Clinical values of two estrogen receptor signaling targeted lncRNAs in invasive ductal breast carcinoma

Klinické hodnoty dvou lncRNA signální dráhy estrogenového receptoru u invazivního duktálního karcinomu prsu

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Summary

Background: Invasive ductal carcinoma (IDC) is the most frequent type of breast cancer (BC) in women, with a high clinical burden due to its high invasive properties. Despite of quickly emerging new data regarding the molecular heterogeneity of invasive cancers, far less is known about the molecular patterns among cases of IDC. An expanding body of evidence has demonstrated that dysregulation of long noncoding RNAs (lncRNAs) is involved in the heterogeneity feature of BC. ***Methods:*** In this study, we analyzed the expression levels of two novel lncRNAs LOC100288637 and RP11-48B3 in 51 IDC tissues in comparison with adjacent non-cancerous tissues. And finally, bioinformatic evaluation has been done. ***Results:*** The results of quantitative polymerase chain reaction showed that LOC100288637 and RP11-48B3 were significantly overexpressed in tumor tissues compared to normal samples ($P = 0.0085$ and $P = 0.0002$, respectively). Also, the two lncRNAs were overexpressed in both MDA-MB-231 and MCF-7 BC cell lines, nevertheless, with a higher expression pattern in MDA-MB-231 than MCF7 cell line. Furthermore, LOC100288637 had an elevated expression level in HER-2 positive tumors compared to HER-2 negative tumors ($P = 0.031$). Interestingly, the lncRNA RP11-48B3.4 was upregulated in IDC subjects with the age at menarche < 14 years compared to patients with the age at menarche ≥ 14 ($P = 0.041$). It was observed in another result that lncRNA RP11-48B3.4 is significantly upregulated in tumors with a lower histological grade compared to tumor samples with higher grades ($P = 0.047$). And finally, using bioinformatic evaluation, we found a predicted interaction between RP11-48B3.4 and mRNA zinc finger and BTB domain containing 10 (ZBTB10). ***Conclusion:*** Altogether, our findings suggest that these lncRNAs with potential oncogenic roles are involved in the pathogenesis of IDC with clinical significance and they may therefore serve as novel markers for the diagnosis and treatment of IDC.

Key words

invasive ductal breast carcinoma – prognosis – long noncoding RNAs – LOC100288637 – RP11-48B3

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Souhrn

Východiska: Invazivní duktální karcinom (invasive ductal carcinoma – IDC) je nejčastějším typem karcinomu prsu (breast cancer – BC) u žen s vysokou klinickou zátěží v důsledku svých vysoce invazivních vlastností. I přes to, že se rychle objevují nová data týkající se molekulární heterogenity invazivních karcinomů, mnohem méně je známo o molekulárních vzorcích IDC. Stále se zvyšující množství důkazů ukázalo, že heterogenní povaha BC souvisí s dysregulací dlouhých nekódujících RNA (long noncoding RNAs – lncRNAs). ***Metody:*** V této studii jsme analyzovali hladinu exprese dvou nových lncRNAs, LOC100288637 a RP11-48B3, v 51 tkáních IDC, které jsme porovnali s přilehlými nekancerózními tkáněmi. Nakonec bylo provedeno bioinformatické hodnocení. ***Výsledky:*** Výsledky kvantitativní polymerázové řetězové reakce ukázaly, že LOC100288637 a RP11-48B3 byly v nádorové tkáni významně overexprimovány, a to v porovnání s normálními vzorky ($p = 0{,}0085$ a $p = 0{,}0002$). Tyto dvě lncRNAs byly také overexprimovány jak v buněčné linii MDA-MB-231 tak v MCF-7 BC, ovšem v buněčné linii MDA-MB-231 byl pozorován vyšší vzorec exprese než u MCF7. Navíc LOC100288637 měl zvýšenou hladinu exprese u HER-2 pozitivních nádorů v porovnání s HER-2 negativními nádory ($p = 0{,}031$). Je zajímavé, že lncRNA RP11-48B3.4 byla upregulována u subjektů s IDC a menarche ve věku < 14 let, a to v porovnání s pacientkami s menarche ve věku ≥ 14 let ($p = 0{,}041$). Při jiném výsledku bylo pozorováno, že lncRNA RP11-48B3.4 je významně upregulována u nádorů nižšího histologického grade v porovnání se vzorky nádorů s vyšším grade ($p = 0{,}047$). A nakonec jsme pomocí bioinformatického hodnocení našli předpokládanou interakci mezi RP11-48B3.4 a mRNA „zinc finger and BTB domain containing 10". ***Závěr:*** Naše zjištění svědčí o tom, že tyto lncRNAs, které potenciálně hrají onkogenní roli, jsou klinicky významně zapojeny do patogeneze IDC a mohou tedy sloužit jako nové markery pro diagnostiku a léčbu IDC.

