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Research Article

ROLE OF LONG NON CODING RNAS (HOT AIR) AS A BIOMARKER IN BREAST CANCER

[1,*]Mohy el din Abd el Fattah,[2]Nashwa Nagy El-Khazragy and [3]Neama dahee Hassanen

[1]Suez Canal University, Faculty of science chemistry department, Ismailia,Egypt
[2]Department of Clinical Pathology/Heamatology, Faculty of Medicine-Ain Shams University, Egypt
3 Suez Canal University, Faculty of Science, Chemistry department , Ismailia , Egypt

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Abstract

In recent years, increasing numbers of long non-coding RNAs (lncRNAs) have been identified as playing an important role in breast cancer progression. Here, the long non Coding RNA (HOTAIR) expression was studied as a potential biomarker in primary localized breast cancer. Patient were divided into two groups, the first is the study group, includes 50 patients with primary localized breast cancer while the second control group includes 50 normal females of matched age to the study group. Samples of whole blood were collected by vein puncture in a gel Vacutainer tube and processed for tumor markers study, Carcinoembryonic antigen (CEA) and Cancer antigen (CA-15.3) and the long non Coding RNA (HOTAIR).Tissue samples obtained from study group for histopathological examination and determination of Immunohistochemistry, Estrogen receptors (ER), Progesterone Receptors (PR) and Human Epidermal Receptor-2 (HER2)which have been assessed as risk factors for breast cancer.Our results showed a high significant difference in the expression of Lnc-HOTAIRby two folds up-regulation in breast cancer patients compared to control group. Also we founded no significant association between any of the tumor receptors, and Lnc_HOTAIR gene expression.

Keywords: Breast cancer, Lnc_HOTAIR, Estrogen receptors, Progesterone Receptor.

INTRODUCTION

Breast cancer is the most common tumor found in women worldwide (DeSantis, Ma *et al.,* 2017, Bray, Secker *et al.,* 2018). Although comprehensive treatment for breast cancer includes surgery, chemo therapy, radiotherapy and hormone therapy, its till entails poor outcomes for advanced breast cancer patients (Bleyer and Welch 2012).Lack of efficient biomarkers to indicate prognosis and chemotherapy resistance are therefore two crucial factors that can lead to the progression of disease (Dawson, Tsui *et al.,* 2013, Tang, Zhao *et al.,* 2017). Thus, it is incumbent upon us to discover appropriate diagnostic and prognostic biomarkers. One of these biomarkers may be found in the use of exosomes.Exosomes are small membrane vesicles (30–150 nm) that originate from the endosomal membrane compartment (Regev-Rudzki, Wilson *et al.,* 2013, Wang, Chen *et al.,* 2016) and cells can release exosomes that then persist in body fluids (Dear, Street *et al.,* 2013). Exosomes contain important biologically active species, including miRNA, lncRNA and circRNAs(Thoms, Thomson *et al.,* 2015). Investigators have recently demonstrated that exosomes not only play key roles in cell-cell communication, but also in the progression of tumorigenesis and tumor metastasis (Luga, Zhang *et al.,* 2012, Boelens, Wu *et al.,* 2014). Although exosomes – especially lncRNA – also contribute significantly to tumor metastasis and chemotherapy resistance, they remain largely uncharacterized. Recently, there is an explosive expansion in the understanding of biological function of ncRNA transcripts, exemplified by the significant role of microRNAs (miRNAs) in various human diseases including cancer. Recently, other types of short or long ncRNAs, especially the long noncoding RNAs (lncRNAs), have attracted attention due to their large number and biological significance (Gupta and Weitzman 2010). lncRNAs defined as being at least 200 nucleotides in length, lncRNAs are characterized by their abundant presence in the human genome, as well as their tissue-specific expression patterns. Moreover, lncRNA scan affect various physiological processes such as cell cycle regulation, cell apoptosis and survival, cancer migration and metabolism (Chen, Huang *et al.,* 2011).Long non-coding RNA (lncRNA) genes are an important population of non-coding RNAs with defined key roles in normal development as well as tumorigenesis process. Evidences suggest that they can be classified as tumor suppressor genes or oncogenes according to their functions and expression pattern in tumoral tissues. They have been shown to regulate the plasticity of cancer stem cells(Wang, Mo *et al.,* 2017).Long non-coding RNA Their important roles in the regulation of cancer-related pathways in addition to deregulation of their expression in a number of cancers have suggested that they can be used as markers for cancer detection and prognosis, as well as targets for cancer treatment (Deng, Yang *et al.,* 2017). LncRNAs are a class of non-coding RNAs with biologic function, but which cannot be translated into protein (Lee and Kikyo 2012). HOTAIR is an lncRNA of 2.2 kb in length that is transcribed from the antisense strand of the HOXC gene cluster present in chromosome 12 (Rinn, Kertesz *et al.,* 2007, Bhan and Mandal 2015). Increasing evidence indicates that expression of HOTAIR correlates with malignant tumors, including breast cancer (Gupta and Weitzman 2010, Sørensen, Thomassen *et al.,* 2013), ovarian cancer (Nakayama, Shibazaki *et al.,* 2013), hepatocellular carcinoma (Yang, Zhou *et al.,* 2011) and renal carcinoma (Wu, Liu *et al.,* 2014). Recent studies have revealed that HOTAIR was expressed at a high level in breast cancer tissues compared with adjacent non-tumorous tissues (Gupta and Weitzman 2010, Sørensen, Thomassen *et al.,* 2013), and that over expression of HOTAIR promoted breast cancer cell proliferation, invasion and migration (De Stefani, Boffetta *et al.,* 2007, Chisholm, Wan *et al.,* 2012).Although much has been published on HOTAIR and exosomes few publications mention the relationship between the two. Wang and his colleagues demonstrated that HOTAIR exists in serum exosomes, and serum exosomal HOTAIR has the potential of developing into a diagnostic and prognostic

