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Expression signature of lncRNA APTR in clinicopathology of breast cancer: Its potential oncogenic function in dysregulation of ErbB signaling pathway

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ABSTRACT

A growing amount of evidence has revealed that long noncoding RNAs (lncRNAs) play significant roles in malignancies through different mechanisms especially the competing endogenous RNA (ceRNA). They have also been shown to have the potential diagnostic, prognostic and therapeutic biomarker capacity in oncology research. Recently, lncRNA Alu-mediated p21 transcriptional regulator (APTR) has been proposed as an oncogenic lncRNA in development and clinical outcome of some cancers. However, clinical and molecular importance in breast cancer (BC) is still unclear. The purpose of this study was to examine the APTR expression and its potential roles in BC. The 47 BC tumors and 47 tumor adjacent normal tissues were obtained from the study subjects. Real-time PCR was applied for the analysis of APTR expression in breast tumors compared with paired adjacent normal tissues. Then, we used bioinformatics approach to investigate the potential ceRNA activity of APTR in APTR-microRNAs-mRNAs axes and with focus on ErbB signaling pathway in BC. Our results demonstrated that APTR expression was significantly upregulated in BC tumors compared with the adjacent normal tissues. Besides, APTR over-expression was related to a larger tumors size. Finally, bioinformatics analysis indicated that APTR could influence cell proliferation through dysregulating the oncogenes working in ErbB signaling pathway by sponging some tumor suppressive microRNAs (miRNAs). Current work provided some clues for the involvement of APTR in physiopathology of the breast tumors. However, other aspects of these findings need to be further elucidated by future functional studies.

1. Introduction

Breast cancer (BC) is the most frequent malignancy among women worldwide (Bray et al., 2018). Although many studies have focused on BC pathobiology, detection and treatment, it still remains a health challenge issue for women (Turashvili and Brogi, 2017). Biomedical data suggest that the root cause of this problem is due to the heterogeneous phenotype of breast tumors and solving the problem requires

Abbreviations: BC, breast cancer; lncRNA, long non-coding RNA; miRNA, microRNA; ceRNA, competing endogenous RNA; MRE, miRNA response elements; TCGA, the Cancer Genome Atlas; GTEx, Genotype-Tissue Expression.

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revealing specific molecular changes behind its different phenotypes (Turashvili and Brogi, 2017; Ellsworth et al., 2017). Because of this, various recent works on BC have focused on recent discoveries about the genome function to find key relevant clues (Low et al., 2018). Interestingly, ENCODE (Encyclopedia of DNA Elements) project exposed that the majority of the human genome is transcribed as non-coding RNAs (Consortium EP, 2012). And more importantly, there is body of evidence that in addition to abnormal expression of coding transcripts, BC tumor cells show remarkably altered expression patterns of various types of non-coding RNAs compared with normal condition (Klinge, 2018). Among these dysregulated transcripts, long noncoding RNAs (lncRNAs) have been demonstrated to play pivotal roles in BC development and progression through acting in multiple ways (Zhang et al., 2019).

lncRNAs are a subgroup of non-coding RNAs with >200 nucleotides in length which do not have the capacity to produce protein. These RNAs have emerged as one of the important expression regulators of the other genes by both transcriptional and post-transcriptional mechanisms (Yoon et al., 2013). And due to the advent of high-throughput sequencing methods, thousands of lncRNAs have been proved to be involved in various physiological and pathological conditions (Chiu et al., 2018). Recently, increasing evidence suggests that lncRNAs are involved in cancer initiation and progression by acting as competing endogenous RNAs (ceRNAs) in molecular networks which connect the function of mRNAs with that of noncoding RNAs (Salmena et al., 2011; Abdollahzadeh et al., 2019). In these networks, lncRNAs execute their roles as microRNAs (miRNA) sponges via carrying miRNA response element and consequently result in suppressing miRNAs function through lncRNA/miRNA/mRNA axes (Abdollahzadeh et al., 2019). One example is alu-mediated p21 transcriptional regulator (APTR) lncRNA which is located at 7q21 with 2303 nucleotides in length. Evidence exists that APTR acts as a suppressor of p21 expression through recruiting the polycomb repressive complex 2 (PRC2) to the p21 promoter in human glioblastomas (Negishi et al., 2014). And newly, it is suggested that APTR/miR-132-3p/YAP1 axis contributes to osteosarcoma cell proliferation, invasion and migration (Guan et al., 2019). Therefore, it is proposed that APTR expression is necessary for cell proliferation.

