ORIGINAL ARTICLE

GMJ.2021;10:e2108
www.gmj.ir

Received 2020-12-26
Revised 2021-01-20
Accepted 2021-02-05

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

Farzaneh Darbeheshti[1,2], Hosein Mansoori[3,4], Rasoul Abdollahzadeh[1], Hassan Dastsooz[5], Abdolreza Daraei[6], Maral Mokhtari[7], Hamzeh Salmani[1], Mostafa Davood Abadi Farahani[8], Sedigheh Tahmasebi[4], Yaser Mansoori[3,9]✉

[1] Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2] Breast Cancer Association (BrCA), Universal Scientific Education and Research Network (USERN), Tehran, Iran
[3] Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran
[4] Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[5] Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Via Accademia Albertina, Torino, Italy
[6] Department of Genetics, School of Medicine, Babol University of Medical Sciences, Babol, Iran
[7] Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[8] Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[9] Department of Medical Genetics, Fasa University of Medical Sciences, Fasa, Iran

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

✉ **Correspondence to:**
Yaser Mansoori, Department of Medical Genetics, Fasa University of Medical Sciences, Fasa, Iran
Telephone Number: 071 53357091
Email Address: fums.mansoori@gmail.com

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 shows pivotal 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 (Riboclear 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 AGTCCATCTCTGCCTTCTCT (reverse); B2M (as a housekeeping gene): AGATGAGTATGCCTGCCGTG (forward) and GCGGCATCTTCAAACCTCCA (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. lncRNA-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 lncRNA-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 expres¬sion 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

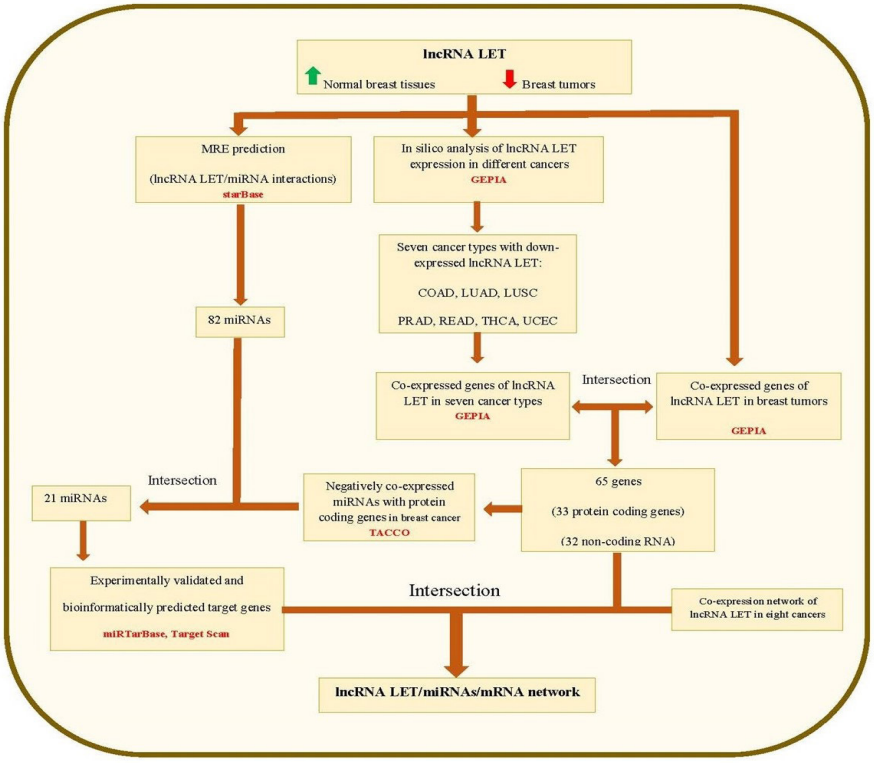


Figure 1. The workflow of bioinformatics analyses

Table 1. Association Between Expression of lncRNA-LET and Demographic and Clinicopathological Features of Studied Patients.

