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Expression analysis of CDKN2C-related lncRNAs in breast cancer

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ABSTRACT

Cell cycle related genes are implicated in the pathogenesis of breast cancer. In the present study, we aimed at identification of the role of a cell cycle related gene and functionally associated long non-coding RNAs in breast cancer. Thus, we studied expression levels of *CDKN2C* gene and three related long non-coding RNAs, namely *PANDAR*, *AATBC* and *PANTR1* in breast cancer samples compared with adjacent tissues. Expression of *CDKN2C* was significantly lower in breast tumor samples compared with control samples (expression ratio (95% CI) = 0.49 (0.3–0.8), *P* values = 0.0049). On the other hand, *PANTR1* was up-regulated in cancerous samples compared with control samples (expression ratio (95% CI) = 4.25 (1.25–14.38), *P* values = 0.033). While *CDKN2C* could separate these sets of samples with AUC ± SD, sensitivity, specificity and P values of 0.75 ± 0.05, 0.68, 0.86 and 0.0002, respectively; *PANTR1* could not distinguish between cancerous and non-cancerous samples (P value = 0.6). AUC ± SD, sensitivity and specificity values for *PANTR1* were 0.53 ± 0.07, 0.97 and 0.36, respectively. There were significant differences in the correlation patterns of *CDKN2C/PANDAR*, *CDKN2C/AATBC* and *CDKN2C/PANTR1* pairs between cancerous and non-cancerous tissues. In fact, expression of *CDKN2C* was correlated with all lncRNAs in cancerous samples, in spite of lack of correlation in non-cancerous tissues. The present study shows possible role of CDKN2C in the pathoetiology of breast cancer.

1. Introduction

CDKN2C gene encodes cyclin-dependent kinase 4 inhibitor C. This protein interacts with CDK4 or CDK6, and prevents activity of the CDK kinases. Therefore, this protein is regarded as a regulator cell cycle transition. Over-expression of CDKN2C has led to suppression of cell growth in an RB1-dependent manner function (Guan et al., 1994). CDKN2C has been found to be altered in a number of cancers. For instance, about one-fourth of pituitary adenomas have exhibited loss of heterozygosity at the *CDKN2C* locus. Moreover, methylation of *CDKN2C* promoter has been detected in about 40% of these tumors (Kirsch et al., 2009). Another study has reported deletion of *CDKN2C* in a proportion of patients with monoclonal gammopathy of undetermined significance, smoldering multiple myeloma, and newly diagnosed multiple myeloma patients. Notably, hemizygous or homozygous deletion of this locus has been associated with poor overall survival. Thus, deletion of *CDKN2C* is regarded as a contributor in the development and clinical course of this cancer (Leone et al., 2008).

This cell cycle-related protein can interact with other biomolecules, particularly long non-coding RNAs (lncRNAs). These interactions have been best recognized in the context of cancer. For instance, *long intergenic non-protein coding RNA 673* (*LINC00673*) has been shown to repress expression of CDKN2C through EZH2-mediated Trimethylation of H3K27, thus participating in the development of squamous cell carcinoma of esophagus (Zhou et al., 2020). This lncRNA has also been shown to be an indicator of poor prognosis in patients with lung cancer. In this

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type of cancer, *LINC00673* silencing has overturned TGF-β associated epithelial-mesenchymal transition (EMT). Notably, expression of *LINC00673* has been found to be down-regulated by miR-150-5p. Moreover, *LINC00673* has the ability to sponge miR-150-5p and influence expression of an important regulator of EMT, namely ZEB1 (Lu et al., 2017). Moreover, in the context of hepatoma, *HULC* has been shown to decrease expression of CDKN2C (Du et al., 2012). Also, *PANDA* could promote G1/S transition in osteosarcoma cells through suppression of transcription of CDKN2C (Kotake et al., 2017).

Based on the critical roles of lncRNAs in the development of breast cancer (Zhang et al., 2019), we aimed at identification of the role of cell cycle-associated lncRNAs in breast cancer. We designed the current investigation to find CDKN2C-related lncRNAs through an *in silico* approach (using https://bio-annotation.cn/lncrna2target/search.jsp.), then assessing their expressions in paired breast tumor samples and adjacent samples. This *in silico* approach is founded on differentially expressed genes after knockdown or overexpression of lncRNAs, and recognizes the targets of a particular lncRNA or for the lncRNAs that target a certain gene (Cheng et al., 2019). Thus, the obtained lncRNAs through this approach are transcriptionally related with CDKN2C.