However, its dysregulation and function in BC is still unclear. The aim of the present study was to compare the expression level of the APTR between paired breast tumor and normal adjacent tissue samples as well as evaluation of its expression with demographic and clinicopathological characteristics of the BC patients. Finally, we adopted a bioinformatics approach to investigate the potential APTR-miRNAs-mRNAs axes in BC through highlighting the ErbB signaling pathway.

2. Material and methods

2.1. Tissue sample collection from participants

In the present study, 47 primary tumors and 47 paired adjacent normal tissues were obtained from the BC patients who had received neither chemotherapy nor radiotherapy before surgery from Shahid Faghihi hospital, Shiraz, Iran. Besides, 10 normal breast tissues, as calibrator samples, were collected from healthy women who undergone mammoplasty surgery. In order to implement suitable conditions for RNA extraction in later stages, fresh tissue specimens were immediately frozen in liquid nitrogen and were stored at −80 °C. Notably, all tumor and paired normal tissues were confirmed by histopathological diagnosis. This study was approved by the local ethical committee at Shiraz University of Medical Sciences (Ethical Code: IR.FUMS.REC.1395.102). Written informed consent was also obtained from all subjects. A standard questionnaire was used for collection of the clinical and demographic documents. Data about clinicopathological and demographic characteristics of the BC patients is shown in Table 1.

Table 1
Expression of APTR in relation to the clinicopathological and demographic variables of the patients. The bold font indicates a statistically significant assosiation

Study variables	Subgroups	N	Expression median	P-value
Age	<50	29	7.4	0.7
	≥50	18	4.7	
Tumor size	≤2	16	6.6	**0.04**
	2–4	22	4.3	
	≥4	9	12.6	
Estrogen receptor	Positive	42	4.7	0.6
	Negative	5	7.6	
Progesterone receptor	Positive	32	4.5	0.1
	Negative	15	7.6	
HER2	Positive	19	5.9	0.5
	Negative	28	4.9	
Histological grade	G1	12	7.8	0.07
	G2	22	5.5	
	G3	13	3.8	
TNM stage	1/2	32	7.5	0.1
	3	15	3.8	
Lymph node metastasis	Yes	27	7.4	0.5
	No	20	4.5	
Histologic type of invasive carcinoma	IDC	45	5.3	
	ACC	1	38.3	0.1
	ILC	1	0.5	
Age of menarche	≤13	27	4.5	0.7
	≥14	20	7.7	
Age of first full term pregnancy (FFTP)	<25	32	5.1	0.7
	≥25	8	4.2	
Breastfeeding duration (month)	NO	8	9.9	0.6
	≤6	8	3.2	
	6–24	24	5.2	
	≥24	7	7.4	
Menopausal status	Pre	29	7.4	0.4
	Post	18	4.3	
Menopausal age	<50	8	6.9	1.0
	≥50	10	3.3	
Family history of BC	Positive	20	7.5	0.4
	Negative	27	4.7	

IDC: infiltrating ductal carcinoma, ACC: adenoid cystic carcinoma, ILC: invasive lobular carcinoma, BC: breast cancer.

2.2. Total RNA extraction and cDNA synthesis

Total RNA was isolated by TRIzol reagent (Thermo Fisher) according to the manufacturer's instructions and RNA integrity was assessed by gel electrophoresis. The extracted RNA samples were treated with DNase I (Takara, Japan) and their concentration and purity of the all treated RNAs was measured by NanoDrop ND-1000 Spectrometer (Thermo Scientific, Boston, MA). Moreover, the RNA integrity was confirmed by agarose gel electrophoresis. The cDNA synthesis assay was performed by Hyperscript™ kit from GeneAll (Korea, Cat.No: 601-005) according to the manufacturer's instructions.