Klíčová slova

invazivní duktální karcinom prsu – prognóza – dlouhé nekódující RNA – LOC100288637 – RP11-48B3

Introduction

Breast cancer (BC) is the second cause of death among women worldwide [1]. BC is a highly heterogeneous disease which is characterized by different phenotypic features, diverse response to existing therapy and unpredictable clinical outcome [2]. Invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) are the most common variants of the BC [3]. IDC, also known as infiltrating carcinoma, is accounted for about 80% of all invasive BC [4]. The relevant molecular mechanism of IDC is not elucidated and thus the optimal management of IDC has become increasingly complex. Further investigation is urgent to identify effective approaches for the diagnosis and treatment of IDC [5].

Long noncoding RNA (lncRNA) are a class of RNA molecules longer than 200 nucleotides [6]. They regulate gene expression at the transcriptional, post-transcriptional, and epigenetic levels and also play an important role in pathological processes such as neurological disorders, diabetes, tumors [7,8] and more. Recent studies have clarified vital roles of several lncRNAs in BC pathobiology. Accordingly, lncRNAs could be a potential biomarker for BC prognosis, diagnosis, and therapeutic management [9]. Although cell- specific expression of lncRNAs and their role in regulating the expression of oncogenic and tumor suppressor genes have been shown in recent investigations, their underlying molecular mechanisms in IDC remains undetermined [10–12]. In recent years, a number of lncRNAs including LOC100288637 and RP11-48B3 were found to be associated with abnormal estrogen receptor (ER) signaling pathway in BC, suggesting that lncRNAs can be applied as prognosis biomarker in patients with breast cancer [13,14]. AS mentioned previously, the information on their roles in IDC remains unclear. Therefore, in the presented study, we assessed the expression levels of LOC100288637 and RP11-48B3 in patients with IDC as well as BC cell lines and analyzed their correlation with clinicopathological parameters.

Materials and methods

Study population

A total of 51 invasive ductal carcinoma breast cancer patients who had received no chemotherapy or radiotherapy were included in this study. All subjects were selected from individuals referred to Faghihie Hospital affiliated to Shiraz University of Medical Sciences. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. The demographic, reproductive and clinical information as well as the pathological data of tumor samples were obtained from each participant in the current study. Tab. 1 shows the characteristics of the study participants. The participants were 27–68 years old, 15 (30%) participants were aged < 40 years, and 36 (70%) were ≥ 40 years old. Among the 51 participants, 36 (70%) were parous and 15 (30%) were nulliparous. Regarding the menopausal status, 4 (8%) participants were premenopausal and 47 (92%) were in postmenopausal status. Moreover, the participants were divided into 2 subgroups based on the age at menarche of < 14 or ≥ 14 years. The age at the first full term pregnancy and breast-feeding duration were also recorded. Parous participants were divided into 2 subgroups according to the age at the first full-term pregnancy of < 25 or ≥ 25 years.

Breast tissue sampling

Fresh breast cancer tissues and their adjacent noncancerous tissues were snap-freeze in liquid nitrogen after resection and stored at –80 °C until RNA extraction.

Estrogen receptor, progesterone receptor and HER2 determination

The estrogen receptor (ER), progesterone receptor (PR), and HER2 status were determined according to the patient's histopathological data, following immunohistochemistry (IHC) staining. If ≥ 1% of tumor cells show positive ER/PR staining, the ER/PR interpretation is positive. The IHC HER2 score 3+ was considered positive as well.

Cell culture

For the purpose of this study, we chose MCF7 and MDA-MB-231 (triple negative) human breast cell lines. All cells were obtained from the cell bank of Pasteur Institute of Iran, and cultured in RPMI 1640 medium (Sigma 42 Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C in 5% CO_2 at 95% humidity.

RNA isolation and real time polymerase chain reaction

Total RNA was extracted from all tissue samples and cell lines using the TRizol as was recommended by the manufacturer (Life Technologies, Carlsbad, CA) and the final concentration was quantified using a NanoDrop at 260 and 280 nm. In our experiment, 500 ng RNA was used for each sample with a purity range 1.8–2 for both 260/280 and 260/230 nm.

The RNA integrity was confirmed by gel electrophoresis and to remove the probable DNA contamination, the total RNA was treated with DNase (Takara Bio Inc, Otsu, Japan) according to the manufacturer's instruction. The cDNA synthesis was carried out using approximately 500 ng of total RNA with the Prime Script-RT kit (Takara, Japan) according to the manufacturer's protocol. In the next step, real time polymerase chain reaction (PCR) was carried out in a QuantStudio™ 3 system (Applied Biosystems, USA by Thermo Fisher Scientific) with SYBR Premix Ex Taq II kit (Takara, Japan) according to the manufacturer's protocol. Real-time specific primer pairs used were as follows: RP11-48B3, forward 5′-CAAGCCCTGATCAACTAGGAATA-3′, reverse 5′-GGAAAGTTGGTTGCTGTGTAAG-3′; LOC100288637, forward 5′-CTAAGCCCTGCTTCTGGTATG-3′, reverse 5′-GGAGGCAGATCCAGTTCATTAG-3′; B2M, forward 5′-AGATGAGTATGCCTGCCGTG-3′, reverse 5′-GCGGCATCTTCAAACCTCCA-3′. For each reaction, 10 µl SYBR® Green Master Mix with 0.8 µL (40 nM) Primer 1 and 0.8 µL (40 nM) Primer 2 with 2 µL was used and the final volume was adjusted to a total of 18 µL using distilled H_2O. All the reactions were carried out in triplicates. The real time PCR was performed in the following conditions: at 95 °C for 30 s followed by 40 repetitive cycles at 95 °C for 30 s and then at 60 °C for 30 s. No template controls were included in each run. Relative mRNA expression levels of LOC100288637 and RP11-48B3 were normalized to β2-microglobulin expression level as a housekeeping gene. The expression level (i.e. fold change) for each gene was calculated using the $2^{-\Delta\Delta CT}$ method.