2. Materials and methods

2.1. Breast cancer patients

Expressions of *CDKN2C*, *PANDAR*, *AATBC* and *PANTR1* were enumerated in 40 pairs of breast tumors and their adjacent non-tumoral samples. Control samples were obtained from non-cancerous tissues of the same patients. These samples were collected from Farmanieh and Sina hospitals, Tehran, Iran during 2017–2020. All samples were immediately frozen in liquid nitrogen and then were stored in −70 °C. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Science (IR.SBMU.MSP.REC.1399.332). All participants signed informed consent forms. Tissue samples were acquired during surgical removal of breast tumors prior to any chemotherapy or radiotherapy. Medical records were assessed to acquire clinicopathological and demographic information.

2.2. Experiments

Total RNA was extracted from tumor samples and adjacent tissues using the GeneAll kit (Seoul, South Korea). Then, approximately 60 ng of extracted RNA was transformed to cDNA using the SMOBIO kit (Taiwan). Expressions of *CDKN2C*, *PANDAR*, *AATBC* and *PANTR1* were enumerated in all samples using Ampliqon master mix (Denmark). Based on our previous experiment showing stability of *B2M* expression in breast tissues and their independence from the presence of cancer (Oskooei et al., 2018), we used this gene as the normalizer. Primers sequences are demonstrated in Table 1.

Table 1
Nucleotide sequences of primers.

Gene	Primer sequence		Primer length	Product size (bp)
PANDAR	Forward	TCCCAACAAACAAGGGGTGG	20	134
	Reverse	AGGTCTTGGATTGAGGAACAGG	22	
AATBC	Forward	AAGGCCGGTTATCAACGT	18	151
	Reverse	GCCAGTCCCTCACTGCTCT	19	
PANTR1	Forward	AATCACTGCAATTGAAGGAAAAA	23	207
	Reverse	CCTTGTTTTCCAACCCTTAGACT	23	
CDKN2C (P18)	Forward	ACTGGTTTCGCTGTCATTCA	20	113
	Reverse	GGCAGGTTCCCTTCATTATCC	21	
B2M	Forward	AGATGAGTATGCCTGCCGTG	20	105
	Reverse	GCGGCATCTTCAAACCTCCA	20	

2.3. Statistical analysis

Expression data was analyzed using SPSS v.22.0 (Chicago, IL). Charts and figures were designed using GraphPad Prism version 9.0 (GraphPad Software, La Jolla California, USA). Expressions of *CDKN2C* and three selected lncRNAs in breast tissue samples were calculated using the following formula:

2.4. Efficiency adjusted Ct of B2M - efficiency adjusted Ct of target gene

Relative expression of *CDKN2C* and three selected lncRNAs was appraised in breast tumors and adjacent tissues using the paired *t*-test or Wilcoxon matched-pairs signed rank test. Association between expression of these genes and clinical features was evaluated by Mann-Whitney and one-way ANOVA tests (Kruskal-Wallis). As our sample size was smaller than 50, we used the Shapiro–Wilk test to decide whether or not the data fits a normal distribution. The majority of our qPCR data was not normally distributed; therefore, we used the relevant nonparametric tests for relative expression of studied genes (Wilcoxon matched-pairs signed rank test) and the relevant nonparametric tests for evaluating correlations between expression levels of studied genes (Spearman correlation coefficient). However, the qPCR data of tumor and normal adjacent tissue samples for *AATBC* gene was normally distributed; therefore, we used the relevant parametric test for relative expression of this gene (paired *t*-test). For evaluating association between expression of the studied genes and clinical features, we used nonparametric tests. The Mann-Whitney test was used for comparing means between two independent samples and Kruskal-Wallis test was used for comparing means between more than two independent samples. Receiver operating characteristic (ROC) curve was plotted by the GraphPad Prism v.9 software. P value <0.05 was considered as significant.

3. Results

The study included a cell cycle related gene and three associated lncRNAs. The biological activity of *PANDAR* lncRNA was mainly related with DNA damage response. For other lncRNAs, the biological activities are not well-defined. Table 2 shows information about selected genes in this study.

We have gathered several clinicopathological parameters from medical records of patients. Hormone receptor and HER2 status was assessed by IHC. Table 3 summarizes the clinicopathological data in enlisted patients.

