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ORIGINAL RESEARCH

Long Non-Coding RNA H19 Expression in Leukemia Patients

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Abstract

Objective: *BCR-ABL fusion gene occurs with the formation of translocations in the t(9;22) region of the Philadelphia (Ph) chromosome, which is used as a diagnostic biomarker in Chronic Myeloid Leukemia (CML). These abnormal genetic changes, which cover 15% of leukemias, reach dimensions that threaten human life. Recent studies have determined that thousands of genes expressed in differentiation and development processes contain non-protein-coding RNA with a regulatory role. Of these, the first discovered long noncoding RNA (LncRNA) H19 has been associated with its biological role, cell proliferation, apoptosis and metastasis Therefore, studies have been conducted considering that LncRNA H19 can be used as a biological marker in CML patients.*

Method: *For this study, blood from 72 CML patients over 18 years of age and 64 healthy individuals were used. After RNA isolation of each of these bloods and cDNAs were obtained, the expression levels of the LncRNA H19 gene were analyzed by Real Time PCR method.*

Results: *As a result of the expression study, it was found that LncRNA H19 gene expression increased 4.37 times and was upregulated in Bcr-Abl positive patients (p=0.414683).*

Conclusion: *In this study conducted in Diyarbakır region, we think that LncRNA H19, which is up-regulated in terms of CML profiles, can be used as a biological marker for new treatment applications.*

Keywords: *CML, LncRNA, H19, RT-PCR, BCR-ABL*

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Introduction

CML is a malignant disease that occurs with an uncontrolled increase in the number of leukocytes produced in the blood and bone marrow and generally affects the life of people over 40 years of age negatively[1]**.** BCR-ABL fusion gene occurs with the formation of translocations in the t(9;22) region of the Philadelphia (Ph) chromosome, which is seen in 95% of CML cases[2]. Diagnosis is made by detecting the BCR-ABL fusion gene from blood samples taken from CML cases by RT-PCR method[3]. As a result of the analyzes obtained from the genome studies, it has been determined that thousands of genes contain non-protein-coding RNA with a regulatory role[4]. In addition, LncRNA H19 contributes to the formation and progression of CML in tumor formation of the BCR-ABL gene, which is an oncogene. LncRNA H19 has been identified as functional involvement in the progression of BCR-ABL mediated leukemia[5]. Lnc RNA H19 found in humans has been identified as a maternally expressed imprinted gene with a length of 2.7 kb (NR_002196, 2322 bp RNA) and four small intron regions[6]. Excessive increase in the expression of LncRNA H19 in various cancer types causes uncontrolled proliferation of cells and spread of cancer. Therefore, LncRNA H19 has been shown to be effective in the uncontrolled proliferation of cells, programmed cell death, and the spread of cancer throughout the body[7].

The specific mechanism of lncRNA H19 in CML remains to be elucidated. We think that the results of our study for the expression of LncRNA H19 in CML patients will contribute to further studies.

Materials and Methods

Our study, which was approved by Dicle University, started in August 2022 according to the Declaration of Helsinki and was concluded in February 2023. The study included 72 patients who applied to the Hematology outpatient clinic of Dicle University Medical Faculty Hospitals with the diagnosis of CML or were followed up with a new diagnosis, and 64 healthy individuals without any health problems as the control group were included in the study. Two tubes of 10 ml of blood were taken from the individuals included in the study. Whole blood White Blood Cell (WBC) results of the patients were recorded from the hospital automation system for leukocyte isolation from the blood sample. According to the RNA isolation kit protocol, the required amount of blood samples were taken by looking at the WBC counts, and leukocyte and RNA isolation were performed. The concentration values of the RNAs we obtained were measured in a spectrophotometer.

The cDNA was obtained according to the Ipsogen RT Kit (Qiagen GmbH, 679923) protocol used for cDNA extraction from the RNA isolated samples. After this step, BCR-ABL positive

patients were identified using the Ipsogen BCR-ABL1 Mbcr IS-MMR Kit with the RT-PCR method. The cDNAs of the determined samples were diluted 1/20 and RT-PCR was performed with the LncRNA H19 primer and B2M (Beta-2-microglobulin) as the reference gene, since it showed high stability. Information about the B2M reference gene and H19 primers used in the study were obtained from the National Library of Medicine and are indicated in Table 1[8].