Corresponding Author:** ***Mohy el din Abd el Fattah; Department of Organic chemistry, Suez Canal University, Faculty of science chemistry department, Egypt.

biomarker in laryngeal squamous cell carcinoma (Yuan, Yang *et al.,* 2014). Few studies have depicted the value and function of exosomal HOTAIR in breast cancer patients; however, it remains largely uninvestigated. In the present study, we uncovered the generation of HOTAIR in exosomes and evaluated the diagnostic and prognostic value of breast cancer exosomal HOTAIR. Deregulation of a number of lncRNAs, such as HOTAIR, XIST, MALAT, and H19 has been detected in breast cancer samples and cell lines. In addition, the association between lncRNAs signature and breast cancer patients' survival has been assessed in various studies (Li, Zhai *et al.,* 2016). Here, the expression patterns of lncRNAs in breast cancer, as well as their significance in prognosis and patient treatment are discussed (Zhao, Zhao *et al.,* 2016). HOTAIR "HOX transcript antisense RNA" is a trans-acting lncRNA that regulates different target loci including tumor suppressor genes (Hajjari and Salavaty 2015).Owing to this regulatory mechanism, lots of the studies reported the role of HOTAIR in progression of different cancers such as breast, colon, and gastric cancer (Hajjari and Salavaty 2015).It was found the dysregulation of this long transcript in breast tumors (Gupta and Weitzman 2010). Then, other studies showed that HOTAIR is associated with poor prognosis, metastasis, invasion, and short overall survival of breast cancer. Moreover, it was demonstrated that HOTAIR is a marker for lymphatic metastases in ER-negative patients (Gökmen-Polar, Vladislav *et al.,* 2015).In spite of the great significance of HOTAIR as a biomarker in cancer; only few studies with limited number of samples indicating the differentiated expression of HOTAIR in breast tumors compared to normal tissues. We believe that the differential expression of HOTAIR may indicate the potential role of this lncRNA in cancer initiation and progression (Haemmerle and Gutschner 2015).

MATERIAL AND METHODS

The current study was conducted on 100 individuals at Global Research Labs. They classified into two subgroups. The breast cancer cases constitute 50 patients; samples collected from patients after diagnosis has been confirmed by histopathology based on immune-histochemical analysis as well as Computerized axial tomography (CT scan), mammography and Magnetic resonance imaging (MRI). The patients were compared to

Group II: 50 healthy controls who were matched for age and sex.

Group I: included patients selected from the Ain Shams Internal Medicine hospital, oncology department in the period from March 2018 to March 2019. After taking the approval of research ethics committee of Faculty of medicine, Ain Shams University, Patients were diagnosed as Breast Cancer before receiving any treatment in form of Chemotherapy or Radio-therapy. They were diagnosed on the basis of histopathology and radiological picture such as CT scan.