Relative expression levels of *CDKN2C*, *PANDAR*, *AATBC* and *PANTR1* are shown in Fig. 1.

Table 2
Information about function and location of selected genes.

Name/ Gene ID	Accession number	Location	Description	Biological activity
CDKN2C	NM_001262.3 NM_078626.3	1p32.3	Cyclin dependent kinase inhibitor 2C	Function as a cell growth regulator that controls cell cycle G1 progression
PANDAR	NR_109836.1	6p21.2	Promoter of CDKN1A antisense DNA damage activated RNA	It may regulate the response to DNA damage.
AATBC	NR_026961.1	21q22.3	Apoptosis associated transcript in bladder cancer	–
PANTR1	NR_037883.1	2q12.1	POU3F3 adjacent non-coding transcript 1	–

Table 3
Summary of obtained clinical and demographic information (ER, PR and HER2 status is based on IHC staining).

Case No	Stage	Grade	Tumor Size (cm)	Mitotic Rate	First Pregnancy Age (year)	OCP	HRT	Obesity	Lymph node	ER	PR	HER-2
1	3	2	2	1	17	Positive	Negative	ND	ND	Positive	Negative	−
2	4	2	3	2	17	Negative	Negative	Normal	Positive	Positive	Positive	−
3	3	3	ND	2	22	Positive	Negative	ND	Positive	Negative	Negative	−
4	1	ND	ND	ND	15	Positive	Negative	Normal	Positive	Negative	Negative	+
5	ND	ND	ND	ND	18	Positive	Negative	Overweight	ND	Negative	Negative	−
6	2	2	2.5	1	24	Negative	Negative	Overweight	ND	Positive	Positive	−
7	1	1	1.5	1	24	Negative	Negative	Normal	ND	Positive	Positive	+
8	1	ND	1.5	ND	22	Negative	Negative	Overweight	ND	Positive	Positive	+++
9	2	3	2.5	2	34	Positive	Negative	Normal	ND	Positive	Positive	+++
10	3	1	3	ND	24	Negative	Negative	Normal	ND	Positive	Positive	−
11	2	ND	7	ND	24	Negative	Negative	Normal	ND	Positive	Positive	++
12	1	ND	2	ND	15	Negative	Negative	Normal	Positive	Positive	Negative	+++
13	1	2	ND	ND	23	Positive	Negative	Overweight	Negative	Negative	Negative	+
14	1	2	2	1	20	Positive	Negative	Normal	Positive	Positive	Positive	−
15	1	ND	2	ND	17	Positive	Negative	Normal	Positive	Positive	Positive	−
16	1	2	1.5	2	–	Negative	Positive	Overweight	Negative	Positive	Positive	+
17	1	2	1.8	2	28	Positive	Negative	Overweight	Positive	Positive	Negative	++
18	3	3	1	3	37	Positive	Negative	Normal	Positive	Negative	ND	+++
19	2	1	2	1	27	Positive	Negative	Normal	Negative	Positive	Positive	+
20	4	3	2	3	–	Negative	Positive	Overweight	ND	Positive	Positive	++
21	3	2	2	1	18	Negative	Negative	Overweight	Negative	Positive	Positive	−
22	3	3	1	2	28	Negative	Negative	Overweight	ND	Positive	Positive	+++
23	2	2	2	2	18	Negative	Negative	Overweight	ND	Positive	Positive	−
24	2	3	2	2	19	Positive	Negative	Underweight	Negative	Negative	Positive	−
25	3	1	1.5	1	24	Negative	Negative	Normal	Negative	Positive	Positive	+++
26	2	1	3	ND	14	Negative	Negative	Overweight	Positive	Positive	Positive	++
27	3	2	1.5	1	21	Positive	Negative	Normal	Negative	Negative	Negative	−
28	1	2	2	2	27	Positive	Negative	Overweight	Positive	Positive	Positive	+++
29	3	2	2	1	25	Positive	Negative	Overweight	Negative	Positive	Positive	+
30	3	3	2.8	ND	–	Positive	Negative	Normal	Negative	Positive	Positive	+
31	4	3	2	ND	18	Positive	Negative	Normal	Negative	Positive	Positive	++
32	1	ND	1.5	ND	–	Negative	Negative	Normal	Positive	Positive	Positive	−
33	3	2	2	2	22	Positive	Negative	Normal	ND	Positive	Positive	+
34	2	3	4	3	17	Positive	Negative	Overweight	ND	Positive	Positive	++
35	4	3	2	2	19	Positive	Positive	Overweight	ND	Positive	Positive	+
36	2	3	3	1	18	Negative	Negative	Normal	Negative	Positive	Positive	++
37	1	1	1	2	19	Negative	Negative	Normal	Positive	Positive	Positive	++
38	1	1	0.5	2	15	Positive	Negative	Overweight	Negative	Positive	Positive	+++
39	4	3	5	2	27	Negative	Negative	Normal	Negative	Positive	Positive	−
40	3	3	1	3	23	Negative	Positive	Overweight	Positive	Positive	Positive	++

