult malignancies, especially with respect to therapy. Immunophenotyping, cytogenetic-molecular studies (Mona et al., 2014). However, most of the studies focused on children and therefore a deep molecular characterization of adults is still challenging. Epigenetic changes are of great importance to leukemia development (CHEN et al., 2013). In this study, we have evaluated the prognostic significance of FHIT expression in Adult ALL. In the present study, the mRNA expression of FHIT was analyzed in ALL. As in other tumors, a reduced expression of FHIT was observed in ALL. The present study showed that the expression level of FHIT was altered concordantly in ALL, and that the frequency of FHIT expression. In previous studies, the reported frequency of FHIT alterations in ALL has varied; the expression of FHIT mRNA or protein has been reported to be altered in 20 70% of cases (CHEN et al., 2013; Hallas et al., 1999).

In this study lower FHIT expression was associated with higher WBC, this agree with Shichun Zheng study's (Shichun et al., 2004) who reported that significantly higher WBC counts were associated with FHIT methylation correlates with strongly reduced FHIT mRNA and FHIT protein expression. In this study lower FHIT expression was associated with lymphadenopathy and low level of platelets concentration. This agree with Hagop M. Kantarjian study's (Hagop M. Kantarjian et al.,1999) who decided that Patients with a reduced cellular Fhit expression tended to have a higher incidence of leucocytosis (P = 0.04) and a lower incidence of thrombocytosis (P < 0.01) (Hagop et al., 1999). However, no significant differences were found between low and high FHIT expression groups regarding clinical presentations, sex, hemoglobin concentration, peripheral or marrow blasts, or immunophenotypic subgroups of ALL. This agree with Hagop M. Kantarjian study's (Hagop et al., 1999) who showed that No differences between FHIT-negative and FHIT-positive patient group was observed with respect to gender (male versus female, P = 0.563), age (mean, 63.3 versus 62.2 years; P = 0.447) The present study demonstrated that a relatively low expression of FHIT correlates with CR and RP diagnosis statuses in ALL patients, supporting the hypothesis that the occurrence of ALL is a progressive and multi-staged process, similar to that of other tumors. This is agreeing with Chan X stud's (CHEN et al., 2013). Patients with high gene expression continued CR at a rate significantly higher than those with low FHIT gene expression. Low FHIT gene expression group confer higher resistance to chemotherapy than those with high FHIT gene expression in agreement with Chan X stud's (CHEN et al., 2013). In addition, low FHIT expression was independently predictive for DFS and OS all patients'. In previous studies, low FHIT expression were associated with an unfavorable outcome and inferior long-term survival in adult CN-AML and T-ALL (Alison Walker and Guido Marcucci, 2012) . Thus, FHIT characterizes a more aggressive, immature, highly proliferative, and chemoresistant leukemic phenotype. We have found a significant worse prognosis in patients whose tumors showed an altered FHIT expression this agree with Gemma Toledo study's (Toledo et al.,2004) who decided that the 5-year survival rate of patient with tumors with adequate FHIT expression was significantly higher (85.5%) than in tumors FHIT- (64.7%) (P=0.039).

Our data suggest that FHIT may identify patients with an immature, chemoresistant leukemic phenotype associated with an unfavorable outcome and shorter survival of adult ALL. FHIT expression could better discriminate patients into various prognostic groups and identify patients who might benefit from dose intensified induction chemotherapy. Stratification of adult ALL into 2 distinctive groups of patients with outcome and survival characteristics might in the future facilitate treatment stratification for adult ALL. These data, together with frequent alterations of the FHIT expression in acute lymphoblastic leukemia, suggest its role in the pathogenesis of acute lymphoblastic leukemia.

In summary, the FHIT gene potentially plays a vital

role in the carcinogenesis and development of acute lymphoblastic leukemia. One possible avenue that FHIT inactivation operates through in acute lymphoblastic leukemia progression .Thus, FHIT expression could serve as a useful biomarker in evaluating the biological behavior of FHIT and have clinical utility in devising innovative treatment strategies. A better understanding of FHIT promoter methylation and phenotypic expression will provide new insights into acute lymphoblastic leukemia carcinogenesis, cancer treatment, and feasible chemo preventive measures for the future.

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