RESEARCH ARTICLE

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Fas-Antisense Long Noncoding RNA and Acute Myeloid Leukemia: Is There any Relation?

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Abstract

In recent years, lncRNAs have been considered as potential predictive biomarkers for prognosis of different human cancers. One example is the FAS antisense RNA 1 (FAS-AS1) located in the 10q23.31 region which is transcribed from the opposite strand of the FAS gene. FAS has an important role in regulation of apoptotic pathways and there is an inverse correlation between FAS-AS1 expression level and production of the soluble form of Fas, so that it might have potential as a therapeutic target to improve chemotherapy effectiveness. In the present study we therefore evaluated FAS-AS1 expression in blood samples of de novo AML patients and healthy controls using real-time quantitative reverse transcription-PCR (qRT-PCR). Our results indicated that the expression level of FAS-AS1 lncRNA demonstrated no significant difference between AML patients and healthy individuals. We conclude from the obtained data that FAS-AS1 is not an informative and reliable biomarker for AML diagnosis, although our results need to be confirmed in further studies.

Keywords: Acute myeloid leukemia- long non- coding RNA- FAS- AS1

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Introduction

Acute Myeloid Leukemia (AML: # 601626) is the most common leukemia in adults with a high incidence and mortality rate especially in elderly (Deschler and Lübbert, 2006). AML as an aggressive malignancy is a consequence of aberrant mechanisms of cell cycle that results in the malignant hematopoietic stem cell (HSC) proliferation, differentiation, and accumulation in the bone marrow and blood of patients (Estey and Döhner, 2006). Different aspects of cell cycle process is deregulated in AML patients, however the exact underlying mechanism of the disease is still unclear and different studies revealed that exposure to environmental causes and also the genetic background of the patients are both involved in the etiology of the disease (Buffler et al., 2005). Especially, deregulated expression of genes involved in the highly ordered mechanisms of HSCs lineage commitment is an important etiologic defects that lead to malignant proliferation and accumulation of immature myeloid progenitors (Skalnik, 2002).

As genome wide association (GWA) studies have noted various immune-related LncRNAs in other cancers (Sayad et al., 2017a; Taheri et al., 2017; Sayad et al., 2017b). Recently, a new described relative risk factor for different human cancers are Long non-coding RNAs (lncRNAs) which are important regulators of several biological mechanisms by controlling the target genes expression at the transcriptional or translational level (Geisler and Coller, 2013; Kurokawa, 2011). LncRNAs are introduced to be a pivotal regulatory factor during developmental steps of hematopoiesis (Venkatraman et al., 2013; Aoki et al., 2010; Hu et al., 2011). Among them FAS antisense RNA 1 also known as FAS-AS1 or SAF is a long noncoding RNA located at the 10q23.31 region and is transcribed from the opposite strand of intron 1 of the FAS gene (Yan et al., 2005). As a member of the tumor necrosis factor receptor superfamily the FAS gene is involved in the regulation of apoptotic pathways (Nagata, 1994; Xerri et al., 1998). This receptor is expressed by normal tissue cells such as T and B-cells as well as different tumor cells. Defects in Fas-mediated apoptosis are reported in autoimmune diseases and lympho-proliferative disorders such as AML (Thompson, 1995; Lowe and Lin, 2000; Rudin and Thompson, 1997; Ettou et al., 2012; Hara et al., 2000) and also its increased soluble level is shown to be associated with poor therapeutic outcomes and resistance to chemotherapy (Niitsu et al., 1999). On the other hand, previous studies suggested that there is an inverse correlation between FAS-AS1 level and production of soluble form of FAS that indicated a potential therapeutic targets to improve the chemotherapy effectiveness in B-cell lymphoma (Sehgal et al., 2014).

Regarding to these evidence in the current study, in

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Table1. The Sequences of Probes and Primers That Used in the Real-Time Quantitative RT-PCR for Fas-AS1 lncRNA and HPRT1 Gene

property	HPRT1	FAS-AS1
Forward primer	AGCCTAAGATGAGAGTTC	GAAAAGGTGCCGTTCTTCCG
Reverse primer	CACAGAACTAGAACATTGATA	CTGGCAGTTCTCAGACGTAGG
Probe	FAM-CATCTGGAGTCCTATTGACATCGC-TAMRA	FAM-CGGCTTAACCACTGCTTCGGTGCT-TAMRA

order to find a possible predictive biomarker for AML, it was investigated whether there is a correlation between the expression level of FAS-AS1 lncRNA and AML disease.