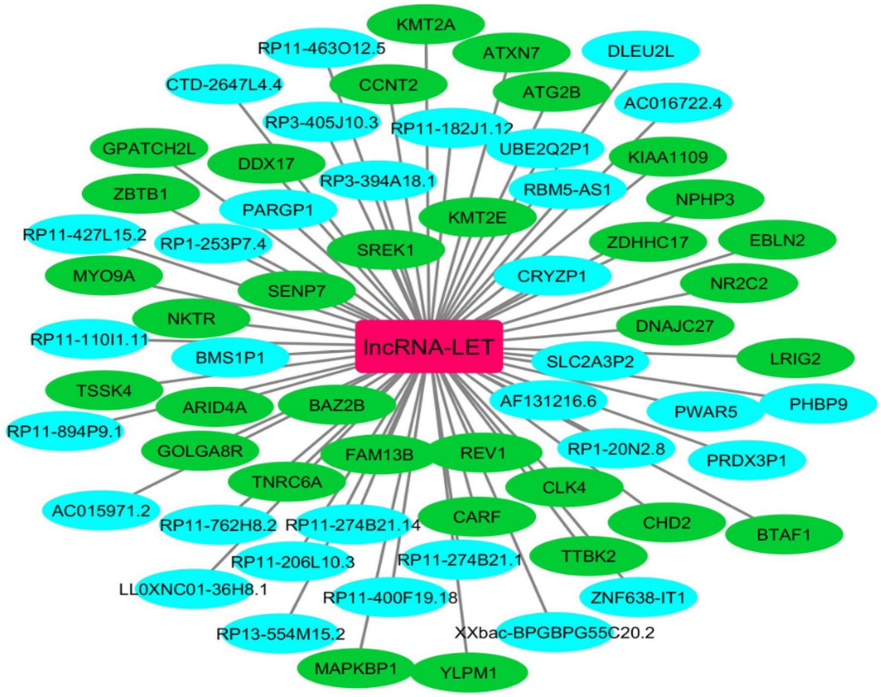
Variables	Subgroups	Number (%)	Median	P-value
Age, y	<50	30 (62.5)	0.06	0.4
	≥50	18 (37.5)	0.03	
Tumor size, cm2	≤2	16 (33.4)	0.05	0.8
	2-4	22 (45.8)	0.04	
	≥4	10 (20.8)	0.05	
Estrogen receptor	Positive	42 (87.5)	0.04	0.2
	Negative	6 (12.5)	0.08	
Progesterone receptor	Positive	32 (66.7)	0.05	0.7
	Negative	16 (33.3)	0.05	
HER2	Positive	19 (39.5)	0.06	0.2
	Negative	29 (60.5)	0.03	
Histologic grade	G1	12 (25)	0.04	0.4
	G2	22 (45.8)	0.06	
	G3	14 (29.2)	0.03	
TNM stage	1/2	33 (68.8)	0.03	0.1
	3	15 (31.2)	0.07	
Lymph nodes metastasis	Yes	28 (58.4)	0.06	0.4
	No	20 (61.6)	0.03	
Histologic type of invasive carcinoma	IDC	46 (96)	0.05	0.2
	ACC	1 (2)	0.35	
	ILC	1 (2)	0.02	
Age of menarche, y	≤13	28 (58.3)	0.02	0.006
	≥14	20 (41.7)	0.07	
Age of FFTP, y	<25	32 (80)	0.04	0.5
	≥25	8 (20)	0.05	
Breastfeeding duration, months	No	9 (18.8)	0.06	0.6
	≤6	8 (16.6)	0.04	
	6-24	24 (50)	0.03	
	≥24	7 (14.6)	0.06	
Menopausal status	Pre	30 (62.5)	0.06	0.8
	Post	18 (37.5)	0.03	
Menopausal age, y	<50	8 (44.5)	0.09	0.02
	≥50	10 (55.5)	0.03	
Family history	Positive	20 (41.6)	0.05	0.5
	Negative	28 (58.4)	0.05	

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.

lncRNA LET Down Expression in Different Cancers

The TCGA data analysis revealed that lncRNA-



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.

