RESEARCH ARTICLE

TET2 Promoter DNA Methylation and Expression in Childhood Acute Lymphoblastic Leukemia

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Abstract

The ten-eleven-translocation-2 (TET2) gene is a novel tumor suppressor gene involved in several hematological malignancies of myeloid and lymphoid origin. Besides loss-of-function mutations and deletions, hypermethylation of the CpG island at the TET2 promoter has been found in human cancers. The TET2 encoded protein regulates DNA methylation. The present study aimed to examine DNA promoter methylation of TET2 in 100 childhood acute lymphoblastic leukemia (ALL) cases and 120 healthy children in southeast Iran. In addition, mRNA expression levels were assessed in 30 new cases of ALL and 32 controls. Our ndings indicated that promoter methylation of TET2 signi cantly increases the risk of ALL (OR=2.60, 95% CI=1.31-5.12, p=0.0060) in comparison with absent methylation. Furthermore, the TET2 gene was signi cantly downregulated in childhood ALL compared to healthy children (p=0.0235). The results revealed that hypermethylation and downregulation of TET2 gene may play a role in predisposition to childhood ALL. Further studies with larger sample sizes and different ethnicities are needed to con rm our ndings.

Keywords: TET2 - methylation - gene expression - acute lymphoblastic leukemia - Iran

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Introduction

Acute lymphoblastic leukemia (ALL) is the most commonly diagnosed childhood malignancy. It accounts for approximately 25% of cancers in children younger than 15 years of age, and the peak onset occurs at 2-5 years of age (Belson et al., 2007; Eden et al., 2010; Kaatsch, 2010). While the etiology of ALL is generally unknown, it has been proposed that multiple gene alterations and chromosomal rearrangements as well as interplay of multiple environmental and inherited factor plays a critical role in disease development (Belson et al., 2007; Guo et al., 2014; Ma et al., 2014).

Gene methylation is an alternative mechanism of gene inactivation, and numerous tumour suppressor genes (TSG) regulating the cell cycle, apoptosis and cell signaling have been shown to be hypermethylated in hematological malignancies (Chim et al., 2002). The ten-eleven-translocation-2 (TET2) gene belongs to a family of three members (TET1, TET2, and TET3) was originally recognized as a partner for the MLL gene within t(10;11)(p12;q23) translocations in acute myeloid leukemia (AML) (Lorsbach et al., 2003). They encoding for proteins that has a catalytic dioxygenase activity with a putative function in the DNA demethylation process which catalyzes the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-HmC) (Tahiliani et al., 2009), which could epigenetically regulate gene expression by altering methylation-driven gene silencing. TET2 act as a TSG by regulating DNA methylation and epigenetic control of gene expression (Ito et al., 2010).

TET2 gene mutations have been reported as an important event in the pathogenesis of myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), AML and chronic myelomonocytic leukemia (CMML) (Delhommeau et al., 2009; Kosmider et al., 2009a; Kosmider et al., 2009b; Tefferi et al., 2009; Abdel et al., 2010; Nibourel et al., 2010; Makishima et al., 2011; Perez et al., 2012).

Down regulation expression of TET2 triggers increase in number of hematopoietic stem cells (HSCs), enhances their self-renewal capacity in vitro and confers competitive advantage over wild-type HSCs in vivo. The altered differentiation of hematopoietic cells is predominantly skewed toward myeloid lineage, however inactivation of TET2 in mouse models affects both myeloid and lymphoid differentiation (Quivoron et al., 2011).

Hypermethylation of the CpG island in the promoter of TET2 gene was found in 4.4% of patients with Ph-negative MPN (Chim et al., 2010). In contrast, other studies did not recognize hypermethylation at TET2 promoter in MPN, MDS, CMML nor AML (Jankowska et al., 2009;

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Abdel-Wahab et al., 2010; Musialik et al., 2014). So, we proposed that TET2 hypermethylation might happen in childhood ALL. In the present study we evaluated the DNA methylation as well as mRNA expression level of TET2 gene in childhood ALL.

Materials and Methods

Patients

For DNA methylation, a case-control study was done on 100 children diagnosed with ALL and 120 age and sex matched healthy children in Zahedan, southeast Iran. The study design and enrolment procedure have been described previously (Hasani et al., 2014; Hashemi et al., 2014a; Hashemi et al., 2014b). For mRNA expression of TET2 we enrolled 30 new cases of childhood ALL and 32 healthy age and sex matched children.

The project was approved by local ethics committee of Zahedan University of Medical sciences approved the project, and informed consent was obtained from parents of cases and controls.

Promoter methylation

Promoter methylation status was assessed using methylation specific (MSP) technique. Blood samples were collected in EDTA-containing tubes and genomic DNA was extracted from peripheral blood using salting out method as described previously (Hashemi et al., 2010). The CPGenomeTM Direct Prep Bisulfite Modification Kit (Millipor, Germany) was used for modification of genomic DNA with sodium bisulfate according to the manufacturer's recommendation. Modified DNA was stored at -80 °C until it was used for MSP.

