Journal of Biology and Today's World

Journal home page: http://journals.lexispublisher.com/jbtw

Received: 04 November 2016 • Accepted: 07 March 2017

Research

doi:10.15412/J.JBTW.01060203

Polymorphisms in lncRNA HOTAIR and Susceptibility to Acute Myeloid Leukemia in Iranian Patients

Arezou Sayad[1], Abbas Hajifathali[2], Mohammad Taheri[1,3*]

[1] Department of Medical Genetics, Shahid Beheshti University of Medical sciences, Tehran, Iran

[2] Taleghani Bone Marrow Transplantation Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Urogenital Stem Cell Research, Shahid Beheshti University of Medical sciences, Tehran, Iran

*Correspondence should be addressed to Mohammad Taheri, Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; Address: 8th Floor, SBUMS Bldg., Next to Ayatollah Taleghani Hospital, Evin, Tehran, Iran; Post code: 198396-3113; Tel: +982177635431; Fax: +982177635431; Email: Mohammad_823@yahoo.com.

ABSTRACT

One of the recent aspects in the cancer research is about the investigation of the etiological role of long noncoding RNAs as a new regulator for the expression of proto-oncogenes or tumor suppressor genes in cancer. Among them HOX transcript antisense RNA (HOTAIR) is increasingly indicated to be deregulated in different cancers such as AML. According to the important regulatory role the gene in the developing and prognosis of the hematological malignancies we investigate whether there is an association between rs12826786, rs1899663, and rs4759314 common polymorphisms of the gene with AML patients. The results have shown no relation between any of the analyzed Single Nucleotide Polymorphisms and AML either in genotypic frequencies or in haplotype analysis. The data derived from the present study suggested that pathogenic role of the HOTAIR to increase the susceptibility of AML should be considered from other aspects and further analysis are needed to explore the exact mechanism underlying the pathogenesis.

Key words: Acute Myeloid Leukemia, long non-coding RNA, HOTAIR, Polymorphism.

Journal of Biology and Today's World is published by *Lexis Publisher*; Journal p-ISSN 2476-5376; Journal e-ISSN 2322-3308.

1. INTRODUCTION

Acute myeloid leukemia (AML) (# 601626) as a highly malignant cancer is the most common type but with the lowest survival rate of leukemia. AML is a relatively rare disease while its incidence (3.7 per 100,000 persons) and age-dependent mortality rate (2.7 to 18 per 100,000 persons) increased as the population aged. Basically, AML considered to be an elderly diseases with an average age at onset ≥ 65 years that is rarely diagnosed before age 40 (1). AML is a consequence of aberrant cell proliferation and differentiation and deregulated cell surveillance mechanisms that results in an accumulation of myeloid blasts in the bone marrow and blood of patients. The observed diversity in the etiologic aspects of the AML is still a wide area for future research to explore new clues of the mechanisms underlying 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 Acute Lymphoblastic Leukemia (ALL), Multiple Myeloma (MM), Non-Hodgkin Lymphoma (NHL), Hodgkin Lymphoma (HL), previously (2-6). Discovered functional mutations in the noncoding genome and aberrant expression of the lncRNAs in various disease introduced them as a relative risk factor in human disease especially in cancer (2). The indispensable role of the lncRNAs in the regulation of pivotal biological processes such as cell proliferation, differentiation, and apoptosis and investigated altered expression of them in tumor genesis have suggested them as diagnostic and prognostic biomarker or even a therapeutic target for different cancers (3). In this regard, several recent studies have revealed the regulatory role of lncRNAs in different stages of hematopoietic stem cell (HSC) commitment and developmental lineage and have shown that aberrant expression of the lncRNAs due to the disrupting variants could influence the highly ordered mechanisms of HSCs