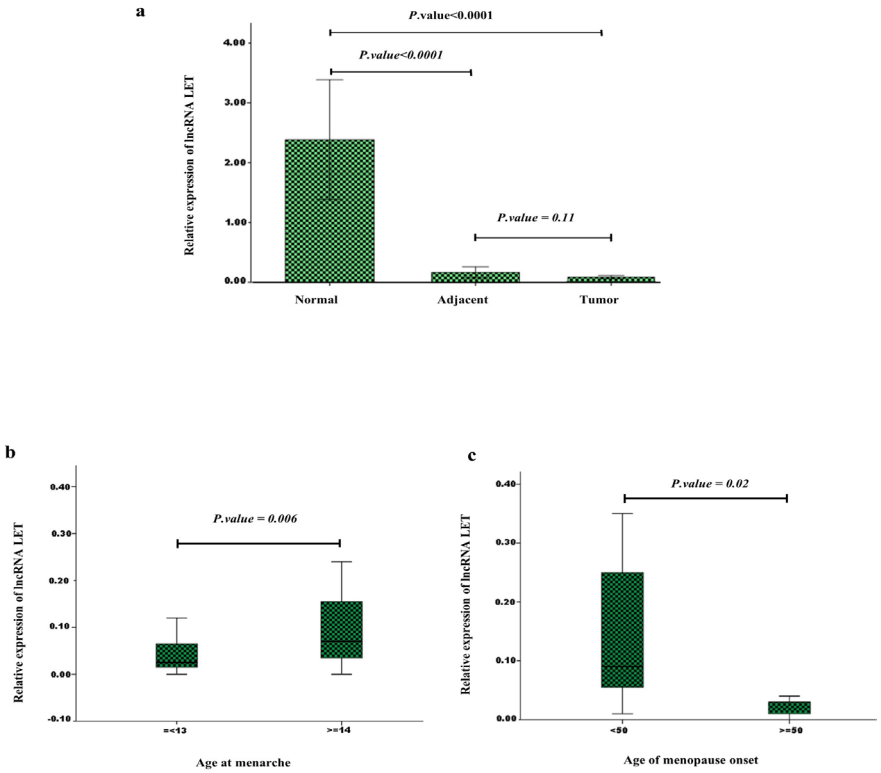


Figure 2. a: The bar graphs of comparison of lncRNA-LET expression among true normal breast tissues, normal-appearing tissues adjacent to tumors, and breast tumors, respectively (from left to right). Error bars represent the standard error of the median. **b** and **c:** lncRNA-LET expression in the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

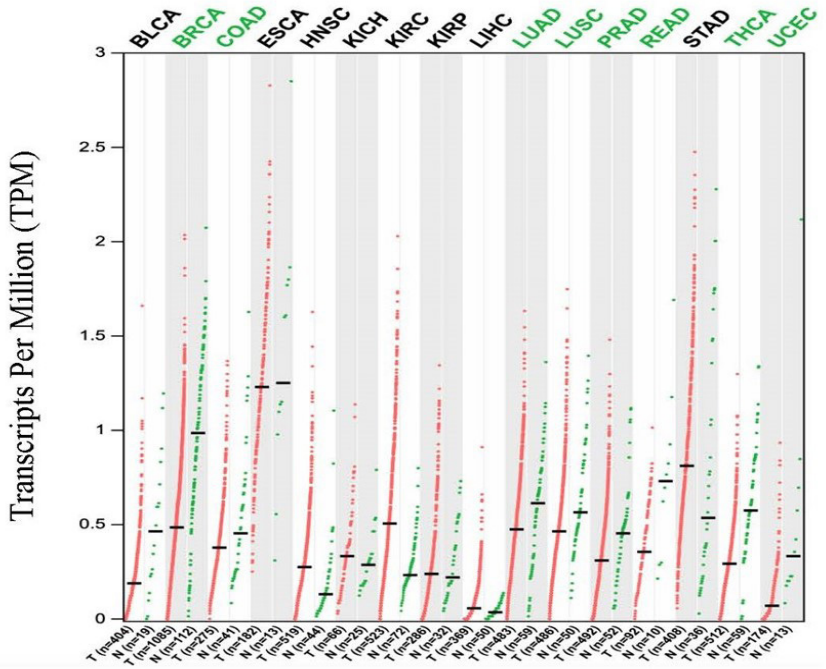


Figure 3. The lncRNA-LET expression across 16 TCGA cancers compared to TCGA normal using GEPIA. It shows the low expression of this lncRNA in BRCA, COAD, LUAD, LUSC, PRAD, READ, THCA, and UCEC. TCGA tumor and its matched normal are given in red and green, respectively. **T:** Tumor; **N:** Normal; **n:** number. X-axis indicate number of tumoral and normal samples.

