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The Predictive Role of LncRNA Molecules (HOTAIR) in Breast Cancer Prognosis

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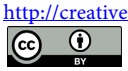

Abstract

Objective: The aim of the present study is to assess the role of the level of change in gene expression of the HOTAIR molecule in breast cancer patients. **Methods**: An assay was conducted for the level of gene expression of the HOTAIR molecule in the plasma of 30 patients and 10 healthy controls, by performing the reverse transcription reaction RT-qPCR, gene expression was evaluated using the Livak equation, tumor characteristics (grade, HER2 receptors, hormonal (HR) receptors) were also studied in the Pathological Anatomy Department and compared between patients. **Results**: The results of the study showed that there was a significant difference ($P < 0.05$) in the level of change in gene expression between the patients and controls. There was a correlation between the level of change in gene expression and tumor grade, positivity for HER2 receptors and negativity for HR receptors. **Conclusion**: It was found that the gene expression level of HOTAIR molecule may have a prognostic significance in the diagnosis of breast cancer and the characteristics of the tumor responsible for increasing tumor virulence and poor prognosis.

Subject Areas

Biochemistry, Cell Biology, Oncology

Keywords

Breast Cancer, HOTAIR, Tumor Grade, HER2 Receptor, HR Receptors

1. Introduction

The part that codes for certain proteins in human cells makes up only 2% of RNAs and although most of them are not translated into proteins, they play an important regulatory role at the level of transcription and gene pre-transcription and non-

coding RNAs can be classified into:

- **Micro RNA**: its length does not exceed 30 nucleotide. **Long non-coding RNA (LncRNA)**: its length is more than 200 nucleotide [1] [2], and empirical evidences concurred on the pathogenetic role of LncRNA molecules in cancers and many diseases [2].

LncRNAs molecules work through a number of epigenetic mechanisms, as they may have a constructive role through which they bind chemical compounds that cause histone changes (histone methylation and histone acetylation) that lead to gene silencing, they may also block some binding sites on the DNA strip, and may play a competitive role with a number of transcription-regulating molecules such as mi-RNA molecules, and affect via these mechanisms on cellular proliferation, tumor development, cellular circuit, the occurrence of metastases and evasion of the immune system [3]. Breast cancer is the most common cancer in women and the most common cause of cancer deaths around the world [4], and it is considered one of the heterogeneous diseases, where there are important molecular differences that modify the therapeutic strategy, such as HER2 and hormonal receptors [5].

In 2000, Perou *et al*. developed a molecular classification based on the gene expression of a number of molecules and was modeled into five patterns: Luminal A, Luminal B, Basal-like (triple-negative), Normal-like and HER2-enriched [6].

The (HOX transcript antisense RNA HOTAIR) molecule is one of the LncRNA molecules that has been studied extensively in breast cancer and consists of 6 exons, located on the chromosome 12q13 between the site HOX11 and HOX12 [7]. The HOTAIR molecule does not express a specific protein, but affects the gene expression of many genes through effects regulating gene expression, and it was found that the high level of gene expression of HOTAIR molecule has an important role in a number of malignancies, the most important of which is Breast Cancer [8] [9]. HOTAIR molecules control the expression of many genes, including the genes regulating the cellular circuit Rb-E2f, CyclinD, CKD6, CKD4, and they also silence many tumor suppressor genes, such as the HOXD gene located on chromosome 2, which is one of the most important genes that inhibit the occurrence of metastases [10], as well as inhibit the effectiveness of a number of genes pathways that inhibit tumor progression by various mechanisms, including PENT, P21, and P53 genes [11].

HOTAIR molecules enhance the effect of certain oncogenes such as HER2, VEGF, vimentin and B-catenin, which are all tumor-stimulating genes [12]-[14]. Among all these effects, the gene expression of the HOTAIR molecule is closely related to the development, grade and recurrence of the tumor, evasion of the immune system, the occurrence of angiogenesis and drug resistance, which pose the greatest challenges in the therapeutic strategy of breast cancer [15] [16].

2. Objectives

Our study aimed to evaluate the role of the level of change in the HOTAIR molecule

and its relationship with tumor characteristics in breast cancer patients.