Materials and Methods

Participants

The present study is done as a case-control analysis which include 25 unrelated AML patients consist of 15 (60%) male and 10 (40%) female and 50 healthy matched controls. All the AML patients have clinically defined diagnosis according to the FAB classification criteria (Bennett et al., 1985). They were recruited from Medical Oncology department of Besat Hospital, Hamadan. As the inclusion criteria, the control individuals were included in the study if they were totally healthy without any cancer or other disease like genetic syndromes or metabolic disorders.

Blood sampling

From each participant 5 ml, peripheral blood was taken in an EDTA tube. Written consent forms were received from all individuals and their complete personal and familial history were obtained. The study was approved by a local Ethical Committee of Hamedan University of Medical Sciences.

Real-time quantitative RT-PCR

The General Hybrid-RTM blood RNA extraction Kit (cat No. 305-101) was used to extract total RNA from blood samples. Applied Biosystems High-Capacity cDNA Reverse Transcription Kits (PN: 4375575) was conducted to synthesize the single strand cDNA according to the manufacturer's instructions. Allele ID 7 (Premier Biosoft, Palo Alto, USA) was applied to design the specific probes and primers. In order to normalize the gene expression level of each sample the expression level of HPRT1 gene was considered as a housekeeping gene. The sequence of probes and primer pairs has been demonstrated in Table 1. Real-time quantitative PCR was carry out in triplicates by using Applied Biosystems TaqMan R Universal PCR Master Mix (PN: 4304449). Corbett Rotor-gene 6,000 machine (Corbett Life Science) was used to run the reactions. The negative control sample was used without cDNA sample as quality control.

Statistical methods

The independent samples t-test was used to analyze the obtained data to examine the differences between both groups. Also, the one-way ANOVA test and Pearson correlation coefficient were used respectively to evaluate the case of a higher number of groups and the correlation between variables. The P value< 0.05 was considered as significant statistically. Analysis was performed using SPSS 18 windows statistical package (Chicago, IL, USA).

Results

Demographic and clinical features

For all the samples clinical information such as age of participants, age of onset and complete blood count (CBC) are described in Table 2. Also, the AML patients and healthy controls were classified into 2 different groups based on their gender.

FAS-AS1 Expression compared the de novo AML patients with healthy controls

The expression level of FAS-AS1 lncRNA was compared among AML patients and healthy controls and the results are demonstrated in Table 3. Totally, no significant statistical difference were found between patients and healthy controls (p-value = 0.7, 95% CI = 0.157-69). We also analyzed the obtained data in two different groups between male and female separately to find any possible correlation among gender, the expression level of FAS-AS1 and susceptibility to AML. The data shown that either there is no significant difference between two groups (P > 0.05).

Discussion

Recently, long non-coding RNAs have been highly regarded as a new regulatory factor for controlling

Table 2. Demographic and Clinical Datad of AML Patients and Healthy Controls

Variables	AML patient	Healthy Control
Female/Male (no. (%))	10(40%)/15(60%)	21(42%)/29(58%)
Age (mean ± SD, Y)	35.1± 3.2	34.9± 3.1
Age range (Y)	22-55	20-58
Age of onset (mean ± SD, Y)	34.8± 4.2	-
WBC (mean ± SD, $\times 10^3$)	47± 3.3	6± 2.4
WBC range (×103)	18-130	04-Jul
Platelet (mean ± SD, $\times 10^3$)	64± 4.9	220± 1.9
Platelet range ($\times 10^3$)	40-250	160-400
Hemoglobin (mean ± SD, g/dl)	8± 3.1	14± 2.3
Hemoglobin range ($\times 10^3$, g/dl)	04-Nov	Dec-18
FAB classification: (no. (%))	25(100%)	
M0	2(8%)	
M1	3(12%)	
M2	6(24%)	
M3	2(8%)	
M4	9(36%)	
M5	3(12%)	