All patients will be subjected to the following:

- Detailed history from each patient, with special reference to present and past family history.
- Full patients Clinical and laboratory data will be collected from patient data sheets.
- Sample collection: 3 ml of whole blood will be collected by vein puncture in a gel Vacutainer tube. The collected samples will be centrifuged at 4000 rpm for 10 minutes at room temperature; serum samples will be stored at at -80ºc for RNA Extraction.
- Laboratory Tests:
  - Tumor markers: Carcinoembryonic antigen (CEA) and Cancer antigen (CA-15.3) will be measured using chemoluminesence technique (*Abbott Laboratories; Germany*).
  - Histopathology: histopathological examination on Formalin fixed Paraffin blocks will be held for diagnosis and scoring.

Sample collection and preparation

3ml of whole blood was collected by venipuncture from all enrolled subjects in gel vacutainer, centrifuged at 4000 rpm for 20 minutes and stored at -20^{o} C until analyzed. Immunohistochemistry: Estrogen receptors (ER), Progesterone Receptors (PR) and Human Epidermal Receptor-2 (HER2) will be examined in tissue using specific polyclonal antibodies TB was formalin-fixed within 4–8 hrs for 6–48 hrs (minimum 6 hrs). IHC was performed, following epitope retrieval, with a polymer based detection system (Envision plus, Dako, Carpinteria, CA) using mouse monoclonal antibodies for ER and PR (Dako, Carpinteria, CA), ER (1D5; 1:50), PR (PgR636; 1:400), and Herceptin kit (HercepTest, Dako, Carpinteria, CA) according to the manufacturer's instructions. For ER and PR, antigen retrieval is performed as follows: sections were deparaffinized and rehydrated with deionized water. They were then heated in citrate buffer (pH 6.0), using an electric pressure cooker for 3 minutes at 12–15 pounds per square inch (PSI) approximately at 120°C, and cooled for 10 minutes prior to immunostaining. All slides were loaded on an automated system (DAKO Autostainer) and exposed to 3% hydrogen peroxide for 5 minutes, incubated with primary antibody for 30 minutes, with labeled polymer (Envision® + dual link) for 30 minutes, 3,3′-diaminobenzidine (DAB) as a chromogen for 5 minutes, and hematoxylin as counter stain for 5 minutes. These incubations were performed at room temperature. Between incubations sections were washed with Tris-buffered saline (TBS). Cover-slipping was performed using the Tissue-Tek SCA (Sakura Finetek USA, Inc, Torrance, CA) cover slipper. Positive controls of known positive tissues (endometrium and breast) and negative controls with primary antibody replaced with TBS are run with the patient/study slides. Nuclear staining in more than 10% of tumor cells was considered positive for ER and PR. Antigen retrieval for HER2/neu using Hercep Test is performed by immersing and incubating the slides in 10mmol/L citrate buffer in a calibrated water bath (95–99°C) for 40 (± 1) minutes. After decanting the epitope retrieval solution, the sections are rinsed in the wash buffer, and later soaked in the buffer for 5–20 minutes prior to staining. The slides are loaded onto the autostainer using the Hercep Test program™ as described in the manufacturers' insert. In the autostainer, the slides are rinsed, followed by 200 µL peroxidase-blocking reagent for 5 minutes, followed by rinsing and then placed in 200 µL primary anti-HER2 protein (or negative control reagent) for 30 minutes, rinsed twice and finally immersed in 200 µL substrate chromogen solution (DAB) for 10 minutes. The slides are counterstained with hematoxylin, and finally coverslipped. HER2 results were determined based on the maximum area of staining intensity, according to the package insert and

ASCO/CAP guidelines as follows: strong circumferential membranous staining in > 30% of invasive carcinoma cells = 3+; moderate, circumferential membranous staining in ≥ 10% of invasive tumor cells or 3+ in ≤ 30% of cells = 2+; weak and incomplete membranous staining in invasive tumor cells= 1+, no staining= 0. Tumors with 0 and 1+ staining were considered negative and cases scored as 2+ equivocal, and 3+ were considered positive, evaluated on 4× and 10× magnifications.

Molecular assay

Extraction and Purification of total RNA, including miRNAs, from whole serum Total RNA was extracted from serum samples using RNeasy Mini Kit; cat no: 74104 (Qiagen, Hilden, Germany).This procedure use the selective binding properties of silica-based membrane with the speed of micro spin technology. High salt buffer system, which allows up to 100µg of RNA longer than 200 bases to bind to the RNeasy silica membrane. Firstly, the biological samples was lysed and homogenized in the presence of highly denaturing guanidine-thiocyanate–containing buffer, which inactivates RNases to ensure purification of intact RNA. Ethanol was added to provide appropriate binding conditions and then the samples were applied to RNeasy Mini spin column where the total RNA binds to the silica membrane and contaminants was washed away. Finally, High-quality RNA was eluted in 30-µl water.TheRNeasy Mini Kit, RNeasy Protect Mini Kit should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions. Preparation of working solutions (prepared freshly)

