Long Non-coding RNA LET Behaves as a Non-coding Signature for Early-Onset Menarche and Late-Onset Menopause in Breast Cancer Patients

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Abstract

Breast cancer (BC) as a major cause of cancer-related death in women, shows a very complex molecular and clinical phenotype, which has reduced the effectiveness of medical interventions. Evidence suggests that long non-coding RNAs (lncRNAs) are responsible for an important part of this complexity. This study aims to assess the expression and clinical implication of lncRNA-low expression in tumor (LET) in the pathobiology of BC. Quantitative real-time polymerase chain reaction was used to measure the expression of lncRNA-LET in breast tumors, adjacent normal-appearing tissues, and normal mammary tissues. Moreover, a bioinformatics approach was applied to uncover the potential lncRNA-LET-mediated sponge regulatory network as LET/miRNA/mRNA crosstalk. Our results revealed that lncRNA-LET was significantly down-expressed in breast tumors and tumor margin normal samples from BC subjects compared with true normal breast tissues obtained from healthy women. The low level of lncRNA-LET was meaningfully associated with early-onset menarche (≤13 years) and late-onset menopause (≥50 years). Moreover, the bioinformatics analyses support that lncRNA-LET could function as a tumor suppressor miRNA sponge. The results indicate that normal-appearing breast tissues can undergo tumor-related molecular changes. Furthermore, they reveal the potential role of the dysregulation in the LET-mediated competing endogenous RNA network in the pathophysiology of BC. [GMJ.2021;10:e2108] DOI:10.31661/gmj.v10i0.2108

Keywords: lncRNA-LET; Breast Cancer; Normal-Appearing Breast Tissue; Bioinformatics; Competing Endogenous RNA
Introduction

Breast cancer (BC) remains the first leading cause of cancer-related death in women [1]. Based on the evidence obtained, this problem goes back to the complex clinical and molecular phenotype resulting from its heterogeneous development. Therefore, it has remarkable clinical utility to further identify the molecular mechanisms of BC initiation and progression as well as uncover new therapeutic targets for BC patients. Recently, long non-coding RNAs (lncRNAs), as a type of non-coding RNAs (ncRNAs), have been revealed to be responsible for the manifestation of various phenotypes of breast tumors [2]. lnc RNAs, which are a subgroup of ncRNAs, have >200 bp length and emerge as gene expression regulators through acting at transcription and post-transcriptional levels [3]. Increasing evidence highlights the roles of lncRNAs in various tumor-related biological processes and their value for becoming biomarkers and therapeutic targets [4]. Interestingly, it is well known that the lncRNA-mediated sponge regulatory network has the predominant effects on the dysregulation of key components of the cancer-driving signaling pathways, as long as competing endogenous RNA (ceRNA) hypothesis is concerned [5].

lncRNA- lncRNA-low expression in tumor (LET; also known as NPTN-IT1) has been recently identified to be down-expressed in several types of solid tumors, including lung cancer, cervical cancer, and nasopharyngeal carcinoma [6]. This lncRNA is an intronic transcript of the neuroplastin (NPTN) gene located at chromosome 15q24.1. It is demonstrated that lncRNA-LET show spvital tumor-suppressive effects through inhibiting hypoxia-mediated metastasis, epithelial-mesenchymal transition, and the Wnt signaling pathway [7]. However, to our knowledge, the function and clinical implications of lncRNA-LET expression with clinicopathological characteristics of BC patients remain unknown.

A piece of evidence indicates that the normal-appearing tissues adjacent to tumors already bear the cancer-related molecular changes, which could reveal the earliest changes leading to carcinogenesis [8]. Hence, we have investigated the expression of lncRNA-LET in breast tumors, tumor-adjacent normal tissues, and true normal breast samples (obtained from healthy women without a history of cancer). Moreover, its association with demographic and clinicopathological characteristics of BC patients has been assessed. Finally, the potential lncRNA LET/miRNA/mRNA interactions map in different cancers has been decoded using a bioinformatics approach.