Table 1. *Information on the B2M reference gene and H19 primers*

Position	RefSeq Number	GeneID	MIM	Symbol	Description
1	NR_002196	283120	103280	H19	LncRNA
2	NM_004048	567	109700	B2M	Beta-2-mikroglobulin

RT-PCR Analysis

For our study, SYBR Green qPCR Mastermix, Human LncRNA H19 qPCR Assay and B2M (LPH28472A) as Reference Gene (House Keeping) were used. During the mixing phase for RT-PCR, 12.5 µl of Sybr Green, 1 µl of H19 primer and 10.5 µl of ultrapure water were prepared per sample. 1 µl of the relevant sample cDNA was added to the prepared total mixture of 24 µl and it was determined as 25 µl. For the RT-qPCR procedure, the PCR protocol was applied as the first heating at 95 °C for 10 minutes and 40 cycles at 95 °C for 15 seconds and 60 °C for 30 seconds. Ct values were determined by evaluating all samples used in the study. GeneGlobe Data Analysis was used for the analysis of the Ct values found. The B2M reference gene was selected to normalize Ct values. Negative Control (NC: Rnase Free Water) was used to see if a contamination had occurred in the samples. Data from the RT-PCR device are shown in Figure 1 below.

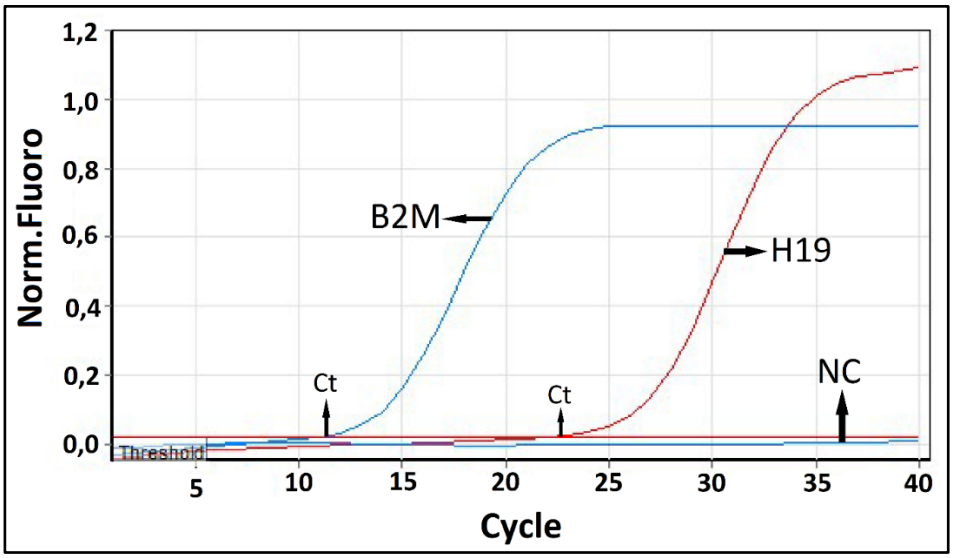

Figure 1. *RT-PCR data*

Statistical Analysis

Fold Change

According to the statistical analysis of our study; Our fold change value, which we found as a result of the comparison of the sick and healthy samples, is 4.37. This is considered to be greater than 1.00, which is accepted as the cut-off value, and significantly increased gene expression according to the p-value < 0.05, and these values are expressed in Table 2. The p-value is based on the deviation between the observed H19 value and the reference B2M, considering the probability data of our study. Statistical analysis was performed online at the GeneGlobe Data Analysis Center[9].

Table2. *Analysis data for H19 and B2M*

Position	Gene Symbol	Fold Change	p-Value
1	H19	4,37	0,414683
2	B2M	1	nan

In our study, the distribution of the change in the expression of LncRNA H19 in CML patients was compared with the data normalized with the B2M gene in RT-PCR analysis. In the figure below, it is seen that B2M, which is used as a reference gene, has not changed since it is located in the central diagonal. The other two diagonals show the threshold of the floor change. Since the point indicated by H19 is outside these thresholds, it was observed that it was upregulated (Figure 2).

