RESEARCH ARTICLE

HOTAIR Long Noncoding RNA is not a Biomarker for Acute Myeloid Leukemia (AML) in Iranian Patients

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Abstract

   Accumulating evidence indicates that lncRNAs may have potential as new biomarkers to predict prognosis of different human cancers. *HOTAIR* lncRNA, transcribed from the human HOX locus, has been suggested to regulate gene expression of important target genes and up-regulation has been noted in malignancies. The role of HOX transcript antisense RNA in acute myeloid leukemia (AML) was investigated in the present case control study. *HOTAIR* expression was evaluated in blood samples of twenty five de novo AML patients and fifty healthy controls using real-time quantitative reverse transcription-PCR (qRT-PCR). Our results demonstrated no significant differences in *HOTAIR* lncRNA expression level between AML patients and healthy individuals. The obtained data indicate that *HOTAIR* is not an informative and reliable biomarker for AML diagnosis, although our results should be confirmed in further studies.

Keywords: Acute myeloid leukemia- long non-coding RNA- *HOTAIR*

Introduction

   Acute myeloid leukemia (AML) is an aggressive malignancy that caused by malignant transformation of hematopoietic stem cells and accumulation of immature myeloid progenitors in the peripheral blood (PB) and the bone marrow (BM) (Döhner et al., 2015). AML is the most common acute leukemia disorder in adults, with a prevalence of 38 cases per 100,000 adults (Estey and Döhner, 2006). Although, development of AML is influenced by exposure to environmental causes a requisite heterogeneous genetic background is involved. The highly ordered developmental process of hematopoietic stem cells (HSCs) lineage commitment is tightly organized by different regulatory factors that control gene expression of involved pathways (Skalnik, 2002). Aberrant gene expression of each component of fundamental cellular mechanisms results in deregulated proliferation, differentiation, and self-renewal that lead to malignant proliferation and accumulation of immature blood cells in the disease. As genome wide association (GWA) studies have noted various immune-related genes located in the 6p21.3 region with several hematopoietic malignancies, we investigated the association of HLA-A, -B, -DRB1 alleles with Non-Hodgkin Lymphoma and HOTAIR gene with other cancers, previously(Sayad et al., 2014; Taheri et al., 2017; Sayad et al., 2017; Khorshidi et al., 2017).

   Long non-coding RNAs (lncRNAs) are important regulators of several biological processes that can control gene expression at the transcriptional or translational level (Geisler and Coller, 2013; Kurokawa, 2011). Particularly, lncRNAs have a pivotal regulatory role in hematopoiesis during different steps of HSCs development (Venkatraman et al., 2013; Aoki et al., 2010; Hu et al., 2011). One of the best-defined lncRNAs is the HOX transcript antisense RNA (HOTAIR) that is transcribed from the HOXC gene located at 12q13.13 region. HOTAIR is a trans-acting lncRNA that recruits a histone methyltransferase, PRC2 complex to target different genes to modifying and suppressing them epigenetically and regulate the expression of different tumor suppressor genes (Hajjari and Salavaty, 2015). The expression level of HOTAIR itself is described as a potential diagnostic or prognostic biomarker in various cancers (Flynn and Chang, 2014).

   Regarding to the functional importance of the HOTAIR in different cancers, in the current study, we investigated the involvement of HOTAIR in hematopoiesis and consequently in AML development, we examined whether its expression level show a correlation with the AML diagnosis.

Materials and Methods

Participants
   In the current case-control study 25 unrelated AML patients include 15(60%) male and 10(40%) female and

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Table1. The Sequences of Probes and Primers

property	HPRT1	HOTAIR
F-primer	AGCCTAAGATGAGAGTTC	AGACGAAGGTGAAAGCGAACC
R- primer	CACAGAACTAGAACATTGATA	CCCTCTGCCACGTTTGTTCC
Probe	FAM-CATCTGGAGTCCTATTGACATCGC-TAMRA	FAM-AACTGCCGCCTTGCTCCCTTGCC-TAMRA

50 healthy matched controls were examined. All the AML patients have clinically defined diagnosis according to the FAB classification criteria (Bennett et al., 1985). The sample was defined as CD34 positive when greater than 20 % blasts were positively stained. Complete remission, secondary AML, childhood AML, and post-treatment patient samples were excluded from our study. In control group, the samples had no any story of cancer and any viral infection recently.

They were recruited from Medical Oncology department of Besat Hospital, Hamadan, Iran. 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. The expression level of HPRT1 gene was as considered as a housekeeping gene to normalize the gene expression level of each sample. The sequence of probes and primer pairs has been demonstrated in Table 1. Real-time quantitative PCR was carried out in triplicates by using Applied Biosystems TaqMan R Universal PCR Master Mix (PN: 4304449). Corbett Rotor-gene 6000 machine (Corbet Life Science) was used to run the reactions. The negative control sample was used without cDNA sample as quality control.