2.3. Quantification of the levels of target lncRNAs by real time PCR

The real-time PCR assay was conducted using BioFACT™ SYBR Green (cat no.: DQ383-40h, South Korea). The β2-microglobulin (B2M) gene was used as the housekeeping gene for normalizing data. The real time PCR reaction assays were accomplished in total reaction volume of 20 μl reaction mixture comprising the 1 ng of cDNA, 5 pmol of each primer, and 10 μl of 2× SYBR Green PCR mix. Also, performance conditions of thermal cycling included an initial stage of activation for 30s at 95 °C followed by 40 cycles at 95 °C for 20s and 60 °C for 30 s. All reactions were performed in duplicate. The sequences of primers for real-time PCR were APTR forward 5′-GGTAGTCGATTGATGGGAAGTG-3′, APTR reverse 5′-ATTCCACTGGCTCTTGTTCC-3′; B2M forward 5′-AGATGAGTATGCCTGCCGTG-3′, and B2M reverse 5′-GCGGCATCTTCAAACCTCCA-3′. The normal breast tissues were used as calibrator to

obtain the relative threshold cycle (ΔCt), and the relative expression was calculated by $2^{-\Delta\Delta CT}$ method.

2.4. Bioinformatics predictions

In order to investigate upregulation of APTR gene in tumor tissues as far as competing endogenous RNAs (ceRNAs) is concerned, a bioinformatics approach was applied. The workflow of bioinformatics analyses has been summarized in Fig. 1. In the first step, the experimentally validated APTR-miRNA interactions were assessed by LncBase version 2 (Paraskevopoulou et al., 2012). We used the tools of LncBase database to survey the experimentally supported miRNA recognition elements (MREs) on desired lncRNAs derived from both available publications and the 153 AGO CLIP-Seq libraries. All complementary sites for seed region of miRNAs were detected in APTR sequence (Supplementary Fig. 1). Next, APTR/miRNAs/mRNAs networks in ErbB signaling pathway was constructed based on Tarbase (experimentally validated miRNA-mRNA interactions) (Karagkouni et al., 2017), DIANA mirPath (Vlachos et al., 2015), and miRmap (Vejnar et al., 2013) databases. It should be noted that the miRNA-mRNA interactions, which were obtained from TarBase, have been validated by miRmap database in an attempt to reduce the number of false positive interactions. The mRNAs with more than two miRNA interactions were selected for further analyses and the extracted subnetwork was illustrated by cytoscape software (Smoot et al., 2010). Finally, GEPIA web server was used to investigate expression of APTR and downstream genes in APTR/miRNAs/mRNAs axes in breast tumors compared with normal tissues based on the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) data (Tang et al., 2017). Enrichr web server was used for functional annotation based on GO Biological Process 2018. P-value <0.05 was considered to be as significant enrichment (Kuleshov et al., 2016).