OCP: Oral contraceptives; HRT: Hormone replacement therapy; ND: Not determined; ER: estrogen receptor; PR: progesterone receptor.

Expression of *CDKN2C* was significantly lower in breast tumor samples compared with control samples (expression ratio (95% CI) = 0.49 (0.3–0.8), P values = 0.0049). On the other hand, *PANTR1* was up-regulated in cancerous samples compared with control samples (expression ratio (95% CI) = 4.25 (1.25–14.38), *P* values = 0.033) (Table 4).

Then, we evaluated diagnostic power of *CDKN2C* and *PANTR1* in differentiation of breast cancer samples from controls (Fig. 2).

While *CDKN2C* could separate these sets of samples with AUC ± SD, sensitivity, specificity and *P* values of 0.75 ± 0.05, 0.68, 0.86 and 0.0002, respectively; *PANTR1* could not distinguish between cancerous and non-cancerous samples (P value = 0.6). AUC ± SD, sensitivity and specificity values for *PANTR1* were 0.53 ± 0.07, 0.97 and 0.36, respectively. Thus, *CDKN2C* and *PANTR1* had high specificity and sensitivity values, respectively.

We also evaluated correlation between expression levels of *CDKN2C*, *PANDAR*, *AATBC* and *PANTR1* in both cancerous and non-cancerous tissues (Table 5). There were significant differences in the correlation patterns of *CDKN2C/PANDAR*, *CDKN2C/AATBC* and *CDKN2C/PANTR1* pairs between cancerous and non-cancerous tissues. In fact, expression of *CDKN2C* was correlated with all lncRNAs in cancerous samples, in spite of lack of correlation in non-cancerous tissues.

We also assessed association between expression levels of *CDKN2C*, *PANDAR*, *AATBC* and *PANTR1* in tumoral tissues and clinical data (Table 6). Expressions of *PANDAR* and *PANTR1* were associated with clinical stage (*P* values = 0.024 and 0.01, respectively), with lowest expression levels detected in stage I. Moreover, expression of *PANTR1* was associated with mitotic rate (P value = 0.048). Finally, expression of *AATBC* was lower in overweight patients compared with those with normal weigh (*P* value = 0.028).

There was a positive association between histological grades and mitotic rates ($\chi 2 = 12.28$, P value = 0.015) and a positive association between clinical stages of patients and tumor size ($\chi 2 = 11.2$, *P* value = 0.01). Also, there was a positive association between clinical stages and histological grades of patients ($\chi 2 = 16.2$, *P* value = 0.01). Finally, there was a positive association between estrogen receptor and progesterone receptor status. ($\chi 2 = 14.48$, *P* value = 0.001).

4. Discussion

We have measured expression of *CDKN2C* gene and three related lncRNAs, namely *PANDAR*, *AATBC* and *PANTR1* in breast cancer samples compared with control samples. Expression of *CDKN2C* was significantly lower in breast tumor samples compared with controls. Inactivation of CDKN2C by point mutations has been shown to participate in the dysregulation cell growth in breast cancer cells (Lapointe et al., 1996). Moreover, mutations in this gene have been detected in a small percentage of breast cancer samples (Blais et al., 1998). Although we detected down-regulation of *CDKN2C* in breast cancer samples, we did not assess the underlying cause of this down-regulation. Future studies are needed to find whether down-regulation of this gene is due to point mutations, loss of heterozygosity or promoter methylation.