Procedure

- 350 µl of RLT buffer were added to an Eppendorf
- 200 µl of serum were added to the buffer
- 550 µl of 70% ethanol were added.
- The mixture was mixed by pipetting and 700 µl of it were transferred into RNeasy Mini spin column.
- Mixture was centrifuged for 30 seconds at ≥8000 x g, and the flow-through was discarded
- The rest of the mixture was transferred into the spin column, then it was centrifuged for 30 seconds at ≥8000 x g, and the flow-through was discarded.
- 700 µl of RW1 buffer were added to the spin column, then it was centrifuged for 30 seconds at ≥8000 x g, and the flow-through was discarded.
- 500 µl of RPE buffer were added to the spin column, then it was centrifuged for 30 seconds at ≥8000 x g, and the flow-through was discarded.
- 500 µl of RPE buffer were added to the spin column, then it was centrifuged for 2 minutes at ≥8000 x g, and the flow-through was discarded.
- The RNeasy spin column was placed in a new 1.5 ml collection tube.
- 30 µlRNase-free water were added directly to the spin column membrane, and centrifuged for 1 min at ≥8000 x g to elute the RNA, then RNA was stored to be used later Reverse transcription of mRNAs and miRNAs.RNA samples that were extracted from patients was reversibly transcribed using miScript II RT kit; (Qiagen; Hilden; Germany) (Cat no. 218161).The miScript II RT Kit, miScript SYBR Green PCR Kit, and miScript PCR Starter Kit are shipped on dry ice. The kits, including all reagents and buffers, should be stored immediately upon receipt at –20ºC in a constant-temperature freezer.

RNA templates were thawed on ice, and centrifuged briefly to collect residual liquid from the sides of the tubesReverse transcription master mix was prepared by adding 2 µl of 5x miScriptHiFlex Buffer, 1 µl of 10x miScript Nucleics Mix, 1 µl of RNase-free water, and 1 µl of miScript Reverse Transcriptase Mix5 µl of RNA template were added to each eppendorf contained master mix, mixed gently, and were centrifuged. The mixtures were incubated for 60 minutes at 37o C. The mixtures were incubated for 5 minutes to inactivate reverse transcriptase. Mixtures were transferred undiluted to -20o C freezer to be stored till applying real time PCR

Quantitative real time polymerase chain reaction (qRT-PCR)qPCR amplification of Lnc_HOTAIR: Relative Lnc-RNA expression of the candidate Lnc_HOTAIR was analyzed by using using RT2 LncRNA PCR primer assay (Qiagen, Germany) (Cat. no. 330520) and Hs_ACTB_1_SG QuantiTect Primer Assay cat no: 249900, ID: QT00095431 as housekeeper gene (Qiagen, Germany). All samples were analyzed using the 5-plex Rotor-Gene Real-Time PCR Analyzer (Qiagen, Germany). RT2 long non-coding RNA (lncRNA) qPCR Assays are shipped at ambient temperature but must be stored at –20°C upon arrival. When stored under these conditions and handled correctly, the product can be kept for at least 1 year from date of receipt without reduction in performance.The RT2 SYBER Green Mastermix, RT2 LncRNAqPCR, and cDNA synthesis reaction were briefly centrifuged for (10-15 s) to bring the contents to the bottom of the tubes, The PCR components were then prepared in a nuclease-free tube The PCR components mix were briefly centrifuged and the tubes were placed into the real-time cycler. The real-time cycler was then programmed according to table 10, depending on the real-time cycler used

Calculations

Moreover, the expression levels were normalized to B-actin levels as a reference gene. The relative expression level (fold change) for HOTAIRwas then calculated using the equation $2^{-\Delta\Delta Ct}$ test control as follows:

Relative quantitation (RQ) = $(2)^{-\Delta\Delta CT}$

Where $\Delta\Delta C_T = (C_{T\ HOTAIR} - C_{T\ B\text{-}actin})_{patient} - (C_{T\ HOTAIR} - C_{T\ B\text{-}actin})_{control}$

Statistical Data Management and Analysis: The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 22 for windows; SPSS Inc, Chicago). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

RESULTS

Demographic data

The current study was enrolled on 100 subjects, they classified into two subgroups. The breast cancer cases constitute 50 patients; samples collected from patients after diagnosis has been confirmed by histopathology based on immune-histochemical analysis as well as Computerized axial tomography (CT scan), mammography and Magnetic resonance imaging (MRI). The patients were compared to 50 healthy controls that were matched for age and sex.