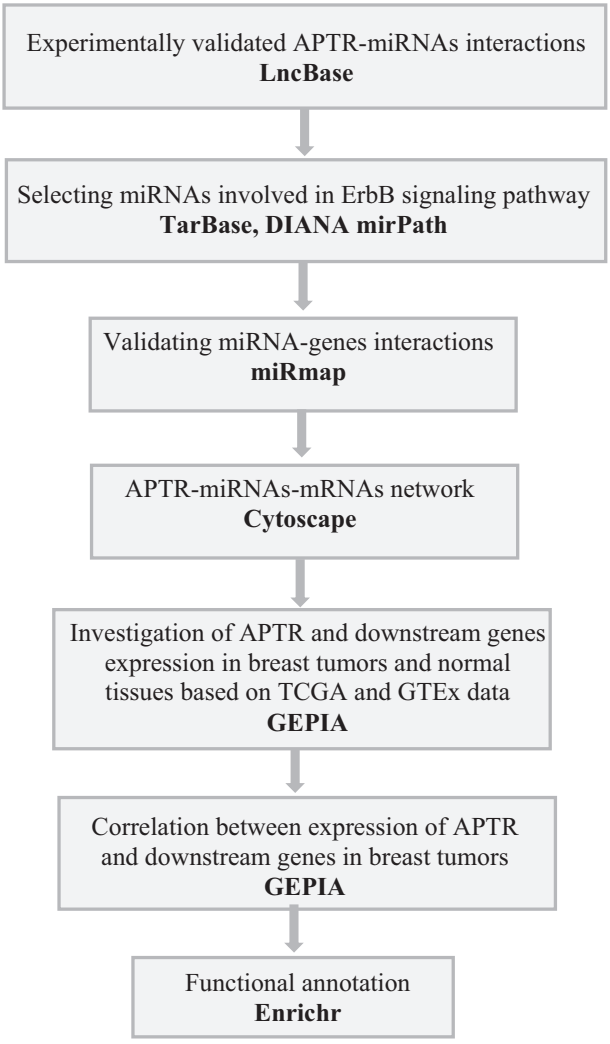

Fig. 1. The workflow of bioinformatics analyses.

2.5. Statistical analysis

In this study, statistical analyses were carried out by SPSS 21 software package. Numerical values were presented as median and 95% confidence interval (CI) and qualitative data as frequency and percentage. The Mann–Whitney test was conducted to compare the APTR relative expression between tumor samples and paired adjacent normal tissues. The association between the expression of APTR and clinicopathological and demographic characteristics were calculated by *t*-test or ANOVA and alternative non-parametric tests. P-value less than 0.05 was considered as a statistically significant level.

3. Results

3.1. APTR expression in tumor and paired normal tissues as well as its expression level in relation to clinicopathological and demographic characteristics of the patients

As it is shown in Fig. 2A, the statistical evaluations showed that the levels of APTR were significantly higher in tumors (Median = 5.3) than normal samples (Median = 0.6) (P-value <0. 0001). In addition, Kruskal-Wallis Test indicated that increased expression of APTR is significantly associated with higher tumor size (Fig. 2B). In this study, APTR expression is not significantly related to other clinicopathological and demographic characteristics of the patients. Altough, increased expression of APTR shows a trend toward higher grade tumors, it is not statistically significance level (P-value = 0.07). The details of clinicopathological and demographic characteristics of the patients and the expression level of lncRNA APTR are summarized in Table 1.

3.2. Bioinformatics prediction of potential APTR-miRNAs-mRNAs axes involved in ErbB signaling pathway APTR-miRNAs-mRNAs network

We observed bioinformatically that APTR, as a miRNA sponging RNA, has several MREs on its own sequence. According to LncBase Experimental v.2 and DIANA miRpath, 12 ErbB signaling-related miRNAs could be sponged by APTR. Furthermore, based on Tarbase and miRmap, 31 ErbB signaling-related mRNAs are regulated by these miRNAs (Supplementary Fig. 2). When identifying a stronger APTR-miRNAs-mRNAs network, we found downstream genes that are potentially regulated by more than two miRNAs. The achieved network indicated 10 miRNAs (miR-4328, miR-5006-3p, miR-26a-5p, miR-32-5p, miR-132-3p, miR-188-3p, miR-19a, miR-19b, miR-450b-5p and miR-139-5p) are not only targeting some ErbB signaling-related genes but also could be sponged by lncRNA APTR. Among them, miR-5006-3p and miR-4328 had three and two MREs on the APTR sequence, respectively. The data obtained on these findings are shown in Fig. 3. In addition, we found that the five protein encoding genes including CRKL, MAP2K4, PTK2, ABL2 and GAB1 were downstream of the APTR-miRNAs-mRNAs axes and were potentially regulated by a set of miRNAs mentioned above. And finally, based on the TCGA and GTEx data, the expression analysis of APTR and its downstream genes in breast tumors compared with normal tissues showed that the APTR, CRKL, MAP2K4, PTK2 and ABL2 had a tendency toward higher expression in tumors, although this observation was not at a significant level (Supplementary Fig. 3).