Table 3. Fas-AS1 Expression in Comparing of de Novo AML Patients and Healthy Controls

Fas-AS1 expression	Control no.	AML patient no.	p-value	Expression ratio	Std. Error	95% CI
Total	50	25	0.7	1.201	0.213-19.4	0.157-69
Male	29	15	0.1	1.286	0.29-16.71	0.198-52
Female	21	10	0.8	1.187	0.26- 18.241	0.213- 57

the expression of their neighboring genes and their consequent roles in various human diseases, especially cancer. In this regard, several recent studies have been reported the relation between aberrant expression of lncRNAs and deregulation of different stages of highly ordered mechanisms of hematopoietic stem cells (HSCs) commitment and developmental lineage (Venkatraman et al., 2013; Hu et al., 2011; Guo et al., 2015).

Among them the FAS-AS1 is an antisense lncRNA that transcribed from the FAS gene locus at chromosome 10 and is involved in a lncRNA-regulated mechanism of cell death program during human erythropoiesis. FAS-AS1 is reported to inversely influence the production of soluble form of FAS receptor (sFAS), which binds to Fas ligand as an anti-apoptotic protein and regulate the induction of Fas-mediated apoptosis in B cell lymphomas (Sehgal et al., 2014). also an increased expression of Saf lncRNA is observed during human erythroid maturation that is related to reduction of surface expression of Fas receptor and is important for terminal erythroid differentiation to maintenance the RBC production (Villamizar et al., 2016).

This evidence suggested that increased expression of FAS-AS1 may be a potential therapeutic target for lymphoma by increasing the sensitivity of lymphoma cells to induced apoptosis by chemotherapy agents and reduce the chemo resistance of patients. On the other hand, the expression level of the FAS-AS1 lncRNA may also considered as a predictive biomarker to estimate the chemotherapy responsiveness.

In this regards at the present study we investigate the expression level of the FAS-AS1 lncRNA in AML patients in comparison to healthy control group. The results shown that the expression level of the FAS-AS1 lncRNA was not significantly different in the blood sample of AML patients versus controls. In addition, exploring the correlation of the expression of this gene with patients considering the two sex group also shown no significant difference between male and female.

Our result is in consistent with previous study which analyzed the expression level of the FAS-as1 is multiple myeloid (MM) patients as the second common hematological malignancy and reported no significant difference in the expression of the gene between the MM patients and healthy controls (Sedlarikova et al., 2016). But our data is in contrast with the result of another study that reported a significant decrease in the expression level of the FAS-AS1 in breast cancer tissue compared to adjacent breast tissue and normal breast cells (Zhou et al., 2016). The obtained data of the current study revealed that the FAS-AS1 expression level may not considered as an adequate predictive biomarker for the diagnosis of AML but our results may be influenced by the limitation of the sample size and it suggested that the results be confirmed in future studies.

In conclusion, overall, our data revealed that the FAS-AS1 mRNA expression level has no significant difference between AML patients and healthy controls. However, due to the importance of FAS-AS1 expression level as potential therapeutic target to increase the effectiveness of chemotherapy, further analyses are needed to confirm the real correlation between FAS-AS1 expression and AML.

Conflict of interest

None.

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References

Aoki K, Harashima A, Sano M, et al (2010). A thymus-specific noncoding RNA, Thy-ncR1, is a cytoplasmic riboregulator of MFAP4 mRNA in immature T-cell lines. *BMC Mol Biol*, **11**, 99.

Bennett JM, Catovsky D, Daniel MT, et al (1985). Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Int Med*, **103**, 620-5.

Buffler PA, Kwan ML, Reynolds P, Urayama KY (2005). Environmental and genetic risk factors for childhood leukemia: appraising the evidence. *Cancer Invest*, **23**, 60-75.

Deschler B, Lübbert M (2006). Acute myeloid leukemia: epidemiology and etiology. *Cancer*, **107**, 2099-2107.

Estey E, Döhner H (2006). Acute myeloid leukaemia. *Lancet*, **368**, 1894-1907.

Ettou S, Audureau E, Humbrecht C, et al (2012). Fas expression at diagnosis as a biomarker of azacitidine activity in high-risk MDS and secondary AML. *Leukemia*, **26**, 2297.

