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Involvement of *FAM170B-AS1*, *hsa-miR-1202*, and *hsa-miR-146a-5p* in breast cancer

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Abstract.
BACKGROUND: *FAM170B-AS1* is usually expressed low in all organs except for testicular tissues. No study was performed to explore its role in breast cancer (BC). Contradictory results were reported about *hsa-miR-1202* and *hsa-miR-146a-5p* in BC.
OBJECTIVE: The present study aimed to explore the involvement of *FAM170B-AS1* in BC using bioinformatics predictive tools, followed by a practical validation besides exploring the impact of *hsa-miR-1202* and *hsa-miR-146a-5p* in BC.
METHODS: This study enrolled 96 female patients with BC, 30 patients with benign breast diseases (BBD), and 25 control subjects. The expressions of circulating *FAM170B-AS1, hsa-miR-1202, and hsa-miR-146a-5p* were quantified using qRT-PCR. These ncRNAs' associations, predictive, and diagnostic roles in BC were statistically tested. The underlying miRNA/mRNA targets of *FAM170B-AS1* in BC were bioinformatically predicted followed by confirmation based on the GEPIA and TCGA databases.
RESULTS: The expression of *FAM170B-AS1* was upregulated in sera of BC patients and *hsa-miR-1202* was upregulated in sera of BBD and BC patients while that of *hsa-miR-146a-5p* was downregulated in BC. These *FAM170B-AS1* was significantly associated with BC when compared to BBD. *FAM170B-AS1* and *hsa-miR-1202* were statistically associated with the BC's stage, grade, and LN metastasis. *FAM170B-AS1* and *hsa-miR-146a-5p* gave the highest specificity and sensitivity for BC. KRAS and EGFR were predicted to be targeted by *FAM170B-AS1* through interaction with *hsa-miR-143-3p* and *hsa-miR-7-5p*, respectively. Based on the TCGA database, cancer patients having mutations in *FAM170B* show good overall survival.
CONCLUSIONS: The present study reported that for the first time, *FAM170B-AS1* may be a potential risk factor, predictive, and diagnostic marker for BC. In addition, *FAM170B-AS1* might be involved in BC by interacting with *hsa-miR-143-3p/KRAS* and *hsa-miR-7-5p/EGFR* through enhancement or repression that may present a new therapeutic option for BC.

Keywords: Bioinformatics, diagnostic, EGFR, KRAS, risk factor

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Abbreviations

AUC	Area under the curve
BBD	Benign breast diseases
BC	Breast cancer
BP	Biological process
CA15.3	Carbohydrate antigen 15.3
CC	Cellular component

CEA	Carcinoembryonic antigen
CDC14A	Cell division cycle 14 homolog
CI	A Confidence interval
DCI	Ductal carcinoma in situ
EMT	Epithelial to mesenchymal transition
ER	Estrogen receptor
FAM170B-AS1	Family with sequence similarity 170 member B-antisense RNA1
GEPIA	Gene expression profiling integrative analysis
GO	Gene Ontology
GTEx	Genotype-Tissue Expression
HCC	Hepatocellular carcinoma
HER-2	Human epidermal growth factor receptor-2
HPA	Human protein atlas
IDC	Invasive ductal carcinoma
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDAP	LncRNA-disease association prediction
LN	Lymph node
LncRNA	Long non-coding RNA
MF	molecular function
miRNA	Micro RNA
mRNA	Messenger RNA
ncRNAs	Non-coding RNAs
NSCLC	Non-small cell lung cancer
OR	Odds ratio
PPI	Protein-protein interaction
PR	Progesterone receptor
ROC	Receiver operating characteristic curve
TCGA	The cancer genome atlas
TNBC	Triple negative breast cancer
TNM	Tumor-node-metastasis
VIF	Variance inflation factor
β	Standardized coefficient

1. Introduction

Being the most common cancer globally among women makes breast cancer (BC) the first cause of cancer-related deaths [1]. About 2.2 million new cases are diagnosed with BC. Therefore, BC accounts for 11.7% of all cancer incidence [2]. In 2019, about 59% of women diagnosed with BC were $>$ 60 years old. By 2030, this percentage is expected to be elevated to 70% BC incidence in patients aged 70 years and older [3]. In women, most clinical breast changes are benign diseases (BBD). Only 3–6% of cases with these benign diseases are transformed into BC. These changes are more common in women of childbearing age; their maximum peak was observed at 30 and 50 years old [4,5]. About 50 and 25% of all women aged over 30 years old have mastalgia, fibrocystic changes, and fibroadenomas, respectively. The risk of dedifferentiation among most benign breast changes is low. However, complex cysts carry a 23–31% risk of transforming into malignancy, 16% for papillary lesions, and 7% for radial scars [6].