differentiation (4-6). It is estimated that around 45-50% of AML patients are etiologically classified as cytogenetically normal (CN-AML) which are more likely to have causative variation in the coding or noncoding genome that serves as oncogenic factors. Previously it was revealed that altered expression pattern of lncRNAs in AML is associated with recurrent de novo mutation (7). One of these proposed lncRNAs for AML is the HOX transcript antisense RNA (HOTAIR) which has been shown to be increased in leukemic cells lines and primary AML blasts HOTAIR is located on 12q13.13 within the locus of the Homeobox C (HOXC) gene cluster and is co-expressed with these genes while it is involved in the repression of the transcription of HOXD genes (8). According to the diagnostic and therapeutic potential of the HOTAIR, we investigate the association of 4 cancer-related single nucleotide polymorphisms in Iranian AML patients to in comparison to healthy controls.

2. MATERIALS AND METHODS

2.1. Participants

In this case/control study 602 individuals were involved include 202 Iranian de novo AML patients and 400 controls who were ethnically, age and sex matched and were without personal or familial backgrounds of cancer or autoimmune disorders between January 2014 and July 2016. All the case samples were obtained from the Medical Oncology department of Besat Hospital, Hamadan. The patients were diagnosed with AML by oncologist according to the revised French–American–British (FAB) classification (9). Table 1 is shown the main demographic and laboratory characteristics of the patients.

Table 1. Demographic and clinical datad of AML patients.

Variables	AML patient
Female/Male (no. (%))	85(42%)/117(58%)
Age (mean ± SD, Y)	33.7 ± 2.9
Age range (Y)	19-65
Age of onset (mean ± SD, Y)	33.4 ± 2.8
WBC (mean ± SD, $\times10^3$)	50 ± 7.3
WBC range ($\times10^3$)	15-150
Platelet (mean ± SD, $\times10^3$)	51 ± 3.8
Platelet range ($\times10^3$)	30-300
Hemoglobin (mean ± SD, g/dl)	8.3 ± 1.8
Hemoglobin range ($\times10^3$, g/dl)	4.2-11.5
FAB classification: (no. (%))	202 (100%)
M0	16 (8%)
M1	22 (11%)
M2	51 (25%)
M3	20 (10%)
M4	75 (37%)
M5	18 (9%)

The exclusion criteria were consisting of patients with remission, secondary AML, childhood AML, and post-treatment. 5 ml peripheral blood samples were collected from each individual. This study was approved by the local Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1395.383). All of the individuals gave an informed written consent agreeing to participate in the present study.

2.2. DNA Extraction and Genotyping

DNA was extracted from blood samples for all the individuals using the standard salting out method. Genotyping for 3 SNPs rs12826786, rs1899663, and rs4759314 were conducted by means of tetra-primer amplification refractory mutation system PCR (Tetra-ARMS-PCR) the detailed descriptive information of the SNPs are indicated in the Table 2.

Table 2. Descriptive information of rs1899663, rs12826786, rs4759314 of *HOTAIR* gene.

SNP	Position	Minor Allele	MAF	MAC	Type	Gene
rs1899663	Chr12:53967210	T	0.25	1271	Intron variant	
rs12826786	Chr12:53961717	C	0.36	1791	Upstream gene variant	*HOTAIR*
rs4759314	Chr12:53968051	G	0.10	476	Intron variant	

The pair primers using for PCR were designed by PRIMER1 (10). PCR reaction was performed using Taq

(2x) red master mix (Ampliqon, Denmark) in a FlexCycler (Analytik Jena, Germany). The cycling PCR protocol was composed of an initial denaturation at 94ºC for 4minutes, followed by 35cycles of 94ºC for 45seconds, annealing temperature for 45seconds and 72 ºC for 60 seconds, with a final extension of 72 ºC for 5 minutes. Specific annealing temperatures were 45°C.