Table 1. Comparative analysis between the expression levels of Lnc_TUG1 in different studied groups

	Studied groups		Mann-Whitney test	
	Control N=50 Median [range]	Breast cancer N=50 Median [range]	Statistics	P value
Lnc_ HOTAIR expression [log^{10}]	0.7 [0.03 – 2.1]	1.2 [0.5 – 2.4]	U=96	p=0.001 [HS]

U: Mann Whitney test value;*= test is significant at level <0.01; ǂ= test is significant at level<0.05

Table 2. Prediction potential of Lnc_HOTAIR in breast cancer patients (ROC Curve)

Parameter	AUC	95% CI	P value	Cut-off value	Sensitivity (%)	Specificity (%)
Breast cancer /Controls	0.92	0.8– 0.98	0.001	1.3	76	84

AUC: Area under the curve; ROC: Receiving operator Characteristics, CI: Confidence interval

We test the normality for age; the data was normally distributed. For this reason, we represent the data in mean value and standard deviation form. Matched age and gender was observed between Breast cancer patients and healthy control. The breast cancer patients (n=50) had a mean age of 50±11.2 compared to healthy control group which were younger (mean: 51 ±10), and range from 30 to 50. The study represents the distribution of different predisposing factors that may contributes to development on breast cancer, they include: age, menopausal status, Diabetes Mellitus and hypertension and positive family history for cancer, higher frequency of breast cancer group had negative family history for cancer (80%), age >50 years (56%), Hypertensive (68%) and diabetic (70%). Premenopausal status was predominant (52%) among the breast cancer group. Expression of the Lnc-HOTAIR in the studied subjects

The expression of Lnc_HOTAIR biomarker; was compared in de novo breast cancer patients versus the non-cancerous tissue. The data normalization was tested by normality test which shows that the biomarker expression is not normally distributed; thus, a Mann-Whitney statistics test for non - parametric values was applied. Our results showed a high significant difference in the expression of Lnc-HOTAIR ($p<0.01$). The Lnc_HOTAIR gene expression is up-regulated by two folds in in breast cancer patients (median: 1.2; range: 0.5 – 2.4) compared to control group (median: 0.7; range: 0.0.3 – 2.14) (Table 1). Data is illustrated in Table 1 and Fig 1

Diagnostic potential of Lnc_HOTAIR in breast cancer patients

In order to assess the diagnostic value of Lnc_HOTAIR in breast cancer; a Receiving operating characteristics curve was plotted. When comparing the expression level of Lnc_HOTAIR in breast cancer patients with healthy controls; we found that Lnc_HOTAIR is a good diagnostic biomarker in breast cancer; at optimum cut-off value of 1.3; the biomarker has a sensitivity of 76% and 84% specificity to differentiate Breast cancer patients from controls (AUC:0.92; p=0.001). Data are illustrated in Table 2, figure 2. Comparative analysis between different Clincopathological features of breast cancer patients for the expression of Lnc_HOTAIR. The breast cancer patients were categorized according to demographic criteria into standard and high risk groups; the expression of Lnc-HOTAIR was compared in both categories for each variable. The median value was used in the analysis because results are not normally distributed; therefore a Mann-Whitney test was conducted. Higher expression levels of Lnc_HOTAIR was significantly associated with advanced histopathological stage), and histopathological grade ($p<0.05$).

On the other hand, no significant association was demonstrated for the Lnc_HOTAIR expression between histopathological type (invasive ductal carcinoma vs medullary carcinoma and sarcomas. Considering, the tumor receptors for breast cancer, the estrogen receptors, progesterone receptors and Her2 receptors are assessed as risk factors for breast cancer. No significant association was detected between any of the tumor receptors, and Lnc_HOTAIR gene expression ($p<0.05$). Data are illustrated in figures (Fig 3, 4, 5 and 6).