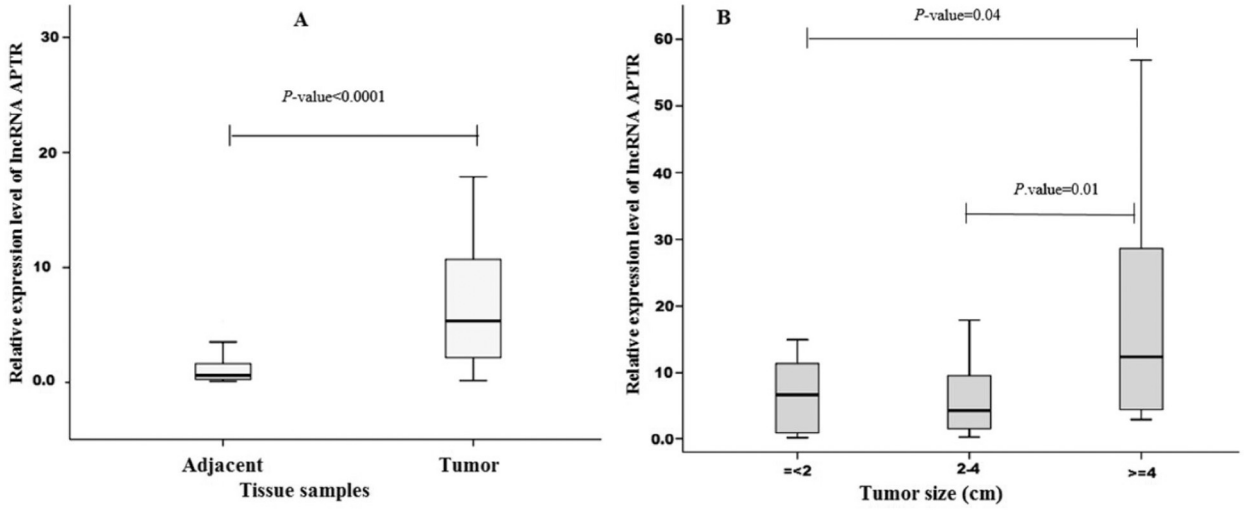

Fig. 2. A: The scatter plot of comparison of APTR expression between tumor samples and paired adjacent normal tissues. B: The boxplot of comparison of APTR expression between the different tumor size groups. P.value was calculated using the Mann–Whitney test.

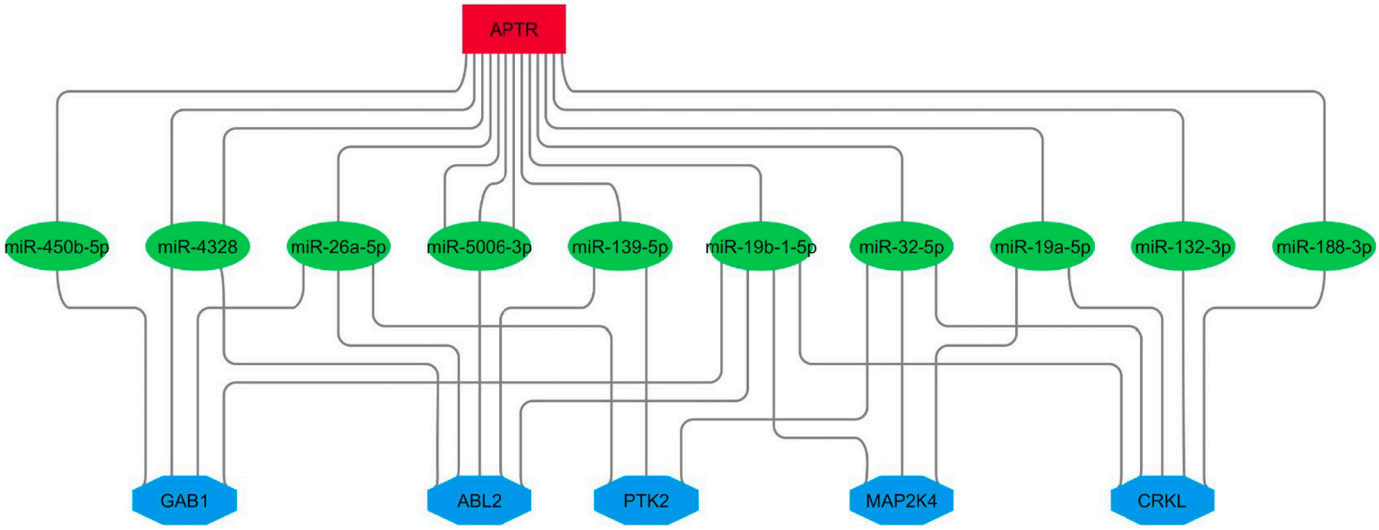

Fig. 3. The extracted axes from APTR/miRNAs/mRNAs network involved in ErbB signaling pathway, including mRNAs which show more than 2 interactions with miRNAs. The nodes in green represent microRNAs, the nodes in blue are mRNAs and node in red is lncRNA. The number of edges between APTR and miRNAs indicates number of complementary sites in APTR sequence for miRNA seed region. The number of edges between miRNA and mRNA indicates complementary sites in mRNA 3′UTR for miRNA seed region.