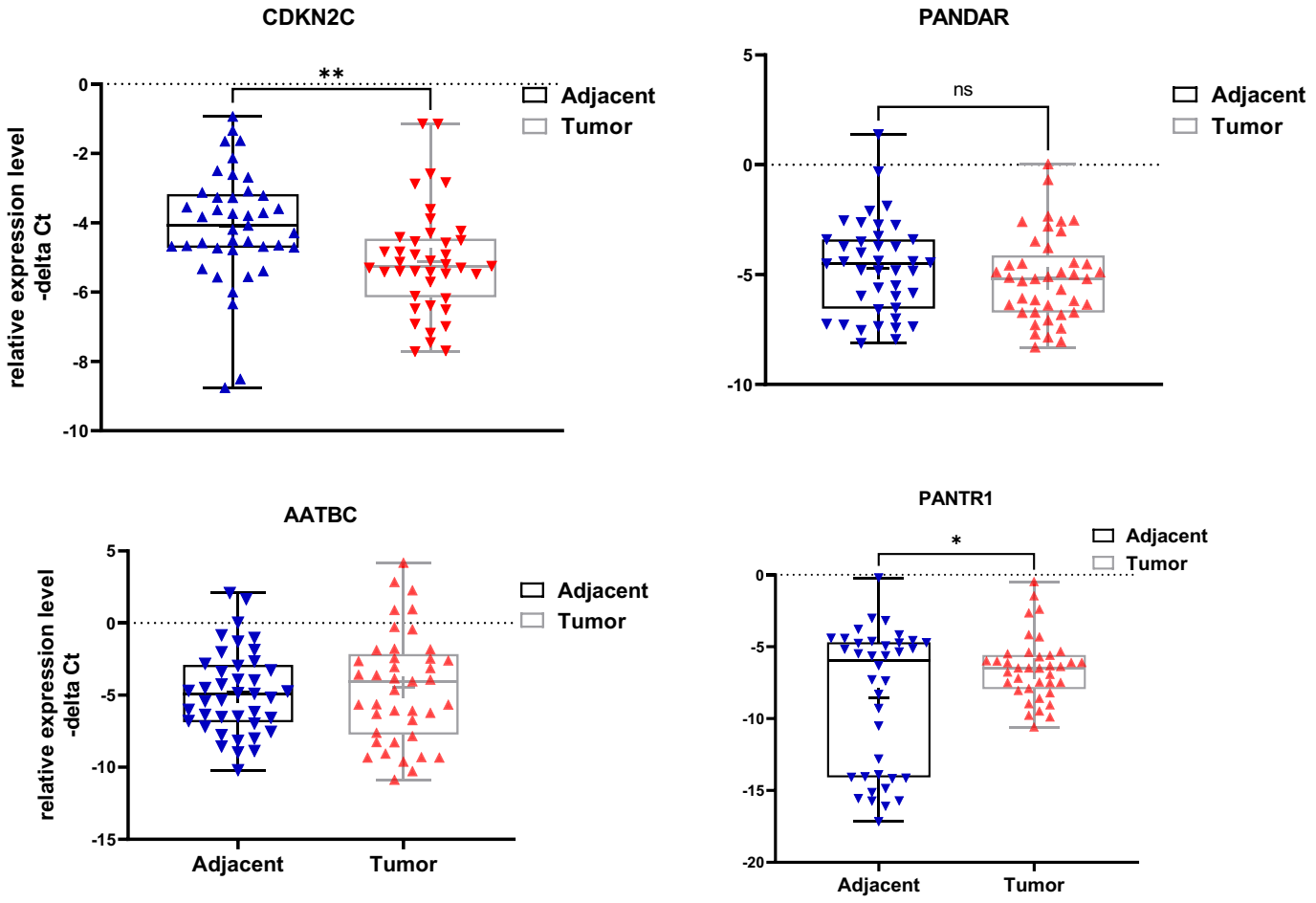

Fig. 1. Relative expressions of *CDKN2C*, *PANDAR*, *AATBC* and *PANTR1* in breast cancer samples compared with adjacent samples.

Table 4
The results of expression study of *CDKN2C* and three related lncRNA genes in breast cancer samples compared with adjacent non-cancerous tissues. The expression ratio of each gene is shown as mean and 95% Confidence interval.