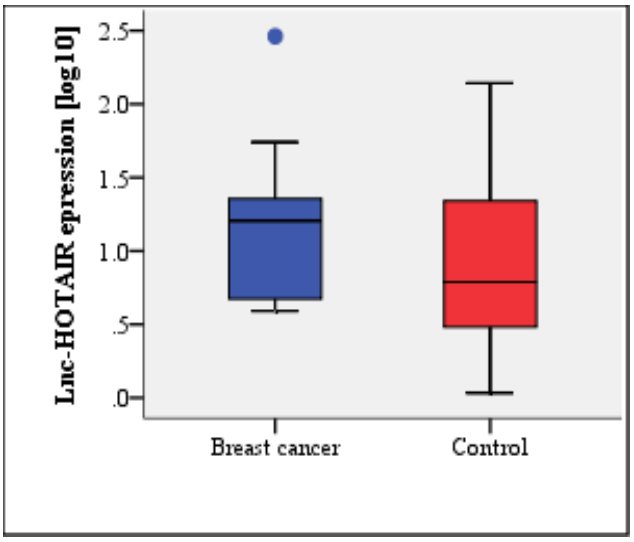

Figure 1. Expression levels of Lnc_HOTAIR in Breast cancer patients and Healthy controls

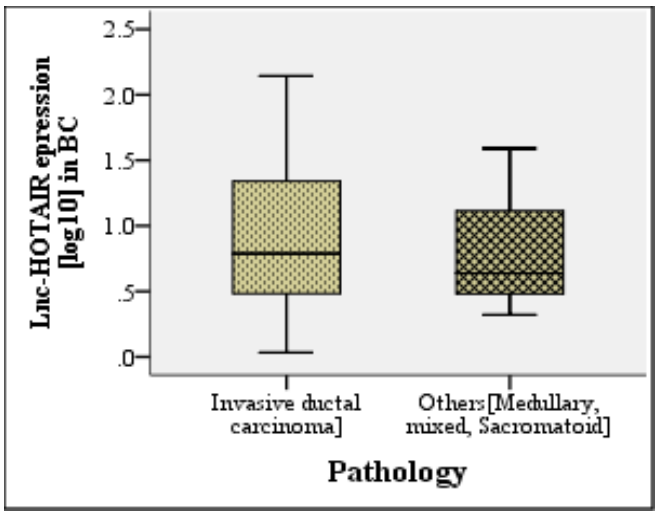

Figure 2. Boxplot graph illustrating non- significant association between the expressions of Lnc-HOTAIR in BC patients with different histopathological types

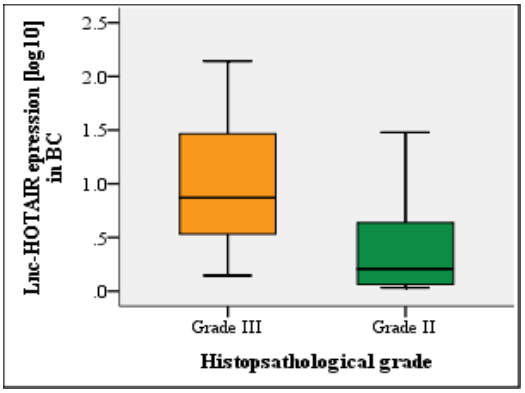

Figure 3. Boxplot graph illustrating a significant association between the expressions of Lnc-HOTAIR in BC patients with different histopathological grades

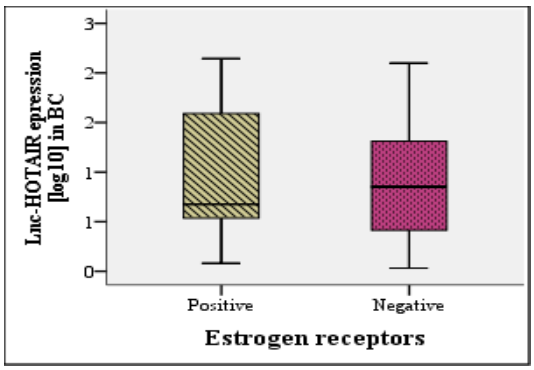

Figure 4. Boxplot graph illustrating non- significant association between the expressions of Lnc-HOTAIR in BC patients with Estrogen receptors

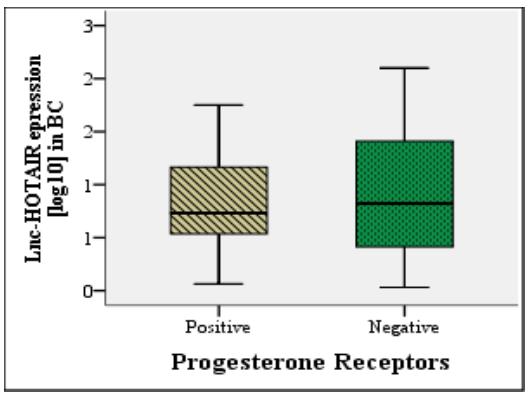

Figure 5. Boxplot graph illustrating non- significant association between the expressions of Lnc-HOTAIR in BC patients with progesterone receptors