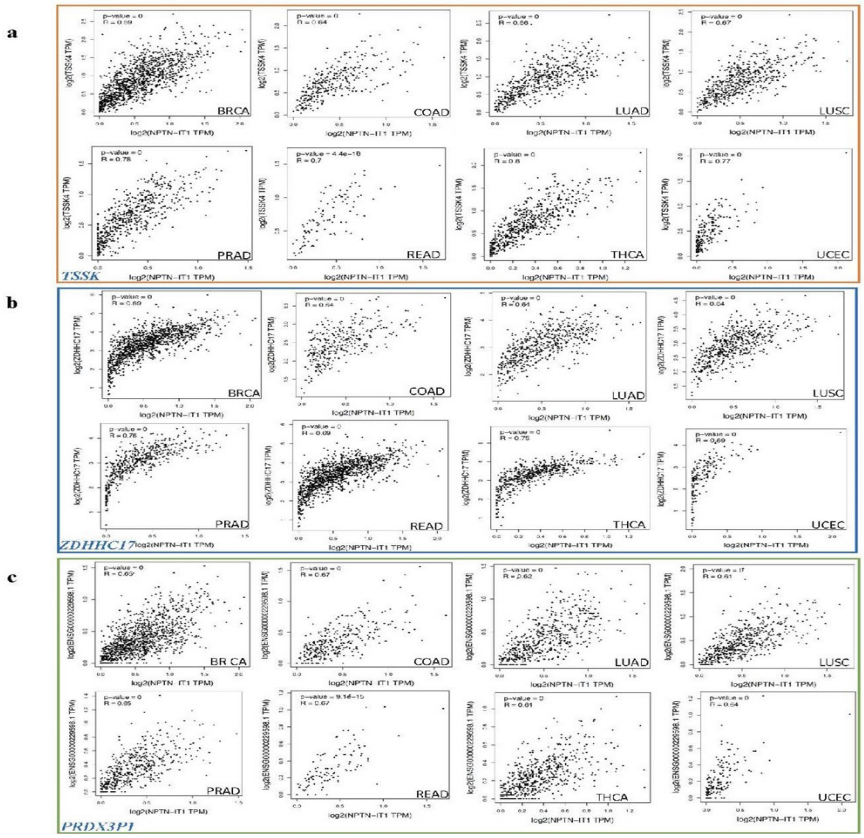


Figure 4. Strong to very strong positive correlated lncRNA-LET genes in BRCA, COAD, LUAD, LUSC, PRAD, READ, THCA, and UCEC cancers, including two protein-coding genes TSSK4(**a**) and ZDHHC17 (**b**) and one pseudogene PRDX3P1(**c**). The graphs have been retrieved from GEPIA.