Materials and Methods

1. Study Population

In this study, 48 paired tumors and adjacent non-tumoral tissue samples were obtained from BC subjects referred to Shahid Faghihi hospital, Shiraz, Iran. BC patients who participated in this study had not received radiotherapy and/or chemotherapy before surgery. In addition, 48 normal mammary tissues were collected from healthy individuals who had undergone cosmetic mammoplasty. These participants had no personal and/or family history of BC and any other types of cancers. The fresh tissue specimens were immediately put into liquid nitrogen and transferred to refrigerator (-80°C) for later use. The demographic and reproductive characteristics of patients are shown in Table-1. All the participants have signed an informed consent regarding their specimens and clinical information. Also, study protocol was approved by Research Ethics Committees of Fasa University of Medical Sciences (ethical code: IR.FUMS.REC.1397.143).

2. RNA Extraction and cDNA Synthesis

Total RNA was extracted from tumors, adjacent non-tumor tissues, and normal mammary specimens using the TriSol isolation reagent (Invitrogen, Thermo Fisher, USA) according to the manufacturer’s instructions. In order to remove DNA contamination, the extracted RNAs were treated by RiboclearTM (Ribocular plus, 50p, GeneALL, Seoul, South Korea). The integrity and quantity of RNAs were assessed by spectrophotometer and gel
electrophoresis, respectively. The Hyperscript TM kit from GeneAll company (Seoul, South Korea) was used for cDNA synthesis according to the manufacturer’s instructions.

3. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)
The Rotor-Gene 6000 cycler (Corbett Life Science, USA) was used to perform qRT-PCR reactions. A 10 µl BioFACT™ master mix including SYBR Green (South Korea), along with 2 µl of cDNA, 1 µl of each primer, and 6 µl DNase-free dH2O was used for per 20 µl reaction volume.
The specific primer sequences included lncRNA-LRT: GGCTCTGTGGGATCAGTTATG (forward) and AGTCCATCTCT-GCCTTCTCT (reverse); B2M (as a housekeeping gene): AGATGAGTATG-CCTGCCGTG (forward) and GCGG-CATCTTCAAACCTCCA (reverse). All reactions were performed in duplicate according to 40 cycles of 95°C for 15 seconds and then 60°C for 30 seconds.

4. Statistical Analysis
The data are presented by the mean and standard deviation for numerical data or median and percentage for qualitative data. The Kruskal-Wallis test was applied to compare lncRNA-LET expression among three sample groups, including tumors, adjacent non-tumor tissues, and normal mammary tissues. The association of lncRNA-LET expression with demographic and clinicopathological characteristics of BC patients was assessed by nonparametric tests, including Mann-Whitney and Kruskal-Wallis. SPSS v. 21 statistical software (SPSS Inc., Chicago, IL, USA) was run for data processing. The P-value less than 0.05 was considered as a statistical significance.

5. In silico Analyses
5.1. Investigation of lncRNA-LET Expression in Different Cancers
Using GEPIA webserver (extracting RNA sequencing expression data of tumors and normal samples from the TCGA data), we investigated the lncRNA-LET expression across TCGA tumors compared to their matched normal data. In our analysis, cancers with the reasonable number of normal TCGA samples were included. These cancers were bladder urothelial carcinoma (BLCA); invasive breast carcinoma (BRCA); colon adenocarcinoma (COAD); lung adenocarcinoma (LUAD); lung squamous cell carcinoma (LUSC); prostate adenocarcinoma (PRAD); rectum adenocarcinoma (READ); thyroid carcinoma (THCA); uterine corpus endometrial carcinoma (UCEC). For differential expression analysis, we considered ANOVA with |Log2FC| cutoff: 1 and p-value cutoff: 0.01.

5.2. IncRNA-LET Co-expressed Genes in Cancers and Consensus Approach
Using GEPIA, we looked for co-expressed genes for human lncRNA-LET in cancers with altered expression of this lncRNA. For this analysis, firstly, we investigated genes with a similar pattern of expression to lncRNA-LET in BRCA (since our study was focused on this cancer). Secondly, we investigated the correlation between the top 100 genes and lncRNA-LET in other cancers with altered expression of this lncRNA. The common co-expressed genes between BC and seven other cancers were picked out for further investigation. It is presumed that these common co-expressed genes might be downstream of the lncRNA-LET/miRNA/gene axes as long as the ceRNA network was concerned.