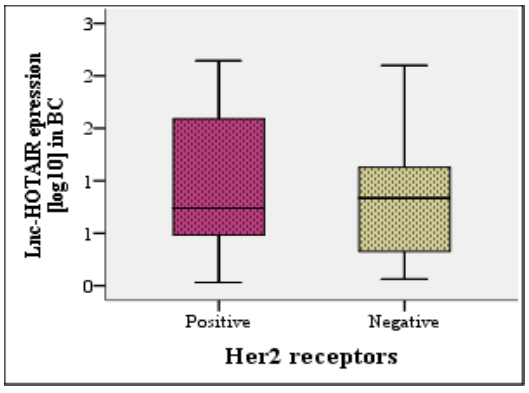

Figure 6. Boxplot graph illustrating non- significant association between the expressions of Lnc-HOTAIR in BC patients with Her2 receptors

DISCUSSION

Breast cancer (BC) is the most prevalent cancer type in women and a leading cause of cancer mortality in the world. Breast cancer is a very heterogeneous disease, and its histological classification is mainly based on the expression of hormonal receptors such as estrogen receptor (ER), progesterone receptor (PR), and ERBB2 receptor (HER2) (Lakhani, Ellis *et al.,* 2012). With respect to gene expression, BC is classified into five molecular subtypes including luminal ER positive (luminal A and luminal B), HER2 enriched, basal-like (also known as triple-negative breast cancer), and normal breast-like subtype (Sørlie 2004). Long non-coding RNA's role in cancer has been widely described, highlighting their capability to influence cell cycle regulation, cell proliferation, trans-differentiation, survival, immune response, metastatic progression, and therapeutic response (Guttman, Amit *et al.,* 2009). Moreover, many lncRNAs are transcriptionally regulated by key tumor suppressors or oncogenes (Huarte, Guttman *et al.,* 2010, Zheng, Do *et al.,* 2014). In cancer, lncRNAs are mainly involved in chromatin remodeling (Sanchez Calle, Kawamura *et al.,* 2018).

Being LncRNAs expressed in a specific manner in a type of cancer and regulating fundamental processes during tumor progression, they could represent not only exceptional diagnostic, prognostic, and predictive markers but also potential therapeutic targets. Many lncRNAs have been associated with BC, and most of them interfere with crucial processes during BC carcinogenesis (Youness and Gad 2019, Zhang, Hu *et al.,* 2019). LncRNA HOTAIR and Its Role in CancerHOX transcript antisense RNA (HOTAIR) is an lncRNA 2158 bp long, consisting of 6 exones, located on chromosome 12q13.13between HOXC11 and HOXC12 genes (Rinn, Kertesz *et al.,* 2007). Itspromoter contains binding sites for many transcription factors, such as AP1, Sp1, ERE elements, HRE elements, and NF-kB(Bhan and Mandal 2015). HOX transcript antisense RNA (HOTAIR) is a key regulator of chromatin status and a mediator of transcriptional silencing (Bhan and Mandal 2015).HOTAIR is overexpressed in epithelial ovarian cancer tissues and correlates with International Federation of Gynecology and Obstetrics (FIGO) stage, histological grade of the tumor, lymph node metastases, and poor survival (Qiu, Lin *et al.,* 2014). In cervical cancer tissues, HOTAIR is associated with clinical-pathological features, lymph node metastases, and prognosis (Huang, Liao *et al.,* 2014).Moreover, the detection of circulating levels of HOTAIR is strongly associated with advanced tumor disease, lymph nodes metastases, and poor survival in cervical cancer patients (Li, Wang *et al.,* 2015). For this study, we used a variety of public microarray databases and computation algorithms for the selection of genetically and functionally linked RNAs involved in breast cancer, at epigenetic level. We evaluated the expression level of HOTAIR in breast breast cancer patient compared to normal matched female group. The current study was conducted on 100 individuals at Global Research Labs. They were divided into two groups. Group I: included patients selected from the Ain Shams Internal Medicine hospital, oncology department in the period from March 2018 to March 2019. After taking the approval of research ethics committee of Faculty of medicine, AinShams University, a written informed consent was obtained from each patient parent after informing her about the steps of the procedure and the expected effects. Patients were diagnosed as Breast Cancer before receiving any treatment in form of