We established a nested methylation-specific polymerase chain reaction (MSP) for assessment promoter methylation of TET2. The primers are shown in table 1. For nested PCR, in each 0.20 ml PCR reaction tube, 1 µl bisulfite-converted DNA, 1 µl of each primer (10 µM) and 17 µl ddH2O were added to each tube of HotStart PCR PreMix (AccuPower, Bionner, South Korea). The PCR conditions were set as follows: 95ºC for 5 min, 30 cycles of 95ºC for 30s, 56ºC for 30 s, and 72ºC for 30 s and a final extension step of 72ºC for 5 min. The PCR product (596-bp) was used as a template for MSP assay. MSP was performed using two pairs of specific primers; one pair for methylated and the other for unmethylated TET2 promoter sequence (table 1). In each 0.20 ml PCR reaction tube, 0.5 µL nested PCR product, 1 µl of each primer (10 µM) and 10 µl of 2X Prime Taq Premix (Genet Bio, Korea) and 7 µl ddH2O were added. MSP was subjected to an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 30 s at 95 °C, annealing at 56°C for 30 s, and extension at 72°C for 30 s with a final extension at 72°C for 5 min. The PCR product electrophoresed onto 2.5% agarose gels containing 0.5 μg/ml ethidium bromide and observed under UV light. The amplicon size for methylated and unmethylated were 211-bp (Figure).

TET2 expression

Total mRNA was extracted from whole blood using commercial available kit (Thermo Scientific GeneJET RNA Purification Kit) and cDNA synthesis was done using AccuPower CycleScript RT PreMix Kit (BIONNER, Cat. No. K-2046) in a final volume of 20 µL.

We quantified the mRNA expression of a target gene (TET2) and an internal control gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by real-time PCR using the SYBR Green 2X RealQ Plus master mix (Amplicon; Bio, Korea) on the ABI quantitative Real-Time PCR system (Applied Biosystems). The primers are shown in table 2.

In each 0.20 ml PCR reaction tube, 1 μl of cDNA, 1 μl of each primers and 10 μl 2X RealQ Plus Master Mix Green High Rox (Amplicon Bio, Korea) and 7 μl ddH2O were added. The PCR conditions were set as follows: 95˚C for 6 min, 40 cycles of 95˚C for 40 s, 63˚C for 40 s, and 72˚C for 35 s and a final extension step of 72˚C for 10 min. Results are presented as expression fold change of patients respective to controls according to the 2-ΔΔCt method.

Statistical analysis

We used SPSS 20 software to do statistical analysis. Data were analyzed using independent sample t-test for continuous data and Fischer exact test or χ^2 test for categorical data. We computing the odd ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analysis to obtain the association between methylation status and ALL. A p-value less than 0.05 were considered to be statistically significant.

Results

Our investigation involved 100 childhoods ALL (54 male, 46 female; age: 5.5 ± 3.6 years) and 120 healthy children (58 male, 62 female; age: 5.6 ± 2.9 years). There was not any significant between the groups concerning sex and age (p=0.419 and 0.761, respectively).

Promoter DNA methylation of TET2 gene in ALL and controls was shown in table 2. The findings revealed that 14.0% of the cases were unmethylation, 70.0% were partial methylation and 16.0% were methylation. While in controls, the frequency distribution of unmethylation, partial methylation and methylation were 30.0%, 59.2%, and 10.8%, respectively. The frequency distribution of methylation status was statistically significant different between the groups (χ^2=8.25, p=0.0162). The partial methylation as well as methylation (Partial+Present) significantly increased the risk of ALL in comparison

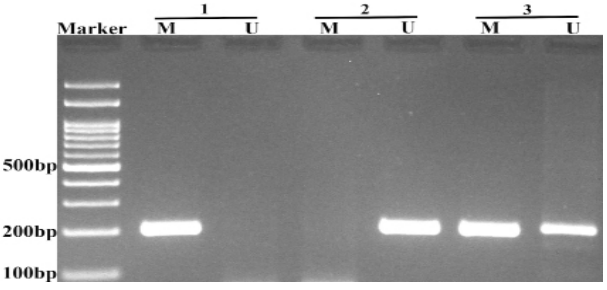

Figure 1. TET2 Methylation Assessed by the MSP-PCR Method. M: DNA marker; Lane 1, MM; Lane 2, UU; Lane 3, MU

Table 1. Promoter DNA Methylation Primers Used

Gene	Primers (5`->3`)	Amplicon size (bp)
Nested	F: GTGAGGGTAGTTTAGGTTTTTATTG	596
	R: ACTACAAAATTTACTCCCCAATCC	
TET2 M	F: CGGAGCGGGAGGAGGTCGGGGC	211
	R: GTCTATTCTCATCACTCAACGAAACCG	
TET2 U	F: TGGAGTGGGAGGAGGTTGGGGT	211
	R: ATCTATTCTCATCACTCAACAAAACCA	
TET2 qRT-CR	F: ATACCCTGTATGAAGGGAAGCC	197
	R: CTTACCCCGAAGTTACGTCTTTC	
GAPDH qRT-CR	F: GAAGGTGAAGGTCGGAGTC	226
	R: GAAGATGGTGATGGGATTTC	