5.3. Negatively Correlated miRNAs with IncRNA-LET Co-expressed Genes
We looked for correlation between the protein-coding genes (having a positive correlation to lncRNA-LET in our studied cancers) and miRNAs in BRCA using TACCO webserver (extracting TCGA data, http://tacco.life.nctu.edu.tw/). Then, we selected miRNAs with negative Pearson's r or Spearman's ρ correlation to these genes. The miRNAs with two following criteria were retrieved to construct the lncRNA-LET/miRNAs/mRNAs regulatory network: 1) complementarity between the seed region of the miRNA and lncRNA-LET sequence, 2) potential interaction between miRNA and lncRNA-LET co-expressed genes. The potential
molecular interactions between lncRNA-LET and miRNAs were found through StarBase [9], a database that predicts the function of ncRNAs in ceRNA regulatory networks. The experimentally validated or bioinformatically predicted miRNA-mRNA interactions were achieved by miRTarBase and TargetScan databases, respectively [10, 11].

5.4. LncRNA-LET/miRNAs/mRNAs Network
Finally, the ceRNA regulatory network involving the central function of lncRNA-LET as a sponge was constructed by Cytoscape software (Institute for Systems Biology (ISB), Seattle, WA). The workflow of bioinformatics analyses is summarized in Figure-1. The Enrichr webserver was run to functional enrichment analysis of the protein-coding genes in the network.

Results

The Expression Investigation of lncRNA-LET in Normal Breast, Tumoral, and Tumor’s Adjacent Normal Tissues
The expression level of lncRNA-LET was determined in 48 pairs of breast tumors and adjacent normal tissues as well as 48 normal mammary tissues using qRT-PCR. As it is shown in Figure-2a, the median of lncRNA-LET expression has the lowest level in tumor tissues and the highest level in normal mammary tissues. Its expression in tumor’s adjacent normal tissues was more than tumors and less than normal mammary tissues (P=0.11 and P<0.0001, respectively). It should be noticed that the expression of lncRNA-LET shows a significant downregulation not only in tumors but also in tumor’s adjacent normal tissues compared with normal mammary tissues (P<0.0001).

The Association of lncRNA-LET Expression with Demographic and Clinicopathological Characteristics of BC Patients
Our data reveal a significant association between low expression of lncRNA-LET and early menarche (age ≤13 years, P=0.006, Figure-2b). Furthermore, the low expression of lncRNA-LET shows a significant association with late-onset menopause (age ≥50 years, P=0.02, Figure-2c). The details of association

![Figure 1. The workflow of bioinformatics analyses](image-url)
Table 1. Association Between Expression of lncRNA-LET and Demographic and Clinicopathological Features of Studied Patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Subgroups</th>
<th>Number (%)</th>
<th>Median</th>
<th>P-value</th>
</tr>
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<tr>
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<td>30 (62.5)</td>
<td>0.06</td>
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<td></td>
<td>≥50</td>
<td>18 (37.5)</td>
<td>0.03</td>
<td></td>
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<td></td>
<td>≤2</td>
<td>16 (33.4)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Tumor size, cm2</td>
<td>2-4</td>
<td>22 (45.8)</td>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>≥4</td>
<td>10 (20.8)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor</td>
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<td>42 (87.5)</td>
<td>0.04</td>
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<tr>
<td></td>
<td>Negative</td>
<td>6 (12.5)</td>
<td>0.08</td>
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</tr>
<tr>
<td>Progesterone receptor</td>
<td>Positive</td>
<td>32 (66.7)</td>
<td>0.05</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>16 (33.3)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>HER2</td>
<td>Negative</td>
<td>29 (60.5)</td>
<td>0.03</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>G1</td>
<td>12 (25)</td>
<td>0.04</td>
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<tr>
<td></td>
<td>G2</td>
<td>22 (45.8)</td>
<td>0.06</td>
<td>0.4</td>
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<tr>
<td></td>
<td>G3</td>
<td>14 (29.2)</td>
<td>0.03</td>
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<tr>
<td></td>
<td>1/2</td>
<td>33 (68.8)</td>
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<td>15 (31.2)</td>
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<tr>
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<td>0.06</td>
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<td></td>
<td>No</td>
<td>20 (61.6)</td>
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<tr>
<td>Histologic type of invasive carcinoma</td>
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<tr>
<td></td>
<td>ILC</td>
<td>1 (2)</td>
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<td>≤13</td>
<td>28 (58.3)</td>
<td>0.02</td>
<td>0.006</td>
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<td>≥14</td>
<td>20 (41.7)</td>
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<td></td>
<td>&lt;25</td>
<td>32 (80)</td>
<td>0.04</td>
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<td>Age of menarche, y</td>
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<td>0.5</td>
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<td></td>
<td>No</td>
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<tr>
<td></td>
<td>≤6</td>
<td>8 (16.6)</td>
<td>0.04</td>
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</tr>
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<td>Breastfeeding duration, months</td>
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<td>0.03</td>
<td>0.6</td>
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<td></td>
<td>≥24</td>
<td>7 (14.6)</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Menopausal status</td>
<td>Pre</td>
<td>30 (62.5)</td>
<td>0.06</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>18 (37.5)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Menopausal age, y</td>
<td>&lt;50</td>
<td>8 (44.5)</td>
<td>0.09</td>
<td>0.02</td>
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<tr>
<td></td>
<td>≥50</td>
<td>10 (55.5)</td>
<td>0.03</td>
<td></td>
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<tr>
<td>Family history</td>
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<td>20 (41.6)</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>28 (58.4)</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