Chemotherapy or Radio-therapy. Demographic study of both groups shows non-significant deference in age of study group(50.2±11.2) compared to control group(50.5±10.5), In study group patient ≤50 years old were 28(56%), while patient >50 years old were 28(56%), According to menopausal Status 26(52%) were pre-menopausal while 24(48%) were post-menopausal, 40(80%) have no family history while 10(20%) have family history, 35(70 %) were diabetic while 15(30%) were none diabetic, 34(68 %) were not hypertensive while 16(32%) were hypertensive and according to HCV infection 34(68%) were negative for HCV Ab while 16(32%) were suffering from hepatitis C virus. Clinico-pathological characteristics of the breast cancer group show that according to histopathological type46(92%) of study group were suffering from Invasive ductal carcinoma while 4(8%) of other types(medullary, sarcomas), according to Histopathological grade 44(88%) cases defined as Grade II while 6(12%) cases defined as Grade III, Immunohistochemistry study of tumor receptor for study group show that Estrogen receptors were negative for18(36%) of patient while it were positive for 32(64%) of patient, Progesterone receptors were negative for18(36%) of patient while it were positive for 32(64%) of patient and Human epidermal receptor type 2 receptors were negative for18(36%) of patient while it were positive for 32(64%) of patient. Our results in molecular study of Lnc-HOTAIR showed a high significant difference in the expression of Lnc-HOTAIR ($p<0.01$). The Lnc_HOTAIR gene expression is up-regulated by two folds in breast cancer patients (median: 1.2; range: 0.5 – 2.4) compared to control group (median: 0.7; range: 0.0.3 – 2.14)fig( ) (Table 3). In order to assess the diagnostic value of Lnc_HOTAIR in breast cancer; a Receiving operating characteristics curve was plotted. When comparing the expression level of Lnc_HOTAIR in breast cancer patients with healthy controls; we found that Lnc_HOTAIR is a good diagnostic biomarker in breast cancer; at optimum cut-off value of 1.3; the biomarker has a sensitivity of 76% and 84% specificity to differentiate Breast cancer patients from controls (AUC:0.92; p=0.001). our results agrees with(Li P, et al 2018) 58 who reported Negative impact of hyperactivity of HOTAIR has been shown on regulation of miR-141 and miR-326 in glioma cells, as well as suppression of miR-141 in breast cancer cells and with (Bian EB, et al 2016) [57] who reveled that Up-regulation of this molecule has also been correlated to poor prognosis, invasiveness and metastasis of several tumours, including breast58 who reported that Data are illustrated in Table 4, figure 2. Molecular study of median Lnc-HOTAIR gene expression levels in Breast cancer patients presented with different histopathological characteristics show none significant deference in expression levels between Invasive ductal carcinoma(0.6) and medullary, sarcomas(0.8%) with p-value= 0.08 , A significant deference in expression levels between Grade II patient (0.9) and Grade III patient (0.2) with p-value= 0.014 which suggested that Lnc-HOTAIR gene expression has a Grade dependant expression, which agrees(Hossein M 2020) with who reported that considering the critical role of HOTAIR in promoting breast cancer development, through different mechanisms of action, we further discuss the potential relationship of HOTAIR with response to different combinational Therapeutic agents, Molecular study of median Lnc-HOTAIR gene expression levels in Breast cancer patients presented with different tumor receptor show that Lnc-HOTAIR gene expression has no significant deference according to Estrogen receptors result as it was (0.7) for patient with negative result and it was (0.85)for patient with positive result with p-value= 0.84 which does not agrees with (Xue X, 2016)74 Accumulating data indicate the potential role of HOTAIR in prohibiting the effect of several hormonal therapeutic agents. As previously indicated, HOTAIR activity directly fosters ER signalling in ER+ breast cancer cells to develop invasiveness and metastasis. Mechanistically, activity of HOTAIR elevates ER occupancy on chromatin and regulates the corresponding downstream genes, this mechanism further encourages drug-resistance in the cancer patients treated with Tamoxifen (as an ER competitive antagonist). It is proposed that HOTAIR could promote ER activity in the Tamoxifen resistant malignant breast cancer cells with lack of estrogen. Also No significant deference according to Progesterone receptors result as it was (0.7) for patient with negative result and it was (0.8)for patient with positive result with p-value= 0.88 and it is the first time a study has been performed to validate the correlation of HOTAIR and HER2 in breast cancer and we found that there is no significant deference according to human epidermal receptor type 2 receptors as it was (0.7) for patient with negative result and it was (0.8) for patient with positive result with p-value=0

Conclusion

Lnc-HOTAIR gene associated with development of breast cancer as it has a higher expression level in patient with breast cancer in a Grade dependant manner while there was no significant deference with any of the studied tumor receptor, Estrogen, Progesterone and human epidermal receptor type 2 (HER2)which suggest that it is a potential biomarker for breast cancer.

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