2.3. Statistical Analysis

The association of genotype and allele distribution was evaluated using Pearson Chi-square test by comparing genotype and allele frequencies between the AML patients and the control group by means of SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The calculated results were represented by reporting Odd ratio (OR) and 95% confidence intervals (CI) for each SNP. The difference in allelic and genotypic distribution between two groups was considered as significant if the calculated P-value was P≤0.05. The genotype frequency of all 3 SNPs are in the Hardy-Weinberg equilibrium. The haplotype frequencies and their possible association with the disease were calculated using SNPStats online software and the obtained data were reported by describing the D′ and r^2 parameters. These analyses were implemented in SNPStats (11) (http://bioinfo.iconcologia.net/SNPstats).

3. RESULTS AND DISCUSSION

The derived data indicated that the genotypic frequency for all 3 polymorphisms were in agreement with Hardy-Weinberg Disequilibrium P>0.05. There were no were significant difference between the calculated allelic frequencies for all investigated SNPs (rs12826786, rs1899663, and rs4759314) in case and control individuals. Also, association analysis for genotypic frequencies of each SNP has shown no significant relation to the disease. The detailed data for allele and genotype analysis for both patients and control groups are detailed in Table 3.

Table 3. Allele and Genotype frequencies of the HOTAIR *gene* polymorphisms in AML patient and control group

SNP	Allele/Genotype	Patients N(%)	Controls N(%)	P value	OR (95%CI)
	A	332(82.18)	665(83.12)	0.681	0.936(0.683-1.283)
	G	72(17.82)	135(16.88)		
rs4759314	AA	134(66.34)	271(67.75)	0.727	0.938(0.655-1.344)
	AG	64(31.69)	123(30.75)	0.815	1.044(0.725-1.504)
	GG	4(1.97)	6(1.5)	0.663	1.327(0.37-4.755)
	C	227(56.18)	443(55.38)	0.789	1.034(0.812-1.315)
	T	177(43.82)	357(44.62)		
rs12826786	CC	62(30.7)	119(29.75)	0.812	1.046(0.724-1.511)
	CT	103(50.99)	205(51.25)	0.952	0.99(0.706-1.388)
	TT	37(18.31)	76(19)	0.839	0.956(0.619-1.477)
	G	233(57.68)	468(58.5)	0.784	0.967(0.759-1.232)
	T	171(42.32)	332(41.5)		
rs1899663	GG	70(34.66)	148(37)	0.572	0.903(0.634-1.286)
	GT	93(44.03)	172(43)	0.478	1.131(0.805-1.589)
	TT	39(19.31)	80(20)	0.84	0.957(0.625-1.466)

On the other hand, estimated haplotype blocks with at least 0.01 frequencies for all SNPs are summarized in Table 4.

Table 4. Haplotype frequencies of the *HOTAIR* polymorphisms in the case and control group

Haplotypes	Patients N(%)	Controls N(%)	P value	OR (95%CI)
A C G	136(34)	264(33)	0.818	1.03(0.8-1.328)
A T T	121(30)	224(28)	0.48	1.099(0.845-1.43)
A T G	50(12)	96(12)	0.85	1.036(0.719-1.492)
A C T	52(13)	104(13)	0.95	0.989(0.692-1.412)
G C G	20(5)	56(7)	0.167	0.692(0.409-1.17)
G T T	12(3)	32(4)	0.369	0.735(0.374-1.442)
G T G	9(2)	16(2)	0.794	1.116(0.489-2.549)
G C T	4(1)	8(1)	0.987	0.99(0.296-3.307)

Loci chosen for hap-analysis: Site1 (**rs4759314**), Site2 (**rs12826786**), Site3 (**rs1899663**)