HER2: Human epidermal growth factor receptor 2; ILC: Invasive lobular carcinoma; ACC: Adenoid cystic carcinoma; IDC: Infiltrating ductal carcinoma; FFTP: First full-term pregnancy

analyses of lncRNA-LET expression with the clinicopathological and demographic variables are shown in Table-1. We have not observed any significant associations between the expression of lncRNA-LET and clinicopathological features of patients.

*IncRNA LET Down Expression in Different Cancers*

The TCGA data analysis revealed that IncRNA-
Figure 2. a: The bar graphs of comparison of lncRNA-LET expression among true normal breast tissues, normal-appearing tissues adjacent to tumors, and breast tumors, respectively (from left to right). Error bars represent the standard error of the median. b and c: lncRNA-LET expression in the subgroups of the age of menarche and menopause onset in BC patients. Error bars represent the standard error of the median.

Supplementary Figure 1. Co-expression network of lncRNA LET in eight cancers based on TCGA data. The green and blue nodes represent protein-coding genes and lncRNAs, respectively.
Figure 3. The lncRNA-LET expression across 16 TCGA cancers compared to TCGA normal using GEPIA. It shows the low expression of this lncRNA in BRCA, COAD, LUAD, LUSC, PRAD, READ, THCA, and UCEC. TCGA tumor and its matched normal are given in red and green, respectively. T: Tumor; N: Normal; n: number. X-axis indicate number of tumoral and normal samples.

Figure 4. Strong to very strong positive correlated lncRNA-LET genes in BRCA, COAD, LUAD, LUSC, PRAD, READ, THCA, and UCEC cancers, including two protein-coding genes TSSK4(a) and ZDHHC17 (b) and one pseudogene PRDX3P1(c). The graphs have been retrieved from GEPIA.
Figure 5. The potential lncRNA-LET-mediated sponge regulatory network in breast tumors based on experimentally validated and bioinformatically predicted interactions. All the genes are significantly co-expressed with lncRNA-LET in BC as well as COAD, LUAD, LUSC, PRAD, READ, THCA, and UCEC. All the miRNAs show a significant negative correlation with their downstream genes in breast tumors based on TCGA data. The red ellipse shows IncRNA-LET, the blue V-shaped nodes and green rectangles present miRNAs and mRNAs, respectively. The contiguous red arrows present experimentally validated interactions between miRNAs and mRNAs. The solid red lines show bioinformatically predicted interactions between miRNAs and mRNAs. The dashed green lines show bioinformatically predicted interactions between lncRNA-LET and miRNAs.

Supplementary Figure 2. Gene ontology (GO) enrichment analysis of the lncRNA-LET-mediated sponge regulatory network. The top 10 GO based on the Enrichr web server. The longer bar and brighter color represent more significant terms.
LET was significantly down expressed in BC as well as another seven cancer types (Figure-3), including colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC).