Geisler S, Coller J (2013). RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. *Nat Rev Mol Cell Biol*, **14**, 699-712.

Guo G, Kang Q, Zhu X, et al (2015). A long noncoding RNA critically regulates Bcr-Abl-mediated cellular transformation by acting as a competitive endogenous RNA. *Oncogene*, **34**, 1768-79.

Hara T., Tsurumi H, Takemura M, et al (2000). Serum-soluble fas level determines clinical symptoms and outcome of patients with aggressive non-Hodgkin's lymphoma. *Am J Hematol*, **64**, 257-61.

Hu W, Yuan B, Flygare J, Lodish HF (2011). Long noncoding RNA-mediated anti-apoptotic activity in murine erythroid terminal differentiation. *Genes Dev*, **25**, 2573-8.

Kurokawa R (2011). Long noncoding RNA as a regulator for transcription. *Prog Mol Subcell Biol*, **51**, 29-41.

Lowe SW, Lin AW (2000). Apoptosis in cancer. *Carcinogenesis*, **21**, 485-95.

Nagata S (1994). Fas and Fas ligand: a death factor and its receptor. *Adv Immunol*, **57**, 129-44.

Niitsu N, Sasaki K, Umeda M (1999). A high serum soluble Fas/APO-1 level is associated with a poor outcome of aggressive non-Hodgkin's lymphoma. *Leukemia*, **13**, 08876924.

Rudin CM, Thompson CB (1997). Apoptosis and disease: regulation and clinical relevance of programmed cell death. *Annu Rev Med*, **48**, 267-81.

Sayad A, Hajifathali A, Hamideh AA, Roshandel E, Taheri M (2017a). HOTAIR long noncoding RNA is not a biomarker for acute myeloid leukemia (AML) in Iranian patients. *Asian Pac J Cancer Prev*, **18**, 1581-4.

Sayad A, Hajifathali A, Taheri M (2017b). Polymorphisms in lncRNA HOTAIR and susceptibility to acute myeloid leukemia in Iranian patients. *J Biol Today's World*, **6**, 33-7.

Sedlarikova L, Gromesova B, Filipova J, et al (2016). Deregulated expression of long non-coding RNAs UCA1, NEAT1 and BDNF-As in multiple myeloma. *Blood,* **128**, 2073.

Sehgal L, Mathur R, Braun FK, et al (2014). FAS-antisense 1 lncRNA and production of soluble versus membrane Fas in B-cell lymphoma. *Leukemia*, **28**, 2376-87.

Skalnik DG (2002). Transcriptional mechanisms regulating myeloid-specific genes. *Gene*, **284**, 1-21.

Taheri M, Habibi M, Noroozi R (2017). HOTAIR genetic variants are associated with prostate cancer and benign prostate hyperplasia in an Iranian population. *Gene*, **613**, 20-4.

Thompson CB (1995). Apoptosis in the pathogenesis and treatment of disease. *Science*, **267**, 1456.

Venkatraman A, He XC, Thorvaldsen JL, et al (2013). Maternal imprinting at the H19-Igf2 locus maintains adult haematopoietic stem cell quiescence. *Nature*, **500**, 345-9.

Villamizar O, Chambers CB, MO Y-Y, et al (2016). Fas-antisense long noncoding RNA is differentially expressed during maturation of human erythrocytes and confers resistance to Fas-mediated cell death. B*lood Cells Mol Dis*, **58**, 57-66.

Xerri L, Bouabdallah R, Deviard E, et al (1998). Sensitivity to Fas-mediated apoptosis is null or weak in B-cell non-Hodgkin's lymphomas and is moderately increased by CD40 ligation. *Br J Cancer*, **78**, 225.

Yan M-D, Hong C-C, Lai G-M, et al (2005). Identification and characterization of a novel gene Saf transcribed from the opposite strand of Fas. *Hum Mol Genet*, **14**, 1465-74.

Zhou S, SU B, Zhou Y (2016). Expression of FAS-AS1 in breast cancer and its effect on the host gene sFas. *Zhonghua Yi Xue Za Zhi*, **96**, 949-953.