Tab. 1. Demographic and reproductive characteristics of the participant subjects.

Variables	Subgroup	Number	Valid percent
age (year)	< 40	15	29.4
	≥ 40	36	70.6
family history for cancer	positive	26	51
	negative	25	49
marital status	married	43	84.3
	single	8	15.7
parity status	parous	36	70.6
	nulliparous	15	29.4
age at first full term pregnancy (years)	< 25	38	74.5
	≥ 25	10	19.6
abortion history	positive	12	23.5
	negative	37	72.5
breastfeeding experience	positive	37	72.5
	negative	14	27.5
breastfeeding (months)	0–6	20	39.2
	6–24	20	39.2
	≥ 24	11	21.6
age at menarche (year)	< 14	36	70.6
	≥ 14	15	29.4
menstrual cycles	regular	43	84.3
	irregular	8	15.7
menopausal status	pre	4	7.8
	post	47	92.2
oral contraceptive pills consumption	positive	10	19.6
	negative	41	80.4

Bioinformatic analysis

In the current study, we also conducted different bioinformatics analyses, mainly by using the data of the Cancer Genome Atlas (TCGA) [15], to get more information about RP11-48B3.4 and LOC100288637. In this regard, we investigated the expression correlation between these two lncRNAs and mRNAs in the TCGA-BRCA dataset through using TANRIC web server [16]. Subsequently, we used the possible correlated mRNAs for any possible interactions between these mRNAs and lncRNA using the lncRRI search web [17].

Statistical analysis

The data are expressed as the mean ± ± SD and analyzed with SPSS version 20.0 software. $P < 0.05$ are considered statistically significant. The com-

Tab. 2. Pathological data of the evaluated tumor samples from breast cancer patients.

Clinical characteristics	Subgroup	Number	Valid percent
tumor size (cm)	< 2 cm	17	33.3
	2–5 cm	33	64.7
	> 5 cm	1	2
estrogen receptor	positive	45	88.2
	negative	6	11.8
progesterone receptor	positive	34	66.7
	negative	17	33.3
HER-2/neu status	positive	21	41.2
	negative	30	58.8
histological grade	1	11	21.6
	2	25	49
	3	15	29.4
lymph node metastasis	involved	30	58.8
	free	21	41.2

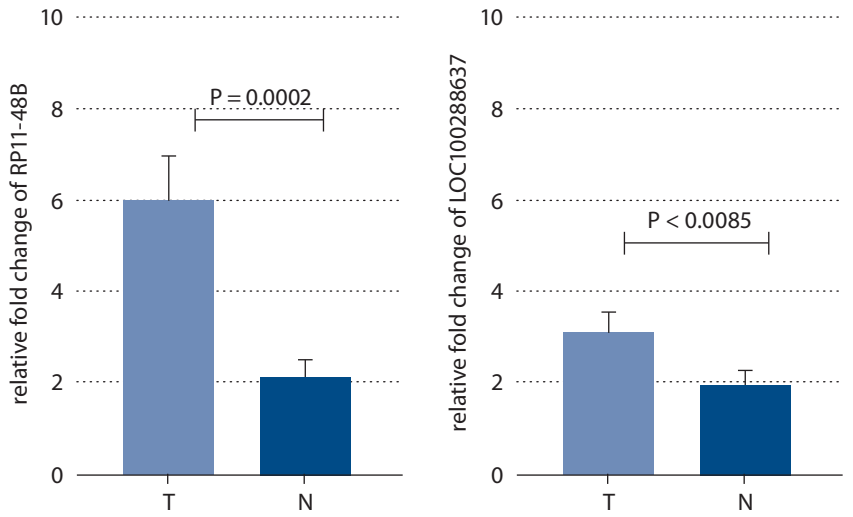

Fig. 1. Expression levels of lncRNAs LOC100288637 and RP11-48B3 in invasive ductal carcinomas and adjacent normal tissues. T and N denote tumor tissue and normal breast samples, respectively.

parisons between groups were made using unpaired Student's t-test or Mann-Whitney test. One-way ANOVA followed by Bonferroni or Kruskal-Wallis tests were used for multiple comparisons. The correlations were analyzed using Spearman's rank correlation coefficient.