Statistical methods

The obtained data was analyzed using the independent samples t-test 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 characteristics

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.

HOTAIR Expression in comparing to de novo AML patients and healthy controls

The expression level of HOTAIR gene 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 = 0.6, 95% CI = 0.102-71). We also analyzed the obtained data in two different

Table 2. Demographic and Clinical Data 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, ×10³)	47± 3.3	6± 2.4
WBC range (×10³)	18-130	04-Jul
Platelet (mean ± SD, ×10³)	64± 4.9	220± 1.9
Platelet range (×10³)	40-250	160-400
Hemoglobin (mean ± SD, g/dl)	8± 3.1	14± 2.3
Hemoglobin range (×10³, g/dl)	04-11	12-18
FAB classification: (no. (%))	25(100%)	
M0	2(8%)	
M1	3(12%)	
M2	6(24%)	
M3	2(8%)	
M4	9(36%)	
M5	3(12%)	

Table3. HOTAIR Expression in Comparing of De Novo AML Patients and Healthy Controls

HOTAIR expression	Control no.	AML patient no.	p-value	Expression ration	Std.Error	95% CI
Total	50	25	0.6	1.131	0.178-16.24	0.102-71
Male	21	15	0.09	1.346	0.315-17.271	0.211-67
Female	29	10	0.7	1.122	0.226- 20.194	0.4- 59

groups between male and female separately to find any possible correlation among gender, the expression level of HOTAIR and susceptibility to AML. The data show that either there is no significant difference between two groups (P > 0.05).

Discussion

As a recently described class of non-coding RNAs, long non-coding RNAs have been revealed to be related with different human diseases, especially cancers. These correlations might be because of the deregulation of lncRNAs in human disease that influence their ability to control gene expression of several components of cellular mechanisms. Among them, HOTAIR is one of the best-studied lncRNA that has been shown to have an oncogenic role in a wide variety of cancers such as breast cancer, colorectal, gastric, cervical cancer and Acute Myeloid Leukemia (Gupta et al., 2010).

In fact, HOTAIR is a trans-acting lncRNA that transcribed from the antisense strand of HoxC gene located on 12q13.13 region and include 6 exons. HOTAIR regulates polycomb-dependent chromatin modification by recruiting chromatin-modifying protein, Polycomb Repressive Complex 2 (PRC2) which is a genome-wide histone methyltransferase that affect gene expression by regulating chromatin states through altering histone H3 lysine-27 trimethylation and enhance the H3K27me3 level of different genes such as tumor and metastasis suppressor genes (Kogo et al., 2011). As an oncogenic molecule, HOTAIR is reported to have a crucial role in the initiation, progression and promoting malignancy of different cancer types (Huang et al., 2014; Nie et al., 2013) that present it as a potential prognostic biomarker in various human cancers. So investigating its expression level can be used to predict the correlated progression of cancer and estimate the survival rate (Cai et al., 2014; Sørensen et al., 2013).

In this regard, we examined the expression of HOTAIR mRNA in the blood samples of in 25 AML patients in comparison with 50 healthy controls to investigate the correlation between this lncRNA expression levels and the disease using quantitative real-time RT-PCR. We also categorized our samples regard to the gender into two separate groups of male and female. Previous reports suggesting that HOTAIR is overexpressed in various cancers such as colorectal cancer (Kogo et al., 2011), hepatocellular carcinoma (Geng et al., 2011), pancreatic cancer (Kim et al., 2013), gastrointestinal stromal tumors (Niinuma et al., 2012), and in ovarian cancer (Gupta et al., 2010, Qiu et al., 2014). In addition, an increased expression of HOTAIR was showed in leukemic cell line and primary AML blasts and the worst clinical outcome and poor prognosis was reported for patients with high HOTAIR expression (Hao and Shao, 2015). Besides, up-regulation of the HOTAIR lncRNA has been shown to play an oncogenic activity in AML by modulating the expression of the c-KIT proto-oncogene through interaction between HOTAIR and miR-193a (Xing et al., 2015) suggesting that malignant proliferation of AML

cells that is caused by up-regulation of HOTAIR might be dependent on the HOTAIR, miR-193a and c-KIT interactions (Gao et al., 2011).