3. Materials and Methods

- **Study design:** a prospective randomized case-control study.
- **Study place and duration:** Lattakia University Hospital between August 2022 and April 2024.
- **Study sample**: a total of 30 recently diagnosed breast cancer patients with 10 healthy controls (negative mammography).

3.1. Inclusion Criteria

1. A newly-diagnosed patient.
2. Age above 19 years.
3. Without any therapeutic interference.

3.2. Exclusion Criteria

1. A history of previous malignancies.
2. A history of chemotherapy or hormonal therapy.
3. A history of autoimmune diseases.

4. Methodology

Blood samples were drawn on EDTA tubes for qPCR testing and tissue biopsies were taken from the patients and sent to the Department of Pathological Anatomy at Lattakia University Hospital to study the characteristics of the tumor (grade, HER2 receptors and HR receptors). HER2 and HR statuses were determined using IHC method for HER2 and Chemoluminuce technique for HR.

RNA extraction: after the samples were collected through EDTA tubes, the samples were deposited at a speed of 12000 g at a temperature of 4C for 10 minutes and then we transfer the float to the working tubes, the triazol kit was used to extract all the RNA according to the instructions included in the kit. The concentration and purity of RNA were confirmed by measurement with the Nano Drop device in the PCR unit of the central laboratory at Lattakia University Hospital and the Atomic Energy Commission in Damascus.

CDNA Synthesis: The Revert AID First strand CDNA kit was used, where 5 ML of RNA from the sample was added to the kit, which contains a random hexamer primer mix that works on complete conversion of the RNA to the CDNA.

Quantitatve reverse transcription

Polymerase chain reaction QPCR:

The change in the level of gene expression of the HOTAIR molecule in patients was assessed by performing a qPCR reaction using the rotor gene (Qiagen, Germany), primers were designed by the Atomic Energy Commission of the company macrogen using the OLIGO program for the HOTAIR molecule:

For word (GGTAGAAAAAGCAACCACGAAGC) reverse (ACATAAACCTCTGTGTGTGAGTGCC). Primers have been designed for GAPDH, which is

from the house keeping gene (HKg): For word (GTGAAGGTCGGTGTGTGAACGG) reverse (GATGCAGGGATGATGTTCTG).

The Rox qPCR Mastermix/Maxima SYBR Green kit was used according to the generalizations mentioned in the kit where the protocol was as follows: setting up 10 minutes at a temperature of 95 C, then 40 heat cycles 15 seconds at a temperature of 95 then 1 minute at a temperature of 60 C. Melting Carve was performed to ensure the quality of the PCR products and then the calculation of gene expression was done using the Livak Equation.

5. Statistical Analysis

Statistical analysis was performed using IBM SPSS program (version 25).

Descriptive data were measured by means with standard deviation (SD). The T-student test was used to compare two groups and the ONE-WAY ANOVA test to compare several groups. Results were considered statistically significant when (p-value < 0.05).

6. Results

The mean fold change of gene expression (F-ch) was 8.14 ± 6.77 in patients compared to 1.017 ± 0.185 in controls with a statistically significant difference (p-value < 0.05).

There were 5 (16.6%) patients in grade I of the tumor, 14 (46.6%) in grade II and 11 (36.6%) in grade III. The mean F-ch of gene expression was 2.157 ± 0.33 in grade I group, 5.61 ± 3.42 in grade II, and 13.60 ± 7.80 in grade III. We found that gene expression had increased with high tumor grade (p-value < 0.05) (Table 1).

Table 1. Comparison according to tumor grade.

Tumor grade		Mean ± SD	P value
Grade I	n:5	2.157 ± 0.33	
Grade II	n:14	5.61 ± 3.42	0.003
Grade III	n:11	13.60 ± 7.80	

There were 18 (60%) patients in HER2 (0) group, 6 (20%) in HER2 (Low) group and 6 (20%) in HER2 (High) group. The means F-ch of gene expression were 4.56 ± 2.46, 8.90 ± 5.76, 17.63 ± 8.23, respectively, which indicated that gene expression increased as HER2 expression elevated (Table 2). In addition, 9 (30%) patients were in HR(−) group and 21(70%) in HR(+) group, the means F-ch of gene expression were 12.78 ± 9.66 and 6.006 ± 4.15 respectively. P-value recorded less than 0.05, which indicated that gene expression increased with negative HR (Table 3).

Table 2. Comparison according to HER2 receptors.