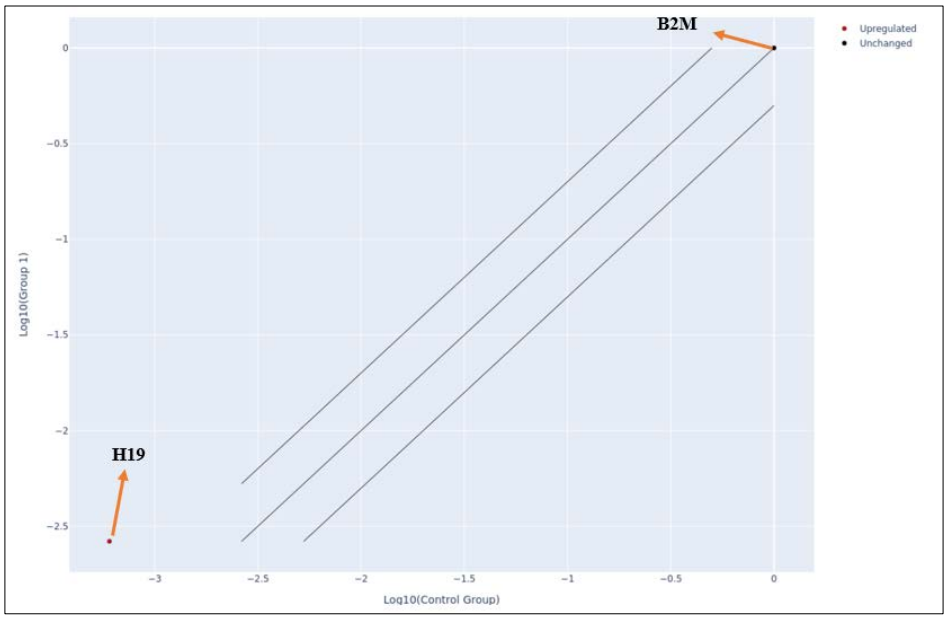

Figure2. *Scatterplot of H19 and B2M*

Results

In the light of previous studies on the H19 gene, the expression of the H19 gene was investigated in 72 CML patients over 18 years of age and 64 healthy individuals who applied to the outpatient clinic for the diagnosis of CML. For this reason, it was determined that the expression of LncRNA H19 was upregulated in individuals with positive Bcr-Abl results.

Discussion

The diagnosis of CML is made by detecting the Bcr-Abl oncogene from the samples taken by applying the RT-qPCR method[3].

In studies on the human genome, it has been determined that thousands of genes contain non-protein-coding RNA with a regulatory role as a result of whole genome and functional analysis[4]**.** Many non-coding miRnas and LncRNAs were found by Klattenhoff et al. It has been stated that lncRNAs are expressed in differentiation and development processes and have important roles in controlling some cellular processes[10].

Thanks to recent technological applications, it has been reported that many transcription factors, miRNAs, RNA-binding proteins and regulators that promote or inhibit tumor growth after transcription, such as lncRNAs, are very important in studies comparing cancer cells and normal cells related to the increase in cancer[11,12,13].

We think that the identification of lncRNA H19, which contributes to tumor formation and is involved in cellular processes during the cancerization process, will contribute to therapeutic targets used in cancer treatment. These non-protein-forming RNAs can be the starting point for the treatment process for the development and prevention of cancer. It can be said that lncRNAs provide answers to questions about the molecular pathology and genetics of cancer formation.

Conclusion

In summary, our data on maternally expressed LncRNA H19 showed upregulation in positive BCR-ABL patients diagnosed with CML. Therefore, we think that it will be beneficial to find out the effects and duties of LncRNAs, including H19, in various types of cancer in humans, and to further elucidate their roles. Therefore, many new findings are needed to determine the expression and targets of other LncRNAs.

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