The results of the association analysis for haplotype blocks were calculated between case and controls and shown no significant difference between the frequency of estimated blocks in case and controls in any of the estimated haplotype blocks. The HOX transcript antisense RNA (HOTAIR) is a noncoding gene that transcribed to a 2.2 kb long noncoding RNA. It is located at 12q13.13 region overlaps the Homeobox C (HOXC) gene cluster while control the gene expression of the HOXD genes on chromosome 2 in trans. lncRNAs are recently described as regulatory factors for fundamental cellular processes. They are especially ascribed to be involved in controlling stem

cell biology such as pluripotent stem cell proliferation, HSCs differentiation and self-renewal through involvement in central signaling pathways (12). Deregulated expression of lncRNAs is revealed to promote tumorigenesis by transcriptionally affecting their target genes, mostly neighboring genes in cis or rarely distant genes in trans (13). Among them one of the well-characterized lncRNAs, HOTAIR is revealed to be overexpressed in different cancers however its oncogenic role in acute myeloid leukemia (AML) still needs to be investigated. The HOTAIR lncRNA was firstly indicated to increase the cancer invasiveness and metastasis through interaction with the Polycomb Repressive Complex 2 (PRC2) a histone H3 lysine 27 (H3K27) methylase that leads to alteration in methylation of histone H3 lysine 27 (H3K27) and as a result, decreased the expression level of HOTAIR–PRC2 target genes (14). The expression of the HOTAIR is up-regulated significantly in AML patients with poor prognosis and decreased survival rate (15, 16). Besides, in AML cells, overexpression of the HOTAIR has been reported to have an oncogenic activity by competitively binding to the tumor-suppressive miR-193a (8). The miR-193a is decreased in primary AML blasts because of its hypermethylated promoter that leads to deregulated expression of the c-KIT proto-oncogene which contributes to increased cell proliferation and tumorgenesis (17). In addition, it is recently suggested that imatinib resistance could be a consequence of deregulated HOTAIR in CML patients by influencing the regulation of PI3K/Akt pathway (18). On the other hand, different studies have reported significantly association between different Single Nucleotide Polymorphisms (SNPs) of the HOTAIR that influenced its expression and function in different types of cancer (19, 20). According to mentioned importance of the HOTAIR in the underlying mechanisms of tumor genesis and also as an effective factor of the invasiveness and prognosis of different cancers especially AML, in the present study an association analysis between 3 different polymorphisms of the HOTAIR noncoding gene include rs4759314, rs12826786, and rs1899663 were done to investigate whether they were associated with an increased risk to developing AML. The association of the rs4759314 with cancer risk was explored in a meta-analysis and reported that G allele may be a risk factor for gastric cancer (21). The rs12826786 the functional SNP in the promoter region of the HOTAIR gene has been revealed to influence the expression of the gene and be associated with the risk of developing gastric cancer and breast cancer (22). Also, the rs1899663 was investigated in a case/control study to explore its association with susceptibility to breast cancer and reported an association between GT + TT genotypes of the rs1899663 and lower susceptibility to breast cancer (23). Despite the previous findings, the result of our study revealed no significant difference between allelic and genotypic distributions of the investigated SNPs between AML cases and healthy individuals and they were associated with an increased risk of the disease neither in genotypic association analysis nor in haplotype analysis. While our data shown no evidence for association of these polymorphisms with AML disease, based on the revealed importance of the HOTAIR in cancer pathology, there may be other causative polymorphisms in linkage disequilibrium with these 3 SNPs association with AML disease and further analysis are needed to explore the relation between the HOTAIR and AML.

4. CONCLUSION

Although, the present study found no association between 3 polymorphisms of the HOTAIR gene and the risk of developing AML in Iranian patients, the physiological importance of the gene in AML could not be ignored. Further studies are needed to found the exact role of the gene in developing AML. Precise recognition of the molecular mechanisms underlying the pathogenic effects of HOTAIR in AML may provide new potential therapeutic targets and also diagnostic strategies for AML patients.

ACKNOWLEDGMENT

Not mentioned any acknowledgment by authors.