Results

Demographics, reproductive and clinical data of the patients

The average patient age was 45.7 with the range 27–68 years. Fifty-one percent of the subjects had a family history of BC and other cancers while 49 % were negative for this variable. Among the 51 enrolled women, 36 participants were parous and 15 cases were nulliparous. The 37 women had experienced breastfeeding in their life and the rest were negative for the breastfeeding experience. Besides, 47 patients were in postmenopausal status and 4 patients were in premenopausal condition. Other demographics and reproductive characteristics of the patients are shown in Tab. 1. Clinically, all 51 tumor samples from BC patients had invasive ductal breast carcinoma. Most tumors were positive for ER (+45 vs. –6) and PR (+34 vs. –17) receptors, but negative for HER-2/neu marker (–30 vs. +21). A total of 33.3% of the tumor samples had a size < 2 cm, 64.7% had 2–5 cm, and 2% had > 5 cm. The tumor size was measured as the largest dimension of the microscopic invasive component in pathologic sections. The data on other tumor features including histological grade and lymph node metastasis (TNM) are indicated in Tab. 2. The tumor grade and TNM stage of the cells were determined by the WHO standard [18].

Expression levels of LOC100288637 and RP11-48B3 in BC tissues and different subgroups of the samples regarding the clinicopathological, demographic, and reproductive characteristics

In the next step, we investigated the expression profile of these two lncRNAs in 51 BC tissues and their adjacent normal tissues. The quantitative PCR (qPCR) results showed that LOC100288637 (P = 0.0085) was significantly upregulated in tumor tissues compared to normal samples. Furthermore, RP11-48B3 had a similar significant overexpression pattern in tumor tissues versus normal tissues (P = 0.0002). Fig. 1 represents the results on relative expression levels of both LOC100288637 and RP11-48B3 in tumor tissues compared to normal samples. In the next step, we compared the expression levels of the two lncRNAs in different subgroups of the patients with the various demographic, reproductive, and clinicopathological features of the subjects (Tab. 3 and 4). The findings showed that the lncRNA RP11-48B3.4 is upregulated in pa-

Tab. 3. Quantitative expression data of target lncRNAs in tumor tissues in relation to demographic and reproductive variables.

		LncRNA RP11-48B3.4			LncRNA LOC100288637		
Clinicopathological characteristics	**Subgroups**	**Mean**	**Std. error**	**P-value**	**Mean**	**Std. error**	**P-value**
age (years)	< 40	6.255	8.430	0.709	2.428	3.129	0.535
	≥ 40	5.850	6.581		3.302	3.908	
family history for cancer	positive	4.946	7.014	0.169	2.804	4.168	0.169
	negative	7.033	7.151		3.296	3.177	
marital status	married	6.336	7.458	0.468	3.196	3.895	0.698
	single	3.9973	4.464		2.235	2.272	
age at first marriage (years)	< 18	4.041	6.103	0.133	3.074	2.958	0.641
	18–24	8.458	8.931		2.543	3.431	
	≥ 24	5.336	4.905		4.931	5.703	
parity	parous	6.915	7.731	0.137	3.1701	3.736	0.694
	nulliparous	3.698	4.713		2.746	3.676	
age at first full term pregnancy (years)	< 25	5.944	7.1748	0.577	2.884	3.106	0.761
	≥ 25	7.201	7.729		4.436	5.599	
abortion history	positive	6.155	5.836	0.709	5.019	5.522	0.161
	negative	3.076	7.653		2.543	2.764	
breastfeeding experience	positive	6.747	7.692	0.213	3.097	3.710	0.883
	negative	3.913	4.814		2.908	3.759	
breastfeeding (months)	0–6	4.562	5.249	0.520	2.551	3.392	0.479
	6–24	6.511	7.155		3.597	4.184	
	≥ 24	7.541	9.714		2.939	3.424	
age at menarche (years)	< 14	9.014	9.597	0.041	3.254	3.490	0.079
	≥ 14	5.971	10.597		2.543	4.209	
menstrual cycles	regular	5.197	6.295	0.223	3.027	3.899	0.437
	irregular	10.116	9.894		3.142	2.418	
menopausal status	pre	4.682	5.997	0.551	4.466	7.786	0.483
	post	6.078	7.219		2.924	3.260	
oral contraceptive pills consumption	positive	6.220	4.416	0.209	4.591	5.728	0.962
	negative	5.908	7.643		2.668	2.977	

tients with age at menarche < 14 years compared to patients with age at menarche ≥ 14 (P = 0.041). The expression of this lncRNA was not significantly different among the different subgroups of the demographic and reproductive variables of the participants. Also, it was not observed any significant data on the difference in expression of the lncRNA LOC100288637 among various levels of the demographic and reproductive characteristics. From the clinicopathological point of view, our analyses indicated that lncRNA RP11-48B3.4 was significantly upregulated in tumors with a lower histological grade (grade 1) compared to tumor samples with higher grades, including grades 2 and 3 (P = 0.047). Regarding LOC100288637, its expression showed a higher level in HER-2 positive tumors than HER-2 negative tumors (P = 0.031). For other different subgroups of the clinical characteristics, any noteworthy results were not found among them in terms of differences in expression of the two studied lncRNAs (Tab. 4). In another of our statistic evaluations, we determined the correlation of LOC100288637 and RP11-48B3 with clinicopathological features of the BC patients. The Spearman's correlation analysis disclosed that the expression level of RP11-48B3 was negatively correlated with histological grade (r = −282, P = 0.045). The Spearman's analysis did not find any other significant correlation between the expression level of these lncRNAs and the other studied variables (Tab. 5).