Table 2. Promoter DNA Methylation of the TET2 Gene in ALL and Controls

Methylation status	Cases n (%)	Controls n (%)	OR (95%CI)	P
Absent	14 (14.0)	36 (30.0)	1	-
Partial	70 (70.0)	71 (59.2)	2.54 (1.26-5.11)	0.0084
Present	16 (16.0)	13 (10.8)	2.09 (0.81-5.44)	0.1466
Partial+Present	86 (86.0)	85 (70.0)	2.60 (1.31-5.12	0.006

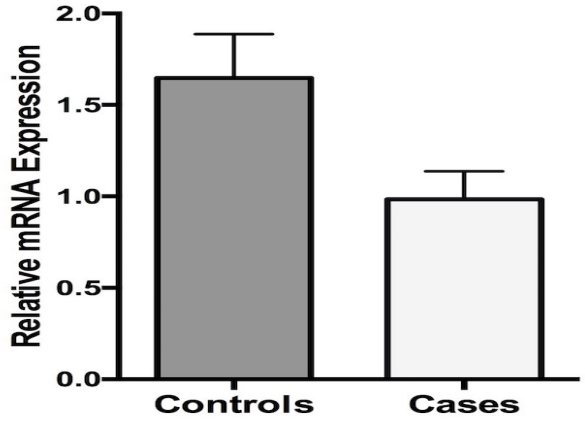

Figure 2. Relative mRNA Expression of TET2 in Childhood ALL and Controls. The mRNA expression level of TET2 was significantly lower in ALL than that of controls (p=0.0235). Statistical analysis was done by Unpaired t-test with Welch's correction

with absent methylation (OR=2.54, 95% CI=1.26-5.11, p=0.0084, and OR=2.60, 95% CI=1.31-5.12, p=0.0060, respectively).

The mRNA expression level of TET2 in cases and controls is shown in figure 2. The finding revealed that TET2 gene was significantly down-regulated in ALL (0.9845 ± 0.1528) compared to healthy children (1.648 ± 0.2397) (p=0.024).

Discussion

Acute lymphoblastic leukemia (ALL) is a multifactorial disease influenced by genetic and environmental factors. DNA methylation at the C5 position of cytosine (5-methylcytosine, 5-mC), documented as key epigenetic modification at CpG dinucleotides, playing critical roles in normal development as well as tumorigenesis (Jones and Baylin, 2007). TET proteins play a critical roles in epigenetic tumorigenesis by converting 5-methylcytosine to 5-hydroxymethylcytosine as an intermediate of active DNA demethylation (Li et al., 2016).

In the present study we investigated the promoter methylation and mRNA expression level of TET2 gene in childhood ALL risk in sample of Iranian population. The findings revealed that that hypermethylation and downregulation of TET2 gene may play a role in predisposition to childhood ALL. The results indicated that methylation status of TET2 gene was significantly higher in ALL than that of healthy children. Furthermore, we found that TET2 gene was significantly down-regulated in ALL compared to healthy children. These findings propose that hypermethylation and downregulation of TET2 gene may play a role in predisposition to childhood ALL.

Though CpG island hypermethylation of tumor suppressor genes has been reported in many human cancers, there are interindividual variations in the susceptibility to methylation (Kang et al., 2008). It has been shown that TET2 expression is downregulated in myelodysplastic syndromes (MDS) and predicts survival outcomes in this disease (Scopim-Ribeiro et al., 2015).

Hypermethylation of the CpG island in the promoter of TET2 gene was found in 4.4% of patients with Ph-negative MPN (Chim et al., 2002). In contrast, other studies did not recognize hypermethylation at TET2 promoter in MPN, MDS, CMML nor AML (Jankowska et al., 2009; Abdel-Wahab et al., 2010).

Aberrant TET2 methylation was previously found in MPN and glioma patients (Chim et al., 2010; Kim et al., 2011). Musialik et al (Musialik et al., 2014) have found no correlation between promoter methylation and mRNA gene expression, however the level of TET2 expression in ALL group was significantly decreased compared to children's normal peripheral blood mononuclear cells and isolated B-cells. They reported that TET2 expression was higher in isolated normal B-cells compared to normal PBMC.

Gene expression influenced by promoter hypermethylation as well as other mechanisms of gene inactivation such as deletion and intragenic mutations. It has been proposed that promoter CpG methylation is an important regulatory process of gene expression.

In summary, the findings of the current study proposed that hypermethylation and downregulation of TET2 gene may play a role in childhood ALL susceptibility. Further studies with large number of samples and different ethnicities are necessary to confirm our findings.

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