FUNDING/SUPPORT

Not mentioned any Funding/Support by authors.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians. 2016;66(1):7-30.
2. Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. Cancer cell. 2016;29(4):452-63.
3. Matjašič A, Glavač D. Long Noncoding RNAs and Tumorigenesis. eLS.
4. Venkatraman A, He XC, Thorvaldsen JL, Sugimura R, Perry JM, Tao F, et al. Maternal imprinting at the H19-Igf2 locus maintains adult haematopoietic stem cell quiescence. Nature. 2013;500(7462):345-9.
5. Hu W, Yuan B, Flygare J, Lodish HF. Long noncoding RNA-mediated anti-apoptotic activity in murine erythroid terminal differentiation. Genes & development. 2011;25(24):2573-8.
6. Guo G, Kang Q, Zhu X, Chen Q, Wang X, Chen Y, et al. A long noncoding RNA critically regulates Bcr-Abl-mediated cellular transformation by acting as a competitive endogenous RNA. Oncogene. 2015;34(14):1768-79.
7. Garzon R, Volinia S, Papaioannou D, Nicolet D, Kohlschmidt J, Yan PS, et al. Expression and prognostic impact of lncRNAs in acute myeloid leukemia. Proceedings of the National Academy of Sciences. 2014;111(52):18679-84.
8. Xing C-y, Hu X-q, Xie F-y, Yu Z-j, Li H-y, Wu J-b, et al. Long non-coding RNA HOTAIR modulates c-KIT expression through sponging miR-193a in acute myeloid leukemia. FEBS letters. 2015;589(15):1981-7.
9. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. Annals of internal medicine. 1985;103(4):620-5.
10. Collins A, Ke X. Primer1: primer design web service for tetra-primer ARMS-PCR. The Open Bioinformatics Journal. 2012;6:55-8.
11. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006;22(15):1928-9.

12. Le Bot N. LncRNAs and self-renewal. Nature Cell Biology. 2012;14(3):237-.
13. Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer discovery. 2011;1(5):391-407.
14. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010;464(7291):1071-6.
15. Wu S, Zheng C, Chen S, Cai X, Shi Y, Lin B, et al. Overexpression of long non‐coding RNA HOTAIR predicts a poor prognosis in patients with acute myeloid leukemia. Oncology letters. 2015;10(4):2410-4.
16. Hao S, Shao Z. HOTAIR is upregulated in acute myeloid leukemia and that indicates a poor prognosis. International journal of clinical and experimental pathology. 2015;8(6):7223.
17. Gao X, Lin J, Li Y, Gao L, Wang X, Wang W, et al. MicroRNA-193a represses c-kit expression and functions as a methylation-silenced tumor suppressor in acute myeloid leukemia. Oncogene. 2011;30(31):3416-28.
18. Wang H, Li Q, Tang S, Li M, Feng A, Qin L, et al. The role of long noncoding RNA HOTAIR in the acquired multidrug resistance to imatinib in chronic myeloid leukemia cells. Hematology. 2016:1-9.
19. Zhang X, Zhou L, Fu G, Sun F, Shi J, Wei J, et al. The identification of an ESCC susceptibility SNP rs920778 that regulates the expression of lncRNA HOTAIR via a novel intronic enhancer. Carcinogenesis. 2014:bgu103.
20. Pan W, Liu L, Wei J, Ge Y, Zhang J, Chen H, et al. A functional lncRNA HOTAIR genetic variant contributes to gastric cancer susceptibility. Molecular carcinogenesis. 2016;55(1):90-6.
21. Liu F, Zhou L, Qiu C, Xia G-f, Zhu P, Luo H. Association of the HOTAIR rs4759314 polymorphism with cancer risk: a meta-analysis. Journal of BU ON: official journal of the Balkan Union of Oncology. 2016;21(4):1016.
22. Bayram S, Sümbül AT, Dadaş E. A functional HOTAIR rs12826786 C> T polymorphism is associated with breast cancer susceptibility and poor clinicopathological characteristics in a Turkish population: a hospital-based case–control study. Tumor Biology. 2016;37(4):5577-84.
23. Yan R, Cao J, Song C, Chen Y, Wu Z, Wang K, et al. Polymorphisms in lncRNA HOTAIR and susceptibility to breast cancer in a Chinese population. Cancer epidemiology. 2015;39(6):978-85.