3.3. Functional annotation analysis

Enrichr web server was used to investigate GO terms associations with five protein coding genes which are shown in Fig. 3 based on GO Biological Process 2018. Detailed results are summarized in Table 2. Results demonstrated that genes function in benefit of tumor proliferation and angiogenesis.

4. Discussion

Although significant advances have been made on various aspects of breast tumors, because of their wide heterogeneous phenotype they do not respond effectively to existing therapeutic interventions. For this reason, BC is still considered as a cancer with high mortality in women (Turashvili and Brogi, 2017; Ellsworth et al., 2017). Therefore, developing the effective therapeutic approaches for this cancer is urgently essential. Accumulating biomedical evidence has revealed that dysregulation of lncRNAs is linked to pathophysiology of the BC and they also

Table 2
Functional enrichment analysis of protein encoding genes including CRKL, MAP2K4, PTK2, ABL2 and GAB1 genes.

Biological process	GO ID	P.value	Combined Score
ErbB signaling pathway	38127	6.30E−07	21.31
Positive regulation of cell migration by vascular endothelial growth factor signaling pathway	38089	0.0019	21.69
Epidermal growth factor receptor signaling pathway	7173	2.30E−07	33.13
Regulation of substrate adhesion-dependent cell spreading	1900024	4.20E−05	17.19
Vascular endothelial growth factor signaling pathway	38084	0.0019	17.04
Negative regulation of anoikis	2000811	0.0039	11.98

Data have been achieved from Enrichr web server. GO: gene ontology.

have the potential to be promising diagnostic, prognostic biomarkers and therapeutic targets (Zhang et al., 2019). Accordingly, detailed understanding the role of lncRNAs and their mechanisms of function at both the transcriptional and translational levels during the BC tumourigenesis might help to further discover the biological features of the breast tumors as well as their clinical uses (Zhang et al., 2019; Arun and Spector, 2019). An interesting and recent discovery related to lncRNAs and BC cancer is the identification of dysregulated lncRNA-related ceRNA regulatory networks, in which lncRNAs act as a sponge for miRNAs regulating the protein-coding mRNAs in BC tumourigenesis and pathogenesis (Abdollahzadeh et al., 2019). According to the suggestions of current BC research, these ceRNA networks appear to be key clues in characterizing the complex aspects of breast tumors, and so identifying more networks in BC may help in revealing how it molecularly and clinically develops. In this regard, some novel dysregulated lncRNAs with ceRNA activity have been reported in the development of various cancers (Qi et al., 2019), however there is no data regarding them in BC (Negishi et al., 2014).

Herein, we indicated an upregulated expression level of the lncRNA APTR in BC tumor tissues compared to paired adjacent normal tissues. In addition, our results revealed that this elevated expression of APTR is significantly linked to a larger tumor size. Previous data regarding the involvement of APTR in BC has not been reported. However, this observation in our study is in line with the previous works whose findings have implied a potential oncogenic role of the APTR in some tumors. For example, Negishi et al. (Yu et al., 2015) demonstrated that APTR represses p21 expression by recruiting the PRC2 complex to the p21 promoter in glioma cell lines. Moreover, APTR was upregulated and activated in hepatic stellate cells (HSCs) in fibrotic liver samples, and APTR silencing could inhibit cell cycle and proliferation in HSCs. In another study, a significant overexpression of the APTR in human osteosarcoma tissues and cell lines has been recently reported. And interestingly, this investigation indicated that lncRNA APTR contributes to osteosarcoma cell proliferation, invasion and migration through APTR/miR-132-3p/YAP1 ceRNA axis and APTR knockdown via siRNA resulted in the reduction of the cell proliferation, invasion and migration of the osteosarcoma cell lines (Guan et al., 2019). Therefore, based on our finding that the expression of APTR is elevated in BC as well as the above observations by various studies on its oncogenic roles in other cancers, it is thought that lncRNA APTR may function as an oncogene in key hallmarks of the BC, partly through via its ceRNA activity.