Tab. 4. Expression levels of the studied lncRNAs in the tumor samples of the patients regarding to the clinicopathological characteristics.

		LncRNA RP11-48B3.4			LncRNA LOC100288637		
Clinicopathological characteristics	**Subgroups**	**Mean**	**Std. error**	**P-value**	**Mean**	**Std. error**	**P-value**
tumor size (cm)	< 2 cm	6.966	8.262	0.169	2.654	3.583	0.639
	2–5 cm	5.612	6.549		3.207	3.828	
	> 5 cm	0.788	0.0		4.349	0.0	
estrogen receptor	positive	5.873	7.411	0.200	3.034	3.625	0.787
	negative	6.686	4.301		3.131	4.500	
progesterone receptor	positive	6.622	7.898	0.549	2.925	3.295	0.905
	negative	4.664	5.0666		3.286	4.471	
HER-2	positive	5.550	7.5420	0.579	3.411	3.897	0.031
	negative	6.262	6.870		1.546	2.212	
histological grade	1	9.647	6.698	0.047	4.042	3.633	0.077
	2	5.497	7.985		2.477	3.937	
	3	4.058	4.816		3.260	3.336	
lymph node metastasis	involved	5.323	6.428	0.438	2.388	2.980	0.079
	free	7.116	8.023		4.049	4.434	

Tab. 5. Correlation of lncRNAs LOC100288637 and RP11-48B3.4 expressions with pathological variables.

	LncRNA RP11-48B3.4		LncRNA LOC100288637	
Variables	**r**	**P-value**	**r**	**P-value**
age (y)	–0.161	0.260	–0.034	0.815
menopausal status	0.084	0.557	0.099	0.489
family history of breast cancer	–0.195	0.171	–0.195	0.171
tumor size (cm)	–0.115	0.422	0.123	0.391
histological grade	–0.282	0.045*	–0.056	0.696
lymph node metastasis	0.127	0.374	0.252	0.184
estrogen receptor	–0.190	0.181	–0.029	0.840
progesterone receptor	0.085	0.554	–0.017	0.906
HER2	–0.078	0.584	–0.043	0.763

* The correlation is significant at the level of 0.05 (two-tailed).

Expression levels of LOC100288637 and RP11-48B3 in MDA-MB-231 and MCF-7 cell lines

For getting more information about involving lncRNAs LOC100288637 and RP11-48B3 in the pathogenesis of IDC, especially metastasis, their expression levels were compared in human BC cell lines MDA-MB-231 and MCF-7 BC. The quantitative PCR (qPCR) data showed that the two lncRNAs were overexpressed in both MDA-MB-231 and MCF-7 BC cell lines; nevertheless, both lncRNAs showed a higher expression pattern in MDA-MB-231 than MCF7 cell line (P = 0.0013 and P = 0.0003, respectively) (Fig. 2).

Bioinformatic evaluations

Through expression correlation analysis, we found some correlations between RP11-48B3.4 and mRNA expression in TCGA-BRCA genes, with negative correlation for mRNA MR1 (r = –0.409, P = 0) and positive correlations for mRNAs *MRPS28* (r = 0.426, P = 0), *NSMCE2* (r = 0.444, P = 0), *POLR2K* (r = 0.436, P = 0), *TCEB1* (r = 0.476, P < 10–47), *UQCRB* (r = 0.418, P = 0), *YWHAZ* (r = 0.464, P = 0), *ZBTB10* (r = 0.567, P = 0), and *ZNF706* (r = 0.471, P = 0) (Fig. 3). Regarding the interaction between the correlated mRNAs and RP11-48B3.4, we found that only zinc finger and BTB domain containing 10 (ZBTB10) were predicted to have interaction with this lncRNA (Fig. 4). It was not any observable interaction between lncRNA LOC100288637 and evaluated mRNAs.

Discussion

Invasive ductal carcinoma (IDC), as the most frequent form of BC in women, has a high clinical burden due to its high invasive properties. Although recent in-

vestigations have made significant advances in revealing some key molecular mechanisms regarding its pathogenesis, it shows a very heterogeneous and complex etiology with many unknown aspects [19,20]. Thus, it is extremely expedient to clarify the underlying molecular mechanisms through which IDC develops. lncRNA transcripts are emerging as key players in cancer initiation and pathobiology of BC, with both oncogenic and tumor-suppressive roles. In this regard, new experimental studies have revealed some novel molecular mechanisms by which lncRNAs involved in BC malignancy, providing a new avenue of investigation for characterizing the different hallmarks of BC [21].