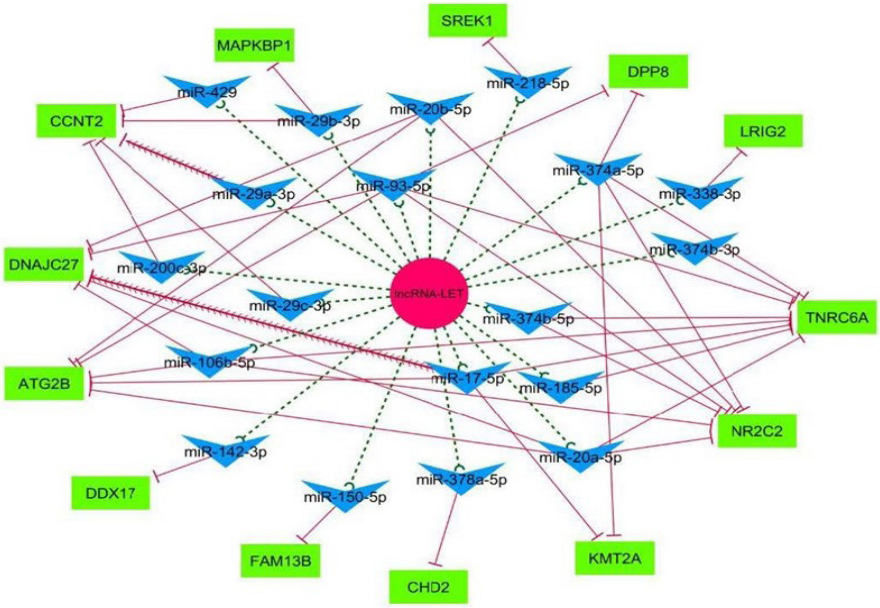
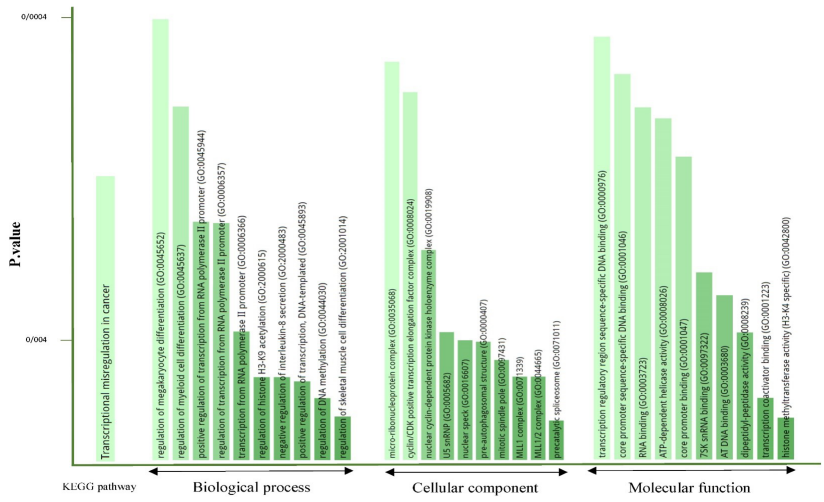


Figure 5. The potential lncRNA-LET-mediated sponge regulatory network in breast tumors based on experimentally validated and bioinformatically predicted interactions. All the genes are significantly co-expressed with lncRNA-LET in BC as well as COAD, LUAD, LUSC, PRAD, READ, THCA, and UCEC. All the miRNAs show a significant negative correlation with their downstream genes in breast tumors based on TCGA data. The red ellipse shows lncRNA-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).

lncRNA-LET Co-expression Network in Cancers

The co-expression analysis for lncRNA-LET in the cancers with significant under-expression of lncRNA-LET uncovered the positive expression correlation of several protein-coding genes and lncRNAs (Supplementary Table-1). Among them, several genes showed a positive correlation (moderate, strong, and very strong correlations) to lncRNA-LET in all eight cancers (genes given in blue in Supplementary Table-1), indicating the major genes and lncRNAs in lncRNA-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 lncRNA LET-mediated Sponge Regulatory Network in BC

According to in silico investigation results, the potential lncRNA-LET/miRNA/mRNA regulatory network has been generated using a combi¬nation of data obtained from lncRNA-LET-miRNA pairs (miRNAs that show negative correlation) with lncRNA-LET co-expressed protein-coding genes) and miRNA-mRNA pairs (mRNAs that show positive correlation with lncRNA-LET, Figure-5). Next, all of the downstream mRNAs were performed to functional annotation. This assessment revealed that the lncRNA-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. lncRNAs, a class of non-protein-coding transcripts, engage in tumorigenesis by modifying the expression profile of cells. lncRNA-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 lncRNA-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 lncRNA-LET in three types of sample groups; tumors, adjacent non-tumor tissues, and normal mammary tissues. In addition, the association of lncRNA-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 lncRNA-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 lncRNA-LET compared with normal mammary tissues. Tumor samples have a lower mean expression of lncRNA-LET rather than adjacent non-tumor tissues, but it does not reach the level of statistical significance. The significant down expression of lncRNA-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 lncRNA-LET/miRNAs/NR2C2 axis shows one of the most interactions in the lncRNA-LET-mediated sponge regulatory network, and lncRNA-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 lncRNA-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, lncRNA-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 lncRNA-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 lncRNA-LET in BC and estrogen-related risk factors in patients.