In the present study, our study shown that the blood level of HOTAIR expression is not significantly different between AML patients and healthy controls (p-value =0.6). Likewise, by comparing HOTAIR expression based on the gender of participants, however, the compared expression level between case and controls was near to be significant p-value = 0.09 in the male group, it was not significantly different in Females p-value = 0.7. However, it should be noted that we cannot definitely mentioned whether there is any difference in HOTAIR level between case and control group and further analysis are needed to confirm our results. Moreover, due to the polygenic pathogenesis of AML disease, to defined the underlying mechanism of the effect of HOTAIR lncRNA on hematopoietic malignancies the lncRNA along with other regulatory factors such as other related genes or miRNAs should be taken into consideration. The obtained data show that HOTAIR expression level could not be considered as a definitive diagnostic or therapeutic biomarker for AML and the correlation between HOTAIR lncRNA and AML still needs to be investigated.

In Conclusion, totally, our results show that the HOTAIR mRNA expression level has no significant difference between AML patients and healthy controls. However, due to the importance of HOTAIR expression level as a potential biomarker for diagnostic and therapeutic purposes in several cancers, we believe that further analyses are needed to provide more evidence about the correlation between HOTAIR and AML.

References

Aoki K, Harashima A, SanoM, 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 Intern Med*, **103**, 620-5.

Cai B, Wu Z, Liao K, Zhang S (2014). Long noncoding RNA HOTAIR can serve as a common molecular marker for lymph node metastasis: A meta-analysis. *Tumour Biol*, **35**, 8445-50.

Döhner H, Weisdorf DJ, Bloomfield CD (2015). Acute Myeloid Leukemia. *N Engl J Med*, **373**, 1136-52.

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

Flynn RA, Chang HY (2014). Long noncoding RNAs in cell-fate programming and reprogramming. *Cell Stem Cell*, **14**, 752-61.

Gao X, Lin J, Li Y, et al (2011). Microrna-193a represses C-Kit expression and functions as a Methylation-Silenced tumor suppressor in acute Myeloid Leukemia. *Oncogene*, **30**, 3416-28.

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.

Geng Y, Xie S, Li Q, Ma J, Wang G (2011). Large intervening non-coding RNA HOTAIR is associated with Hepatocellular Carcinoma progression. *J Int Med Res*, **39**, 2119-28.

Gupta RA, Shah N, Wang KC, et al (2010). Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature*, **464**, 1071-6.

HajjariM, Salavaty, A (2015). HOTAIR: An oncogenic long non-coding RNA in different cancers. *Cancer Biol Med*, **12**, 1.

Hao S, Shao Z (2015). HOTAIR is upregulated in acute Myeloid Leukemia and that indicates a poor prognosis. *Int J Clin Exp Pathol*, **8**, 7223.

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.

Huang L, Liao L-M, Liu A-W, et al (2014). Overexpression of long noncoding RNA HOTAIR predicts a poor prognosis in patients with cervical cancer. *Arch Gynecol Obstet*, **290**, 717-23.

Kim K, Jutooru I, Chadalapaka G, et al (2013). HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. *Oncogene*, **32**, 1616-25.

Kogo R, Shimamura T, Mimori K, et al (2011). Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. *Cancer Res*, **71**, 6320-6.

Kurokawa R (2011). Long noncoding RNA as a regulator for transcription. In Long Non-Coding RNAs. *Springer,* 29-41

Nie Y, Liu X, Qu S, et al (2013). Long non-coding RNA HOTAIR is an independent prognostic marker for nasopharyngeal carcinoma progression and survival. *Cancer Science*, **104**, 458-64.

Niinuma T, Suzuki H, Nojima M, et al (2012). Upregulation Of Mir-196a and HOTAIR drive Malignant character in gastrointestinal stromal tumors. *Cancer Res*, **72**, 1126-36.

Qiu J-J, Lin Y-Y, Ye L-C, et al (2014). Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. *Gynecol Oncol*, **134**, 121-28.

Sayad A, Akbari MT, Mehdizadeh M, Taheri M, Hajifathali A (2014). Hla-A*26 and susceptility of Iranian patients with non-Hodgkin lymphoma. *Iran J Immunol*, **11**, 269-74.

Sayad A, Hajifathali A, Taheri M (2017). Polymorphisms in Lncrna Hotair and susceptibility to acute Myeloid Leukemia in Iranian patients. *J Biol Today's World*, **6**, 33-7.

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

Sørensen KP, Thomassen M, Tan Q, et al (2013). Long non-coding RNA HOTAIR is an independent prognostic marker of metastasis in estrogen receptor-positive primary breast cancer. *Breast Cancer Res Treat*, **142**, 529-36.

Taheri M, Habibi M, Noroozi R, et al (2017). Hotair genetic variants are associated with prostate cancer and Benign prostate Hyperplasia in an Iranian population. *Gene*, **613**, 20-4.

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.

Xing C-Y, Hu X-Q, Xie F-Y, et al (2015). Long non-Coding RNA HOTAIR modulates C-KIT expression through sponging Mir-193a in acute Myeloid Leukemia. *FEBS Lett*, **589**, 1981-7.