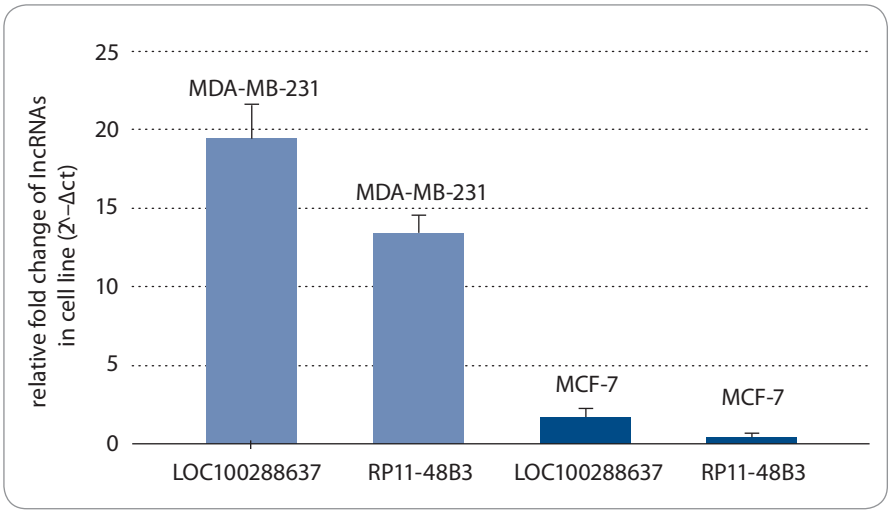

Fig. 2. Relative expression of target lncRNAs in MDA-MB-231 and MCF-7 cell lines compared to normal cell lines.

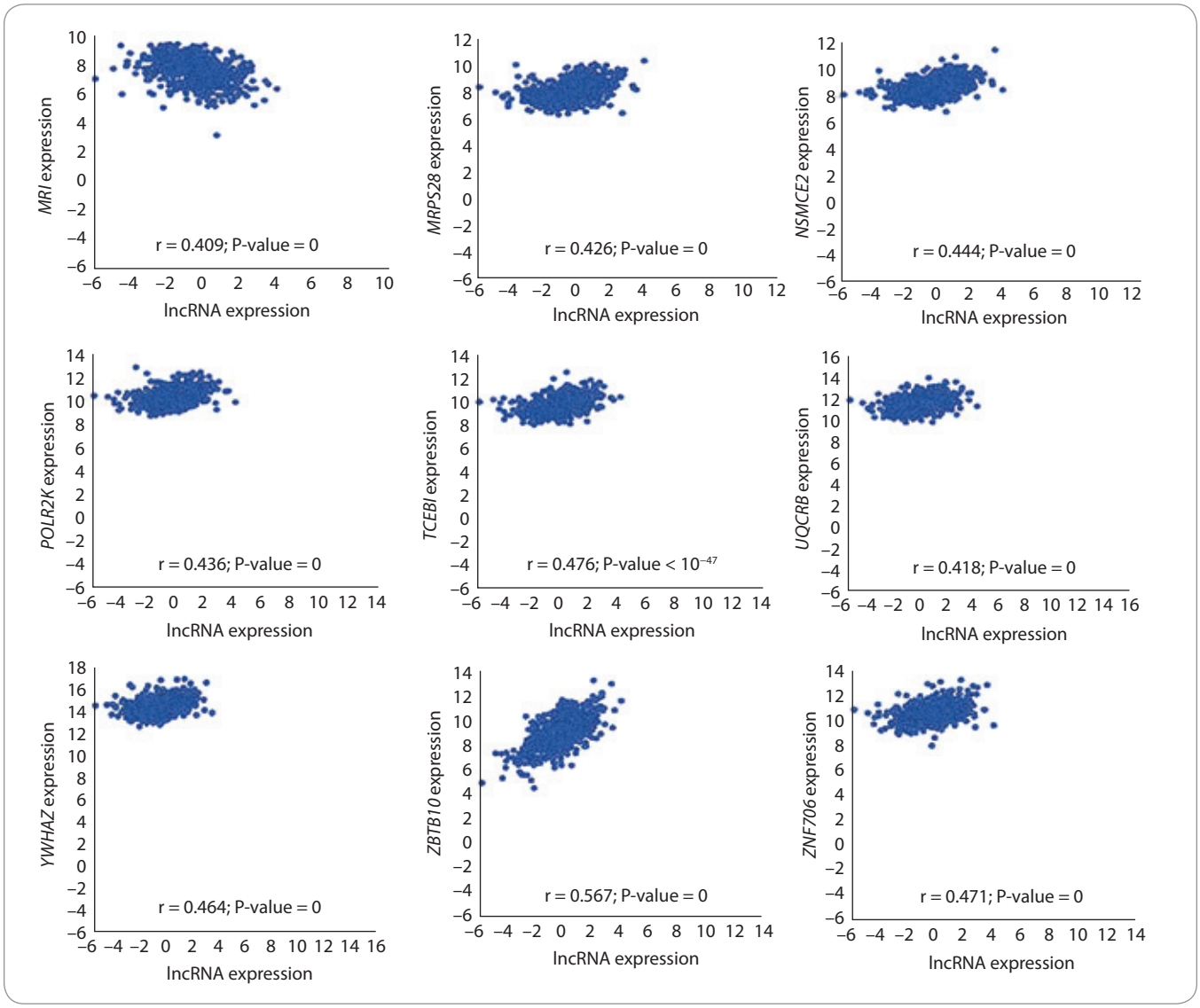

Fig. 3. Correlation analysis between RP11-48B3.4 and mRNAs expression in TCGA-BRCA.