Studied genes	Expression ratio (95% CI)	SEM	P Value
CDKN2C	0.49 (0.3–0.8)	0.34	**0.0049**
PANDAR	0.72 (0.37–1.4)	0.46	0.37
AATBC	1.26 (0.47–3.35)	0.69	0.62
PANTR1	4.25 (1.25–14.38)	0.86	**0.033**

On the other hand, *PANTR1* was up-regulated in cancerous samples compared with control samples. This lncRNA has an oncogenic function in some types of tumors. For instance, *PANTR1* has a functional role in regulation of angiogenic and apoptotic processes in renal cell cancer and its expression is associated with poor clinical outcome (Seles et al., 2020). Moreover, it can promote migration and invasiveness of nasopharyngeal carcinoma cells through enhancing expression of TGF-β1 (Li et al., 2019). *PANTR1* also enhances proliferation of prostate cancer cells through increasing expression of rho-associated protein kinase (Wan et al., 2019). Contribution of *PANTR1* in the pathogenesis of breast cancer might be through modulation of TGF-β1 or rho-associated protein kinase. Thus, up-regulation of this lncRNA in breast cancer samples might affect metastatic ability of these cells.

ROC curve analyses showed that *CDKN2C* can separate malignant tissues from non-malignant ones with diagnostic power of 0.75. However, *PANTR1* could not distinguish between cancerous and non-cancerous samples.

Correlation analyses have shown that there were significant differences in the correlation patterns of *CDKN2C/PANDAR*, *CDKN2C/AATBC* and *CDKN2C/PANTR1* pairs between cancerous and non-cancerous tissues. In fact, expression of *CDKN2C* was correlated with all lncRNAs in cancerous samples, in spite of lack of correlation in non-cancerous tissues. Thus, the presence of cancer can influence the correlation between this cell cycle-related gene and mentioned lncRNAs.

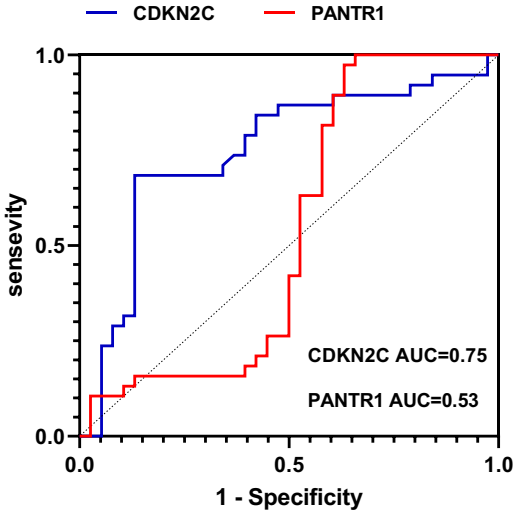

Fig. 2. ROC curves showing diagnostic power of *CDKN2C* and *PANTR1* in differentiation of breast cancer samples from controls.

Table 5
Spearman's correlations between RNA expression levels of genes in breast tumor tissues ($N = 40$) and adjacent non-tumor tissues ($N = 40$).

	PANDAR Tumor adjacent	AATBC Tumor adjacent	PANTR1 Tumor adjacent
CDKN2C (P18)	0.6** 0.02	0.41* 0.28	0.64** 0.27
PANDAR		0.67** 0.47**	0.87** 0.57**
AATBC			0.45* 0.79 **

Expressions of *PANDAR* and *PANTR1* were associated with clinical stage, with lowest expression levels detected in stage I which is compatible with the possible oncogenic roles of these lncRNAs. Consistent with this finding, expression of *PANTR1* was associated with mitotic rate. Finally, expression of *AATBC* was lower in overweight patients compared with those with normal weigh. *AATBC* has been shown to regulate expression of Pinin to enhance metastatic ability of nasopharyngeal carcinoma cells (Tang et al., 2020). Most notably, this lncRNA is regarded as an obesity-related human lncRNA that affects adipocytes plasticity through induction of mitochondrial dynamics and respiration (Giroud et al., 2021). Thus, the observed association expression of this lncRNA and weight of patients with breast cancer might be related to this functional role in the regulation of adipocytes plasticity.

In brief, we reported dysregulation of *CDKN2C* and an associated lncRNA in breast cancer tissues. The present study shows possible role of CDKN2C in the pathoetiology of breast cancer. However, functional studies are needed to find the mechanisms of their participation in this process.

Ethics approval and consent to participant

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. Informed consent forms were obtained from all study participants and from legally authorized representative/next of kin of deceased patients. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences. All methods were performed in accordance with the relevant guidelines and regulations.

Consent of publication

Not applicable.

Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Funding

Not applicable.

Authors contribution

SGF wrote the draft and revised it. MT and AAH designed and

Table 6
Association between expression levels of *CDKN2C* and related lncRNAs in breast cancer samples and clinical data.