Accordingly, we expanded its expression results into bioinformatics ceRNA function data, focusing on its role in important signaling pathways involved in BC, in particular as APTR/miRNAs/mRNAs network regulating ErbB signaling pathway. This signaling pathway is a well-known proliferative pathway in the BC development where several data have shown that ErbB receptor family and its downstream pathways have regulated cell proliferation, angiogenesis and migration (Hardy et al., 2010). The outputs of this analysis indicated that the lncRNA APTR may regulate five target downstream genes, including CRKL, MAP2K4, PTK2, ABL2 and GAB1 through APTR/miRNA/mRNA axis. Besides, through comparing the TCGA breast tumor data with TCGA and GTEx normal data, we observed that mRNAs of the APTR, CRKL, MAP2K4, PTK2 and ABL2 had a tendency toward higher expression level in tumors. In the next step, functional enrichment analysis for these protein encoding genes consistently confirmed their oncogenic roles in positive regulation of cell proliferation and migration as well as negative regulation of anoikis.

Importantly, the CRKL gene produces an adapter protein that is thought to be an oncogene. It plays crucial roles in various cellular biological processes, including cell proliferation, adhesion and migration. The overexpression of CRKL is correlated with progression and malignant proliferation of human BC (Zhao et al., 2013). PTK2, a cytoplasmic protein tyrosine kinase, is over-expressed and activated in several solid tumors, including BC (Rigiracciolo et al., 2019). The protein product of the PTK2 controls invasion and cancer stem cell self-renewal (Sulzmaier et al., 2014). Another member of the target downstream set for APTR was the ABL2 gene, which encodes a non-receptor tyrosine kinase and plays a key role in cell proliferation. It is reported that activation of ABL2 in solid tumors is driven by its enhanced expression due to amplification (Greuber et al., 2013). Furthermore, ABL2 overexpression predicts poor prognosis and is linked to cancer cell migration and invasion in BC (He et al., 2019). Regarding the Grb2-associated binder 1 (GAB1), it is a docking protein that transduces proliferative signals from Met and EGFR tyrosine kinases. And the reported evidence has indicated that the expression of GAB1 is significantly increased in breast tumors compared to normal breast samples. Additionally, its elevated expression is positively linked with the metastasis in BC patients (Wang et al., 2019). Research results concerning the mitogen-activated protein kinase kinase 4 (MAP2K4) have showed inconsistency data. Some studies have revealed its function as an oncogene in cancer progression and invasion (Hübner et al., 2018). However, a number of studies have found that MAP2K4 act as a tumor suppressor gene (Yamada et al., 2002). Consequently, these data highlight the findings of the present study that the lncRNA APTR may play a weighty function in pathophysiology of the BC through interacting with these key cancer-related protein encoding genes.

Additionally, our bioinformatics analysis found that 10 different miRNAs including the miR-4328, miR-5006-3p, miR-26a-5p, miR-32-5p, miR-132-3p, miR-188-3p, miR-19a, miR-19b, miR-450b-5p and miR-139-5p potentially target all the above ErbB signaling-related encoding genes, through binding to their seed regions on the relevant mRNAs. On the other hand, it was indicated that lncRNA APTR could sponge these miRNAs. Thus, this may reveal a potential APTR-miRNAs-mRNAs network in which the APTR acts as ceRNA for sponge of the miRNAs regulating downstream mRNAs whose works are to control ErbB signaling. It is also interesting to note that the lncRNA APTR had three MREs for miR-5006-3p and two MREs for miR-4328, implying a stronger sponging. Notably, evidence from previous research supports that miR-4328, miR-5006-3p, miR-26a-5p, miR-32-5p, miR-132-3p, miR-188-3p, miR-450b-5p and miR-139-5p are downregulated in different cancers, including BC (studies shown in Table 3). Furthermore, the interaction between APTR and miR-132-3p has functionally validated by luciferase assay in the osteosarcoma cell line in which APTR downregulates the miR-132-3p (Guan et al., 2019). Thus, based on these observations it is reasonable to suggest that these miRNAs have tumor suppressive roles in malignancies, and during the BC development, because of increased expression of lncRNA APTR and its oncogenic ceRNA function, expression of these miRNAs is reduced.