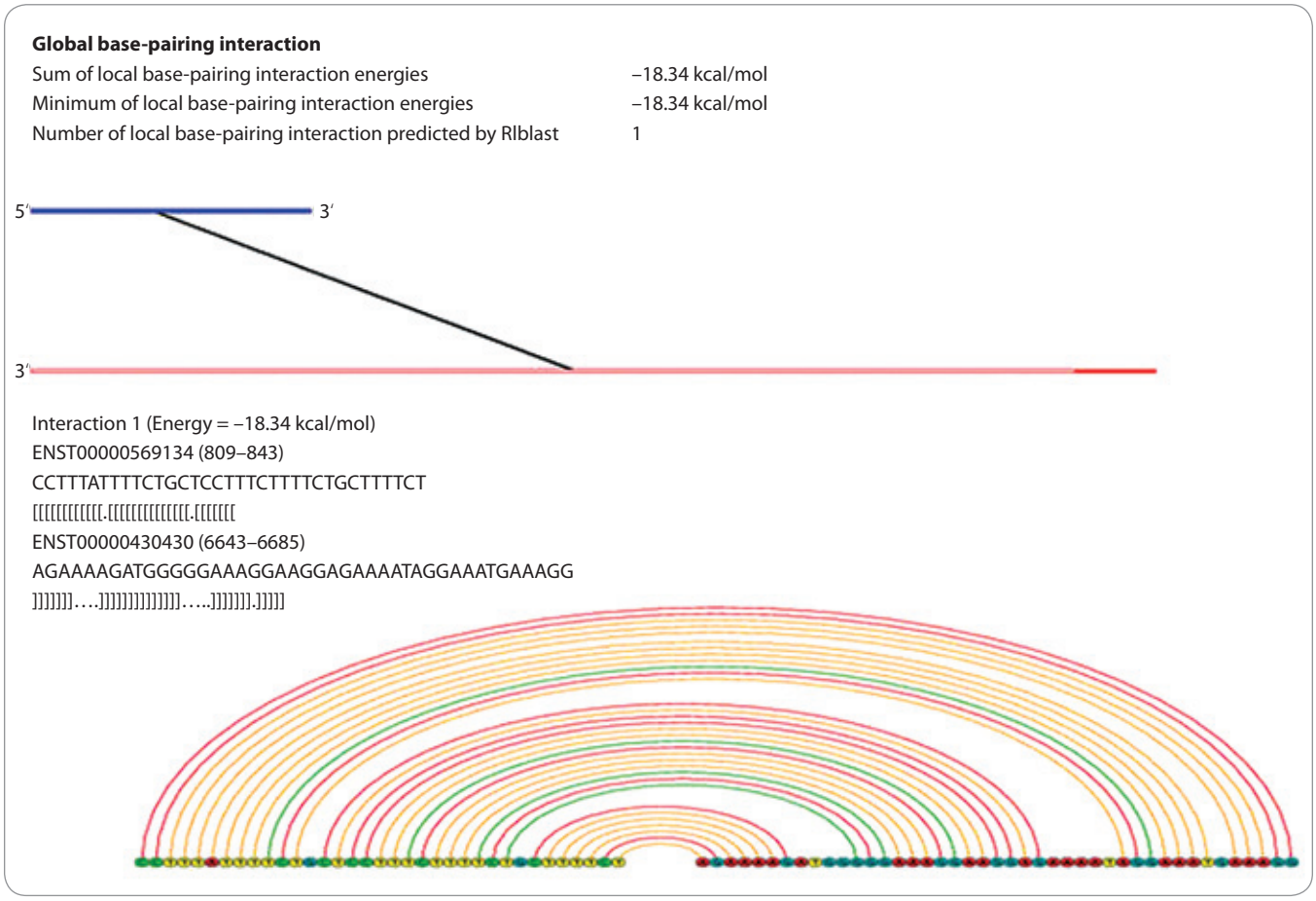

Fig. 4. Prediction of possible interaction between RP11-48B3.3 and ZBTB10.

Moreover, these small noncoding RNAs not only play an important role in BC development, but also have some links with BC risk factors in the breast tissue of healthy women [22,23]. And interestingly, lncRNAs have been shown to exhibit diagnostic and prognostic biomarker properties as well as therapeutic targets for BC [24].