**IncRNA-LET Co-expression Network in Cancers**

The co-expression analysis for IncRNA-LET in the cancers with significant under-expression of IncRNA-LET uncovered the positive expression correlation of several protein-coding genes and IncRNAs (Supplementary Table-1). Among them, several genes showed a positive correlation (moderate, strong, and very strong correlations) to IncRNA-LET in all eight cancers (genes given in blue in Supplementary Table-1), indicating the major genes and IncRNAs in IncRNA-LET co-expression network in cancers (Supplementary Figure-1). For Pearson correlation, we considered $r=0.00–0.19$ as very weak, $r=0.20–0.39$ as weak, $r=0.40–0.59$ as fairly strong (moderate), $r=0.60–0.79$ as strong, and $r=0.80–1.0$ as very strong. Among these co-expressed genes, two protein-coding genes (TSSK4 and ZDHHC17) and one pseudogene (PRDX3P1) show strong and/or very strong correlations in all eight cancers (Figure-4).

**Potential IncRNA LET-mediated Sponge Regulatory Network in BC**

According to in silico investigation results, the potential IncRNA-LET/miRNA/mRNA regulatory network has been generated using a combination of data obtained from IncRNA-LET-miRNA pairs (miRNAs that show negative correlation) with IncRNA-LET co-expressed protein-coding genes) and miRNA-mRNA pairs (mRNAs that show positive correlation with IncRNA-LET, Figure-5). Next, all of the downstream mRNAs were performed to functional annotation. This assessment revealed that the IncRNA-LET-mediated sponge regulatory network could be involved in transcriptional misregulation in cancer (Supplementary Figure-2).

**Discussion**

Despite an astonishing evolution in molecular technologies and their considerable impact on the identification of functional ncRNAs in malignancies, numerous questions regarding their dysregulation, molecular interactions, and functions remain to be addressed. IncRNAs, a class of non-protein-coding transcripts, engage in tumorigenesis by modifying the expression profile of cells. IncRNA-LET has been recently identified as a down-expressed ncRNA in different malignancies, such as nasopharyngeal carcinoma, cervical, bladder, and lung cancers [12, 13]. Accumulating evidence has revealed that the dysregulation of IncRNA-LET plays a pivotal role in cancer progression by regulating gene expression. However, its expression and functions in BC have remained largely unknown. Herein, we have investigated the expression of IncRNA-LET in three types of sample groups; tumors, adjacent non-tumor tissues, and normal mammary tissues. In addition, the association of IncRNA-LET expression with demographic and clinicopathological characteristics of BC patients was assessed. The majority of studies regarding the evaluation of gene dysregulation in cancers compare tumor with normal-appearing tissue adjacent to the tumor as healthy cells. However, it has been determined that adjacent non-tumor tissues do not definitely show the same expression profile as tissues from cancer-free organs [14]. So, we assessed IncRNA-LET expression among tumors, adjacent non-tumor tissues, and normal mammary tissues. Interestingly, our results indicate that both tumors and adjacent non-tumor tissues show a significant down expression of IncRNA-LET compared with normal mammary tissues. Tumor samples have a lower mean expression of IncRNA-LET rather than adjacent non-tumor tissues, but it does not reach the level of statistical significance. The significant down expression of IncRNA-LET in both breast tumor and adjacent non-tumor tissues compared with normal mammary samples was surprising and indicated that normal-appearing breast tissues could undergo tumor-
related molecular changes. Consistently, such tumor-related genetic changes in adjacent non-tumor tissues have been reported in BC and other malignancies [15]. Therefore, although the investigation of gene expression patterns is a powerful method for the molecular reclassification of cancers and helps uncover several predictive and prognostic biomarkers, the most successful application of this evaluation requires the deliberation of selecting samples as the baseline normal tissues. Another finding of this study was the significant low level of lncRNA-LET in the tumor of the patients with early-onset menarche (≤13 years) and late-onset menopause (≥50 years). This finding suggests the possible role of estrogen-related molecular changes regarding lncRNA-LET in BC initiation and/or progression. Epidemiological evidence among women has indicated the contribution of reproductive-related estrogen changes in the cancers derived from hormone-responsive organs [16].

It is well established that early menarche and late menopause are involved, at least in part, in the risk of breast carcinoma. These demographic factors result in being more exposed to estrogen in a lifetime. Moreover, previous studies have documented the roles of lncRNAs in the estrogen pathway as well as BC development [17, 18]. In 2015, Jonsson et al. uncovered that estrogen receptor regulates up to 1000 lncRNAs in the BC cell lines [19]. According to our results and the previous reports, lncRNA-LET is a down-regulated ncRNA with a potential tumor suppressive function. Therefore, the significant association between the down expression of lncRNA-LET in breast tumors and demographic risk factors, including early menarche and late menopause in BC patients could be an intimation concerning a link between estrogen-related molecular changes and BC development.