HER2 receptors		Mean ± SD	P value
(0)	n:18	4.56 ± 2.46	0.004
(Low)	n:6	8.90 ± 5.76	
(High)	n:6	17.63 ± 8.23	

Table 3. Comparison according to HR receptors.

HR receptors		Mean ± SD	P value
(–)	n:9	12.78 ± 9.66	0.001
(+)	n:21	6.006 ± 4.15	

7. Discussion

In our study, by measuring the gene expression of the HOTAIR molecule in the plasma of patients and comparing it with the gene expression in the plasma of controls, it was higher in patients with an important statistical difference, which suggests its role in the development of breast cancer and explains the tumor role through which HOTAIR affects the process of apoptosis, which is considered one of the most inviolable operations over the years of development, and this is manifested in the histological homeostasis caused by the harmony between cell multiplication and death. This process is controlled by two types of proteins, Pro-apoptotic like (Bad, Bax) and Anti-apoptotic like (Bcl1, Bcl2). *Zhao et al*. found that the HOTAIR molecule disrupts this compatibility by controlling certain pathways where it affects the HMG2 gene (one of the main genes contributing to the development and growth of the body). In cancer cells, the gene expression of the HOTAIR molecule increases and this acts to inhibit the miR- 20- 5a molecule, which leads to an increase in the gene expression of HMGA2 [17]. By the same mechanism, *Ding et al*. found that the HOTAIR molecule affects the (Bcl-w) gene (which is the gene opposite to the occurrence of cell death), where HOTAIR overexpression suppresses the effect of miR-601 on the Bcl-w gene and thus enhances the expression of the Bcl-w gene [18]. By studying the correlation between the level of gene expression of the HOTAIR molecule and the tumor grade, it was found that there is a proportionality between them so that the higher the tumor grade, the higher the expression of HOTAIR, this is in line with many experimental studies on cell lines that have demonstrated the role of HOTAIR in maintaining cancer cells in stem state and inhibiting the differentiation process. *Deng et al*. studied the effect of the HOTAIR molecule on a group of genes related to the cessation of differentiation and maintenance of cells in stem state (SOX1, SOX10, OCT4), and found that the HOTAIR molecule activates these genes and stops their inhibition via miR-34a, as the overexpression of the HOTAIR molecule inhibits miR-34a [19]. *Lie et al*. reported that HOTAIR targets the P53 gene (described as the protector of the genome and regulates the cellular cycle) and affects its transcription-inducing binding of the P21 gene encoding protein P21, which inhibits a number

of cyclins and protein kinases, especially CDK2, responsible for the cell transition from phase G1 to phase S [20]. Thus, it prevents the occurrence of cellular hyperproliferation, and in the case of high HOTAIR expression, it silences the P53 gene and stops the transcription of the P21 gene. Our study also clarified the relationship between the level of gene expression and the HER2 receptors as it was found that there is a strong correlation between the level of gene expression of the HOTAIR molecule and the positivity of the HER2 receptors (related to poor prognosis). *Wang et al*. reported the role of the HER2 receptors in stimulating B-catenin, which in turn stimulates the expression of HOTAIR by binding to the LEF/TCF sites in the catalytic region of the HOTAIR molecule [21]. *Yuan et al*. confirmed the existence of a relationship between HOTAIR and HER2, where they found a decrease in HOTAIR expression after treatment with HER2 inhibitors [22]. In the approach of our study on the relationship of the expression level of the HOTAIR molecule and hormonal receptors, it was found that there is a statistically significant relationship between the expression level of HOTAIR and the passivity of hormonal receptors, and this study agreed with *Tang et al*. study who found that high HOTAIR expression is closely related to poor prognosis depending on the molecular classification of breast cancer [23]. Based on previous findings, our study supports the possibility of targeting the HOTAIR molecule to improve therapeutic response.

8. Conclusion

It was found that the gene expression level of HOTAIR molecule may have a prognostic significance in the diagnosis of breast cancer and the characteristics of the tumor responsible for increasing tumor virulence and poor prognosis.

Declarations

Ethical Approval

This research received approval from the Scientific Research Committee at Lattakia University and Lattakia University Hospital.

Disclosures

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Conflict of Interest

The authors declare that there are no conflicts of interest with respect to the pub-

lication of this manuscript.

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