Taken together, our experiment and bioinformatics results, in agreement with the supporting evidence from previous studies, suggest that lncRNA APTR is significantly upregulated in the breast tumors, and mechanistically, it may act as an oncogene in the development of BC through sponging the tumor suppressive miRNAs which controls the ErbB signaling pathway via its ceRNA activity. In turn, this results in upregulating the mRNAs of some oncogenes whose works are to boost ErbB signaling pathway. Most importantly, increased expression of the APTR and its downstream protein encoding genes regulating the molecular ErbB signaling pathway are linked to tumor cell proliferation. Thus, the association of APTR upregulation with tumor size in the present study may be a reflection of such a molecular event, and this makes it to be a potential prognostic biomarker for breast tumors. Although further work needs to be carried out in order to better understand the detailed roles of each of the described components of the potential APTR/miRNAs/mRNAs ceRNA network in BC development.

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Table 3
Summary of previous studies about dysregulation of miRNAs involved in APTR/miRNAs/mRNAs axes (Fig. 3) in different cancer types.

miRNA	Article	Tissue type	Dysregulation
miR-4328	Feng et al. (2017)	Prostate cancer	Down
	Zang et al. (2014)	Esophageal squamous cell carcinoma	Down
	Li et al. (2013)	Keloid fibroblast	Down
miR-5006	Slattery et al. (2016)	Colorectal carcinomas	Down
miR-26a	Ichikawa et al. (2012)	Breast cancer	Down
	Lu et al. (2011)	Nasopharyngeal carcinoma	Down
	Deng et al. (2013)	Gastric cancer	Down
miR-450b-5p	Brockhausen et al. (2015)	Hepatocellular	Down
miR-188-3p	Wu et al. (2014a)	Asopharyngeal carcinoma	Down
	Wang and Liu (2016)	Oral squamous cell carcinoma	Down
	Sun et al. (2010)	Breast cancer stem cells	Down
	Chang et al. (2015)	Triple-negative breast cancer	Up
miR-139-5p	Shen et al. (2012)	Colorectal cancer	Down
	Krishnan et al. (2013)	Breast cancer	Down
	Yonemori et al. (2016)	Bladder cancer	Down
	Song et al. (2014)	Colorectal cancer	Down
	Yue et al. (2015)	Glioma	Down
miR-19a/b	Jia et al. (2013)	Glioma	Up
	Wu et al. (2014b)	Gastric cancer	Up
	Feng et al. (2014)	Bladder cancer	Up
miR-132-3p	Zheng et al. (2014)	Colorectal cancer	Down
	Li et al. (2015)	Lung cancer	Down
	Zhang et al. (2014a)	Breast cancer	Down
	Tian et al. (2016)	Ovarian cancer	Down
	Wang et al. (2014)	Osteosarcoma cells	Down
	Liu et al. (2015)	Hepatocellular carcinoma	Down
miR-32-5p	Guan et al. (2012)	Lung cancer	Down
	Zhang et al. (2014b)	Gastric cancer	Down

CRediT authorship contribution statement

Hosein Mansoori: Data curation, Writing – original draft, Visualization. **Farzaneh Darbehesht:** Data curation, Writing – original draft, Visualization. **Abdolreza Daraei:** Validation. **Maral Mokhtari:** Resources. **Mohammad Bagher Tabei:** Investigation. **Rasoul Abdollahzadeh:** Investigation. **Hassan Dastsooz:** Methodology. **Milad Bastami:** Writing – review & editing. **Ziba Nariman-Saleh-Fam:** Writing – review & editing. **Hamzeh Salmani:** Formal analysis. **Yaser Mansoori:** Project administration, Supervision, Funding acquisition. **Sedigheh Tahmasebi:** Project administration, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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