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THE PREDICTIVE ROLE OF GENE EXPRESSION LEVEL (LNCRNA HOTAIR) IN THE THERAPEUTIC RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN BREAST CANCER PATIENTS

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ABSTRACT

Background: Neoadjuvant chemotherapy is considered before therapeutic strategies in early-staged tumors to reduce size, lymph node metastases and improving the chance of performing conserrative breast tissue surgery. The role of Long non-coding RNA LncRNA molecules has been studied in breast cancer. The HOTAIR molecules is considered one of the first molecules studies, as several experimental studies have clarified its role in disease mechanisms due to the occurrence of hyper-proliferation local invasion tumor, metastasis, and lack of response to pharmaceutical treatment. Our study aims to evaluate the gene expression level of HOTAIR molecule in the plasma and its relationship with complete tissue response to treatment. **Materials and Methods:** An assay was performed for the level of the HOTAIR molecule in the plasma of 30 patients by performing a reverse transcription reaction qPCR. Gene expressional level was evaluated by using LIVAK method The occurrence of a complete histological response was determined through the histology report after surgery. Then a statistical analysis is performed. **Results:** The results showed significant difference (0.005 > p) between the level of gene expression of HOTAIR molecule and the Occurrence of a complete tissue response to treatment.

KEYWORDS: Breast Cancer, HOTAIR, neoadjuvant therapy, LncRNA**.**

INTRODUCTION

The targeted therapeutic strategies used have brought about a remarkable development in reducing the mortality rate during the last 10 years in breast cancer despite the increase in the number of infections. This motivates experimental studies aim to find new molecular studies that contribute to determining tumor or prognosis. They may be targeted therapeutically in promising strategies and with the development of targeted therapies, chemotherapies are still form cornerstones of treatment. Over the past years it has undergone several modifications.

(Neoadjuvant chemo therapy NAC); It was initially used in cases that are not enable to surgery.[1] Currently it is used in early-stage patients to reduce tumor size, lymph node metastases and improving the chance of performing surgery which the breast tissue is preserved as much as possible. Many drug combinations have been tested. Since the participating has started between anthracycline compounds and Taxan compounds in early 2000s, the rate of complete histological remission increased (complete response pathologic (CRP).[2])

However, chemotherapy resistance remains the biggest challenge and the most common cause of poor prognosis in breast cancer. This is due to the heterogeneity of molecular mechanisms that stimulate of un explained drug resistance.

Therefore, many experimental studies are being conducted to find new molecular markers useful in predicting the prognosis of response to NAC this is to faciliate the selection of candidates to benefit from the treatment.

(long non-coding RNA LncRNAs): A type of LncRNAs molecule that is more than 200 nucleotides in length and It doesn't symbolize a specific protein.[3] Several experimental studies have implicated level disorder such as breast[4], oropharynx[5] and ovarian cancer.[6]

Current studies are interested in explaining its role in pathogenic mechanisms of cancer and its relationship to poor prognosis. Experimental evidence has unanimously agreed on its role in causing tumor formation, tumor angiogensis and hyper proliferation.[7] In addition to the

occurrence of drug resistance[8] and the stimulation of metastases.[9] These molecules influence epigenetic mechanisms through which they control gene expression, this is on several levels before gene transcription and at the transcription and this translation also works through this to silence genes and stimulate others.

There are several mechanisms: [I] It can play a constructive role scaffold. It binds chemical compounds and it effects histone.[10] [II] It may work to hide stimulating sites located on a DNA tape.[11] [III] It can play a competitive role in cellular signaling pathways with a number of molecules, The most important to which is miRNA which plays a significant role in regulating gene transcription.[12] Through these mechanisms cellular reproduction, tumor immune-evasion and drug resistance have been affected.[13]

One of the most important molecules currently being studied in breast cancer is (HOX Transcript Antisense Intergenic RNA HOTAIR) which derived from HOX11 and HOX12 gene. It is located on the chromosome 12p13.13.

It was discovered in 2007 and considered one of the genes that has been preserved over hundreds of years.[14,15]

Interventional studies were conducted to clarify the role of its high expression level in breast cancer diagnosis and prognosis.[16,17] In addition to its role in radiation, endocrine and chemical treatment resistance.[17]

They interfere with a number of signaling pathways and stimulate the transcription of a number of oncogenes such as HER2[18], VEGF[19], Vimentin[20] and B-catnin.[21]

It may be linked to a number of chemical compounds that act on histone acetylation thus silencing a number of tumor suppression genes [P53, P21, PENT).[22]

Recently, studies are benig conducted to determine the level of gene expression of the HOTAIR molecule in the patient's plasma in addition to searches its relationship to poor prognosis.[23, 24, 25]

In our study to examine the relationship between the gene expression level of HOTAIR molecule and complete histological response to treatment NAC.

2- MATERIALS AND METHODS

1-2- Sample collection: In this study samples were collected from 30 patients newly diagnosed with breast cancer and candidates for preoperative supportive therapy Neoadjuvant therapy before performing any therapeutic intervention between August 2022 and April 2023 at Tishreen University Hospital, Lattakia (Oncology Center).

Inclusion Criteria

1. Newly diagnosed patient.
2. Age over 19 years.
3. No previous therapeutic interference.

Exclusion Criteria

1. Other previous malignancies.
2. previous chemotherapy or glandular treatment
3. autommune diseases.
4. Age under 18 years.