Parameters	Subclasses	Number of patients (%)	Relative expression level of CDK2NC	*P*-value	Relative expression level of PANDAR	P-value	Relative expression level of AATBC	P-value	Relative expression level of PANTR1	P-value
Clinical stage	I	15 (36.6)	−5.45 ± 0.29	0.63	−6.3 ± 0.28	**0.024**	−5.13 ± 0.86	0.69	−7.9 ± 0.39	**0.01**
	II	9 (22)	−4.55 ± 0.64		−3.88 ± 0.66		−4.15 ± 1.18		−5.23 ± 0.84	
	III	10 (26.8)	−5.05 ± 0.53		−5.01 ± 0.75		−4.31 ± 1.4		−5.87 ± 0.74	
	IV	6 (14.6)	−5.15 ± 0.56		−4.46 ± 0.46		−3.56 ± 1.66		−5.64 ± 0.51	
Histological tumor grade	Low grade	8 (19.04)	−5.12 ± 0.36	0.19	−5.88 ± 0.57	0.33	−3.34 ± 1.23	0.33	−7.22 ± 0.67	0.26
	Moderate	12 (28.5)	−4.72 ± 0.43		−4.82 ± 0.53		−4.28 ± 0.78		−6.3 ± 0.62	
	High grade	14 (33.3)	−5.44 ± 0.43		−4.87 ± 0.48		−5.54 ± 1.03		−5.96 ± 0.54	
Tumor Size (cm)	≤2 cm	25 (57.14)	−5.08 ± 0.24	0.57	−5.19 ± 0.35	0.93	−4.73 ± 0.75	0.46	−6.64 ± 0.4	0.57
	>2 cm	12 (28.57)	−5.15 ± 0.55		−5.07 ± 0.62		−3.9 ± 0.92		−6.02 ± −0.71	
Mitotic Rate	Slowest	10 (23.8)	−4.95 ± 0.37	0.45	−5.07 ± 0.56	0.1	−4.21 ± 1.03	0.48	−6.45 ± 0.71	**0.048**
	Moderate	15 (35.7)	−5.54 ± 0.28		−5.71 ± 0.34		−5.08 ± 0.83		−7.16 ± 0.35	
	quickest	4 (9.5)	−4.27 ± 0.66		−3.97 ± 0.84		−3.35 ± 1.38		−4.77 ± 0.93	
Oral contraceptives	No	20 (47.6)	−4.84 ± 0.3	0.25	−5.03 ± 0.43	0.45	−3.93 ± 0.86	0.42	−6.31 ± 0.51	0.48
	Yes	21 (51.2)	−5.38 ± 0.36		−5.29 ± 0.44		−5.03 ± 0.79		−6.58 ± 0.5	
Obesity	Normal	20 (51.2)	−5 ± 0.34	0.97	−4.97 ± 0.45	0.62	−3.38 ± 0.79	**0.028**	−6.38 ± 0.52	0.9
	Overweight	16 (41.4)	−5.24 ± 0.3		−5.41 ± 0.37		−6.01 ± 0.75		−6.54 ± 0.44	
ER (Estrogen receptor)	Negative	7 (17)	−5.12 ± 0.74	0.64	−4.65 ± 0.82	0.56	−4.47 ± 1.38	0.94	−5.76 ± 0.91	0.6
	Positive	34 (83)	−5.1 ± 0.25		−5.26 ± 0.33		−4.47 ± 0.66		−6.59 ± 0.38	
PR (Progesterone receptor)	Negative	8 (19.5)	−5.47 ± 0.56	0.48	−5.12 ± 0.5	0.69	−3.36 ± 0.97	0.32	−6.43 ± 0.65	0.98
	Positive	32 (80.5)	−5.01 ± 0.26		−5.16 ± 0.36		−4.74 ± 0.69		−6.45 ± 0.41	
HER-2/neu receptor	Negative	15 (36.5)	−5.38 ± 0.41	0.42	−5.2 ± 0.46	0.83	−4.39 ± 0.79	0.89	−6.3 ± 0.52	0.81
	Positive	26 (63.4)	−4.96 ± 0.29		−5.13 ± 0.4		−4.5 ± 0.73		−6.52 ± 0.47	

supervised the study. SE analyzed the data. BMH, AR and MG performed the experiment. All the authors read and approved the submitted version.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

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