In silico analyses highlight the utility of considering the roles of lncRNA-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 lncRNA-LET in different cancers.

Acknowledgments

This study was supported by the Department of Medical Genetics, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran (grant number: 97181). Samples were provided from Shahid Faghihi hospital, Shiraz, Iran. We would like to acknowledge the TCGA, GEO, GEPIA, Enrichr, TargetScan, and miRTarBase databases as well as Cytoscape software for free use.

Conflict of Interest

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Mansoori H, Darbeheshti F, Daraei A, Mokhtari M, Tabei MB, Abdollahzadeh R, et al. Expression signature of lncRNA APTR in clinicopathology of breast cancer: Its potential oncogenic function in dysregulation of ErbB signaling pathway. Gene Rep 2021;23:101116.
3. Prensner JR ,Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov 2011;1:391-407.
4. Oliveira-Mateos C, Sánchez-Castillo A, Guil S, Noncoding RNAs as Regulators of Gene Expression in Pluripotency and Differentiation, in Epigenetics and Regeneration. 2019, Elsevier. 73-105.
5. Xiao B, Zhang W, Chen L, Hang J, Wang L, Zhang R, et al. Analysis of the miRNA–mRNA–lncRNA network in human estrogen receptor-positive and estrogen receptor-negative breast cancer based on TCGA data. Gene 2018;658:28-35.
6. Liu B, Pan C-F, He Z-C, Wang J, Wang P-L, Ma T, et al. Long noncoding RNA-LET suppresses tumor growth and EMT

in lung adenocarcinoma. Biomed Res Int 2016.
7. Yang F, Huo X-S, Yuan S-X, Zhang L, Zhou W-P, Wang F, et al. Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. Mol Cell 2013;49:1083-96.
8. Clare S, Pardo I, Mathieson T, Lillemoe H, Blosser R, Choi M, et al., Abstract P1-03-02:"Normal" tissue adjacent to breast cancer is not normal. 2012, AACR.
9. Li J-H, Liu S, Zhou H, Qu L-H, Yang J-H. starBase v2. 0: decoding miRNA-ceRNA, miRNA-ncRNA and protein–RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014;42:D92-D7.
10. Hsu S-D, Lin F-M, Wu W-Y, Liang C, Huang W-C, Chan W-L, et al. miRTarBase: a database curates experimentally validated microRNA–target interactions. Nucleic Acids Res 2011;39:D163-D9.
11. Maziere P ,Enright AJ. Prediction of microRNA targets. Drug discovery today 2007;12:452-8.
12. Jiang S, Wang H-L, Yang J. Low expression of long non-coding RNA LET inhibits carcinogenesis of cervical cancer. Int J Clin Exp Pathol 2015;8:806.
13. Ma MZ, Kong X, Weng MZ, Zhang MD, Qin YY, Gong W, et al. Long non-coding RNA-LET is a positive prognostic factor and exhibits tumor-suppressive activity in gallbladder cancer. Mol Carcinog 2015;54:1397-406.
14. Chandran UR, Dhir R, Ma C, Michalopoulos G, Becich M, Gilbertson J. Differences in gene expression in prostate cancer, normal appearing prostate tissue adjacent to cancer and prostate tissue from cancer free organ donors. BMC Cancer 2005;5:1-11.
15. Widschwendter M, Berger J, Daxenbichler G, Müller-Holzner E, Widschwendter A, Mayr A, et al. Loss of retinoic acid receptor β expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer. Cancer Res 1997;57:4158-61.
16. Takalkar U, Asegaonkar S, Kodlikeri P, Kulkarni U, Borundiya V, Advani S. Hormone related risk factors and breast cancer: hospital based case control study from India. Breast Cancer 2020;2:
17. Mansoori Y, Tabei MB, Askari A, Izadi P, Daraei A, Bastami M, et al. Expression levels of breast cancer-related GAS 5 and LSINCT 5 lnc RNA s in cancer-free breast tissue: Molecular associations with age at menarche and obesity. Breast J 2018;24:876-82.
18. Niknafs YS, Han S, Ma T, Speers C, Zhang C, Wilder-Romans K, et al. The lncRNA landscape of breast cancer reveals a role for DSCAM-AS1 in breast cancer progression. Nat Commun 2016;7:1-13.
19. Jonsson P, Coarfa C, Mesmar F, Raz T, Rajapakshe K, Thompson JF, et al. Single-molecule sequencing reveals estrogen-regulated clinically relevant lncRNAs in breast cancer. Mol Endocrinol 2015;29:1634-45.
20. Darbeheshti F, Mahdiannasser M, Noroozi Z, Firoozi Z, Mansoori B, Daraei A, et al. Circular RNA-associated ceRNA network involved in HIF-1 signalling in triple-negative breast cancer: circ_0047303 as a potential key regulator. J Cell Mol Med 2021;25:11322-32.
21. Darbeheshti F, Rezaei N, Amoli MM, Mansoori Y, Tavakkoly Bazzaz J. Integrative analyses of triple negative dysregulated transcripts compared with non-triple negative tumors and their functional and molecular interactions. J Cell Physiol 2019;234:22386-99.
22. Kamaliyan Z, Mirfakhraie R, Azizi-Tabesh G, Darbeheshti F, Omranipour R, Ahmadinejad N, et al. The role of FOXC1/FOXCUT/DANCR axis in triple negative breast cancer: a bioinformatics and experimental approach. Mol Biol Rep 2022;1-9.
23. Wang X-L, Wei Y-H, Fu G-L, Yu L. Testis specific serine/threonine protein kinase 4 (TSSK4) leads to cell apoptosis relying on its kinase activity. J Huazhong Univ Sci Technol Med Sci 2015;35:235-40.
24. Ordelman V, The role of zDHHC proteins in cancer. 2021.
25. Greaves J ,Chamberlain LH. New links between S-acylation and cancer. J Pathol