In the current study, we determined the expression levels of two novel ER signaling pathway – targeted lncRNAs LOC100288637 and RP11-48B3 in clinical samples of BC tumors type IDC. To the best of our knowledge, this is the first study to explore the links of expression signatures of these two lncRNAs to IDC type of BC as well as its clinicopathological characteristics. Our results showed a significant overexpression of these two ER-related lncRNAs in IDC tumor tissues compared to normal breast samples. Emerging studies have revealed that dysregulation of ER expression and its signaling pathway is intensely linked to the development and pathophysiology of the BC. Furthermore, provided evidence from different investigations is available for involving some lncRNAs in regulating the ER signaling and their aberrant expressions play key roles in the development of BC malignancy, especially the progression and endocrine-resistance of ER-positive subtype. The findings of the present study are in agreement with the results from a previous array-based study that revealed dysregulation of LOC100288637 and RP11-48B3.4 through regulating signaling pathways in ER+ BC patients. They indicated that the expression pattern of these two lncRNAs were significantly correlated with endocrine resistance-free survival and distant metastasis-free survival as well as disease-free survival of ER+ BC patients. Of note, the most of samples included in the current study were ER+ that is consistent with this observation that the majority of the BC tumors are molecularly fallen into ER+ subtype. Therefore, these observations highlight the clinical significance of these lncRNAs in the ER+ BC subtype via regulating the ER signaling pathway.

Furthermore, our study demonstrated a significant overexpression of the lncRNAs LOC100288637 and RP11-48B3.4 in human BC cell lines, including MDA-MB-231 (TNBC for ER and PR expression, as well as HER2 amplification) and MCF-7 (positive for ER, PR expression). However, the results represented a higher expression of both lncRNAs in MDA-MB-231 cells than MCF7 cells. Thus, it could be thought that they might have oncogenic roles in BC tumorigenesis through dysregulating the ER signaling pathway and also give invasiveness and metastatic properties to BC tumor cells.

Notably, our analyses further showed a significantly increased level of LOC100

288637 in HER-2 positive tumors compared to HER-2 negative samples. This finding is consistent with the results of previous work by Fan and colleagues that indicated the elevated expression level of LOC100288637 was strongly correlated with Her2/neu positive status in BC through next-generation sequencing and bioinformatics. Accordingly, we supposed that this lncRNA may play crucial roles in the pathogenesis of IDC via oncogenic functions. However, its exact mechanism needs more investigation by future studies. Furthermore, we observed a higher expression of the RP11-48B3 in lower grade tumors in comparison with the higher-grade tumors which also correlatively indicated by correlation analysis. Although this may be inconsistent with the abovementioned observation that this lncRNA had a higher expression in MDA-MB-231 cell line with high aggressive, invasive, and poorly differentiated properties, such a finding may occur due to our relatively small sample size or unknown complex nature of RP11-48B3 function during BC tumorigenesis which requires more investigation to disclose.

Interestingly, another result of the current study was that the lncRNA RP11-48B3.4 had an elevated expression pattern in BC patients with the age at menarche < 14 years in comparison to patients with the age at menarche ≥ 14 years. It has been reported that lower age at menarche increases the risk of BC through estrogen-related mechanisms [25,26]; however, little is known about its molecular mechanisms behind the risk of BC. Therefore, it can be suggested that a lower age at menarche may increase the risk of IDC partly through affecting the expression level of estrogen-linked lncRNA RP11-48B3.4. Although, confirmation of such assumptions requires conducting more functional studies.

Lastly, the current study also bioinformatically demonstrated some correlations between lncRNA RP11-48B3.4 and expression of several mRNAs in TCGA-BRCA, including negative expression correlation for mRNA MR1 and positive correlations for mRNAs *MRPS28, NSMCE2, POLR2K, TCEB1, UQCRB, YWHAZ, ZBTB10,* and *ZNF706*. However, only the mRNA ZBTB10 were predicted to have interaction with the lncRNA RP11-48B3.4. Regarding LOC100288637, the results were not detected any evident interaction with given mRNAs. This highlights that the novel lncRNA RP11-48B3.4 may be involved in the pathogenesis of the IDC via interacting with some encoding genes by different mechanisms. There is evidence that lncRNAs play key roles in the development of BC through influencing the expression of other coding and non-coding genes [27]. In this way, one suggested mechanism is competing endogenous RNA (ceRNA) function through which lncRNAs regulate expression of mRNAs via sponging miRNAs in regulatory molecular networks whose roles in cancer development, especially in BC, are emerging with clinical significance [28,29].

Conclusion

The presented study showed the elevated expression levels of LOC100288637 and RP11-48B3 lncRNA in IDC breast tumors as well as BC cell lines have some important significance on their clinical outcome. This suggested them as putative oncogenic markers at the molecular level of IDC. However, it is important to analyze the correlation between their expression as well as progression-free survival time to conclude these lncRNAs as prognostic biomarkers. In our study, it was not possible to do such evaluation, since we used samples from newly diagnosed cancers. These findings can also be useful to candidate these two lncRNAs as targets for BC treatment. Future studies are warranted to analyze the expression of these two lncRNAs in various type of cancers to propose them as candidates of tumor biomarkers in combination with biomarker panels.

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Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Author contributions

A.D, Y.M, and S.I contributed to the conception and design of the research. F.F, S.I, M.D.F, and M.R.M performed the experiments. F.F., S.I, H.D., M.D. F, and Y. M interpreted the results of the experiments, analyzed data, and prepared the figures. S.I, M.R.M, and A.D drafted the manuscript. A.D, H.D, S.M.B.T, and S.I. edited and revised the manuscript. M.B.T and Y.M, made study supervision and technical or material support. All authors read and approved the final manuscript.

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