While this association was not observed in normal adjacent tissues, and it needs further investigation in the large sample size. It is suggested that the down expression of lncRNA-LET might contribute to the estrogen-related tumorigenic mechanism. Growing evidence indicates that the crosstalk among different RNAs (coding and non-coding) at the molecular levels underlies the regulation of gene expression and consequently determines cell functions [20-22]. The ceRNA hypothesis has introduced a novel RNA language in which lncRNAs compete with mRNAs through miRNA response elements (MREs). Herein, we have presumed lncRNA-LET as a sponge RNA in the context of ceRNA network. In order to investigate potential lncRNA-LET/miRNA/mRNA axes, the lncRNA-LET co-expressed genes in eight cancers with the significant down expression of lncRNA-LET were found. Among these co-expressed genes, two protein-coding genes (TSSK4 and ZDHHC17) and one pseudogene (PRDX3P1) showed strong and/or very strong correlations with lncRNA-LET in all eight cancers. TSSK4 is a member of the testis-specific serine/threonine-protein kinase family and is highly expressed in testis. The overexpression of TSSK4 in HeLa cells results in apoptotic bodies, indicating TSSK4 induces apoptosis in vitro [23]. Also, ZDHHC17 is a member of the large ZDHHC gene family that codes the enzymes, which mediate S-acylation (post-translational protein modification). The pivotal roles of these enzymes in normal cellular function are highlighted by their association with a broad range of diseases such as neurological disorders and cancers [24]. The tumor suppressor functions for the ZDHHC family have been reported in previous studies [25, 26]. The strong expression correlation between lncRNA-LET and two potential apoptosis regulator proteins (TSSK4 and ZDHHC17) in different cancers could suggest the function of lncRNA-LET in the regulation of their expression. However, these findings need to be validated by experimental studies. In the next step, experimentally validated and bioinformatically predicted interactions between miRNAs that show negative co-expression with lncRNA-LET co-expressed protein-coding genes in BC were also retrieved and integrated with bioinformatically predicted miRNAs, which have a seed region complementarity to the lncRNA-LET sequence. Finally, we propose the first evidence concerning the lncRNA-
LET-mediated sponge regulatory network. As it is visualized in Figure-5, the IncRNA-LET/miRNAs/NR2C2 axis shows one of the most interactions in the IncRNA-LET-mediated sponge regulatory network, and IncRNA-LET could block the effects of six miRNAs, which probably inhibit NR2C2 expression. NR2C2 acts as a transcription factor that controls the expression of target genes through binding to the DNA hormone response elements [27].

As shown in Figure-5, trinucleotide repeat-containing 6 (TNRC6A) shows the most possible interactions in the context of IncRNA-LET/miRNA/mRNA regulatory axes. The TNRC6A protein family is involved in post-transcriptional gene regulation through the RNA interference mechanisms. The significant down expression of TNRC6A has been reported in gastric, ovarian, lung, and colorectal cancers [28, 29]. Considering all evidence, IncRNA-LET could function as a tumor suppressor ncRNA through sponging and suppressing oncomiRs in mammary tissues. Our analyses have uncovered the probable participation of well-defined oncomiRs in IncRNA-LET-mediated sponge regulatory network such as miR-106 [30], miR-20 [31], miR-17 [32], and miR-374 [33].

**Conclusion**

Our findings highlight the importance of molecular methods in identifying defective normal-appearing cells that are undetectable using the microscope. It seems that using the normal-looking tissue near tumors as a normal baseline tissue does not lead to an accurate judgment. Moreover, we uncovered for the first time the significant association between the low-expressed IncRNA-LET in BC and estrogen-related risk factors in patients. In silico analyses highlight the utility of considering the roles of IncRNA-LET in transcriptional misregulation in cancers and its potential to be a biomarker in various malignancies.

While more researches are needed to confirm our results and uncover the regulatory roles of IncRNA-LET in different cancers.

**Acknowledgments**

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**Conflict of Interest**

The authors declare that they have no competing interests.

**References**