The chemotherapy followed by steps of Cyclosphosphamid/ Doxorubicin/ Docetaxel/ Aparamend/ Neakin/ Poclitaxel. Surgery was performed after supportive / neoadjuvant treatment and determining the presence of complete histological response in patients. Blood samples were drawn into EDTA tubes for testing qPCR.

2-2- RNA extraction: After collecting the samples on EDTA tubes, they were weighted a speed of 12000 g, degrees celsiuse C4 for ten minutes then we transfer the supernatant to the working tubes. A Triazol kit was used to extract all the RNA and this is according to the instructions included in the kit. The concentration and purity of the RNA were confirmed by measuring with a Nano Drop in the PCK unit in the central laboratory at Tishreen university Hospital and Atomic Energy Authority in Damascus.

3-2- cDNA Synthesis: Revert AID first strand CDNA was used, where 5 mk of RNA from the sample was added to the kit, which contains aprimer hexamer primer mix random which works to c convert totalRNA into cDNA.

4-2- Quantitative reverse transcription Poly merase chain reaction Q PCR: The change in HOTAIR gene expression level of patients was evaluated by per forming a qPCR reaction using (Qia gen, Germany, generotor).

primers were designed by He Atomic Energy Authority of mocroyen company by using OLLGO device for the HOTAIR molecule:
For word [GGTAGAAAAAGCAACCACGA].
Reverse [ACATAAACCTCTGTGTGTGAG].
Primers were designed for GAPDH and it was from H kg genes (House keeping gene).
For word:
[GTGAAGGTCGGTGTGTGAACGG].
Reverse:
[GATGCAGGGATGATGTTCTG].

Master mix/maxima/ S YB R Green ROXqPCR kit was used. This is according to the instructions mentioned within the kit where the protocol was as follow: Preparation for 10 minutes at a temperature of 95 C°, then 40 thermal cycles of 15 C°, then 1 minute at 60 C°. Melting care was used to ensure thequslity of PCR

prodests. Then the gene expression calculation was performed using the levak equation $AACT^{-2}$.

RESULTS AND DISCUSSION

AqPCR analysis was performed for the patients to evaluate the change in gene expression of HOTAIR molecule.

The presence of a complete tissue response after surgery was determined and the resulte were analyzed statistically via a SPSS program.

Average difference test between patients who responded to treatment

Report			
F-CH			
Patients	Mean	N	Std. Deviation
Not respondent	9.6600	18	7.53369
Respondent	3.7167	12	2.26796
Total	7.2827	30	6.63264

ANOVA Table							
			Sum of Squares	df	Mean Square	F	Sig.
F-CH* patients	Between Groups	(Combined)	254.327	1	254.327	6.972	.013
	Within Groups		1021.439	28	36.480		
	Total		1275.766	29			

The average change of F-CH in patients who didn't respond to the treatment reached 9.60 (Std 7.53369) while the average change of F-CH in patients responding to treatment reached 3.7167 (Std 2.2679) with statistical significance value was 0.013 which is less than the significance level 0.09.

Therefore, the differences are statistically significant.

We can explain these results through international experimental studies that were conducted on the mechanisms of action of HOTAIR and its interaction on a number of chemical compounds, including: (Anthracycline, Taxan, Gemcitabine and carboplatin.[26],[27]

In a study conducted by L.V and others on the level of HOTAIR molecule expression in the circulation of breast cancer patients who underwent chemotherapy NAC with the participating of drugs between Anthra and Taxan.

They found that the increasing of HOTAIR molecule expression level was accompanied by alack of response to chemo therapy.[28]

An experimental study conducted by Yaun et al on the role of HOTAIR molecule expression level in the resistance on Cisplatine which found that it opposes miR-126 which works to suppress a number of tumor pathways VEGE/P13K/AKT/MRP1 or the pathway BIK3R2/P13K/AKT/MRP1.

These pathways activate the entry of cells from G_1 phase in to S phase, which contributes to tumor development and cellular hyper proliferation and suppress programmed cell death, hence the rise of HOTAIR expression leads to the activation of these on cogenic pathways.[29]

A study by LIU et al about mechanisms of resistance to chemical treatment found that the rise of HOTAIR expression was accompanied with gene suppression P21 which inhibits the confinement of cells in G_0/G_1 phase and inhibits cell death.[30] The HOTAIR also works to silence a number of genes where Fang and et al found that It works on the methylation of islets CGP in the promoter regions of gene transcription HOTAIR.

These genes stimulate cellular differentiation and confine cells to the cellular circut. As well as stimulating programmed cell death.[31] In a study on treatment with Pa Clitaxel conducted by Xiao et. al which works to block cells in G_1 as well as inhibiting the cellular pathway Wnt/contenin.

This study found that the high of HOTAIR suppresses the miR-203-3 pmolecule, which inhibits the cellular pathway Wnt/B-cotenin, therfore the high of HOTAIR expression level activates the a fovementioned pathway. Thus stimulating resistance to treatment with compounds [Paclitaxel].[32] There was also a pole for the high of molecule expression in drug resistance 5 Fu in a study by LI et al. He found that it p act to recruit the compound PCK 2 which inhibits miR-218-2 molecule which lends to activation of Ts/NF-KB, which activates genes Bcl-XL, Bcl2 which inhibits cell death.[33]

CONCLUSION AND RECOMMENDATIONS

Through our current study we found that the high of HOTAIR molecule expression has been valuable for NAC therapeutic response (Neoadjuvant chemotherapy).

Due to the molecular mechanisms of action of HOTAIR in a number of cellular pathways. That affects regulatory genes of cellular circuit. This encourage future studies to develop interventions that conteract the effect of HOTAIR to improve therapeutic response.

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