2014;233:4-6.
26. Yeste-Velasco M, Mao X, Grose R, Kudahetti SC, Lin D, Marzec J, et al. Identification of ZDHHC14 as a novel human tumour suppressor gene. J Pathol 2014;232:566-77.
27. Shyr C-R, Hu Y-C, Kim E, Chang C. Modulation of estrogen receptor-mediated transactivation by orphan receptor TR4 in MCF-7 cells. J Biol Chem 2002;277:14622-8.
28. Kang L, Yang C, Wu H, Chen Q, Huang L, Li X, et al. miR-26a-5p regulates TNRC6A expression and facilitates theca cell proliferation in chicken ovarian follicles. DNA Cell Biol 2017;36:922-9.
29. Muhanhali D, Zhai T, Jiang J, Ai Z, Zhu W, Ling Y. Long non-coding antisense RNA TNRC6C-AS1 is activated in papillary thyroid cancer and promotes cancer progression by suppressing TNRC6C expression. Front Endocrinol (Lausanne) 2018;9:360.
30. Li N, Miao Y, Shan Y, Liu B, Li Y, Zhao L, et al. MiR-106b and miR-93 regulate cell progression by suppression of PTEN via PI3K/Akt pathway in breast cancer. Cell Death Dis 2017;8:e2796-e.
31. Yu Z, Wang C, Wang M, Li Z, Casimiro MC, Liu M, et al. A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. J Cell Biol 2008;182:509-17.
32. Matsubara H, Takeuchi T, Nishikawa E, Yanagisawa K, Hayashita Y, Ebi H, et al. Apoptosis induction by antisense oligonucleotides against miR-17-5p and miR-20a in lung cancers overexpressing miR-17-92. Oncogene 2007;26:6099-105.
33. Xu X, Wang W, Su N, Zhu X, Yao J, Gao W, et al. miR-374a promotes cell proliferation, migration and invasion by targeting SRCIN1 in gastric cancer. FEBS Lett 2015;589:407-13.