The presence of a variety of genetic aberrations against BC makes it a very heterogeneous disease [7]. Hence, BC has five main molecular subtypes identified depending on the level of expression of receptors for estrogen (ER), progesterone (PR), and human epidermal growth factor receptor-2 (HER-2) on the surface of BC cells [7,8]. Some of these subtypes are marked by significantly aggressive clinical course, proliferative activity and growth rate, poor prognosis, early metastasis, different targeted therapies, and risk of disease recurrence [9,10,11,12,13]. Hence, early screening and diagnosing the more aggressive subtypes of BC and identifying suspicious breast tumors are vital to reduce morbidity and mortality [14,15].

Long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) are reported to be associated with different types of cancer [16,17]. LncRNAs can suppress mRNA expression by recruiting histone modification complexes, controlling the alternative splicing, and nuclear import [18]. LncRNAs can interact with miRNAs [19,20,21] to de-repress gene expression by competing with miRNAs for interaction with shared target mRNAs. miRNA binds to its complementary sequences in the 3'-UTR of their target mRNAs and post-transcriptionally represses their translation into protein [22]. Therefore, predicting and validating the lncRNA/miRNA crosstalk can modulate gene expression patterns that drive physiological and pathological processes [23,24,25].

Little is known about the Family with sequence similarity 170 member B-antisense RNA1 (*FAM170B-AS1*; ENSG00000234736). It is located on chromosome 10: 49,121,839-49,151,547 forward strand with four transcripts (https://www.ensembl.org/index.html). Genotype-Tissue Expression portal (https://gtexportal.org/home/) shows the low expression of *FAM170B-AS1* in all body tissues except for the testis. It also reveals that *FAM170B-AS1* is expressed in the breast epithelial (luminal) cells. In 2018, 54 single nucleotide polymorphisms (SNPs) in 21 genes were recognized as

independent and significant determinants of coronary artery disease. *FAM170B-AS1*, one of these 21 genes, was identified for the first time as a possible genetic risk factor for coronary artery disease in Japanese patients [26]. Ke et al. [27] and Zhou et al. [28] reported that *FAM170B-AS1* is one of the differentially expressed genes in the skeletal muscle of diabetic patients and the monocyte-derived dendritic cells of patients with allergic rhinitis, respectively. Recently, bioinformatics analysis was performed by Hu et al. [29] to explore the hub mechanisms of ischemic stroke and illustrated the involvement of *FAM170B-AS1* as one of 406 genes in ischemic stroke. Falcon et al. [30] and Zhang et al. [31] pointed out the participation of *FAM170B-AS1* in hepatocellular carcinoma (HCC) and thyroid carcinoma, respectively, based on the data extracted from the cancer genome atlas (TCGA) database. It was reported that *FAM170B-AS1* may cause homeostasis imbalance in HCC by allowing cancer cells to be affected by oxidative stress or even dysregulating cancer cell division rate [30,32]. Recently, a significant alteration in the frequency of *FAM170B-AS1* was reported and found to be correlated with allograft inflammatory factor 1 in pan-cancer based on bioinformatics analysis [33]. These bioinformatics studies opened a new window to clinical study of the possible roles of *FAM170B-AS1* in several types of cancer.

hsa-miRNA-1202 and *hsa-miRNA-146a* were reported to be associated with cancer progression, positively or negatively. *hsa-miRNA-1202* is transcriped by *MIR1202* gene on chromosome 6: 155,946,797-155,946,879 forward strand. It is reported to be upregulated in breast cancer [34] endometrial cancer [35], and lung cancer [36] while it is revealed to be downregulated in glioma cells [37] and HCC [38]. *hsa-miR-1202* was reported to inhibit the proliferation of glioma cells and induce endoplasmic reticulum stress and apoptosis *via* regulating Rab1A in glioma [37]. Another study reported the inhibitory role of *hsa-miR-1202* on HCC cell migration and invasion by targeting cyclin-dependent kinase 14 [38].

hsa-miR-146a is transcribed by the *MIR146A* gene on chromosome 5: 160, 485, 352-160, 485, 450 forward strand. Wu et al. [39] indicated that *hsa-miR-146a-5p* is downregulated in non-small cell lung cancer (NSCLC) tissues, whereas Wang et al. [40] reported an up-regulation of *hsa-miR-146a-5p* in NSCLC patients. Liu et al. [41] revealed the oncogenic mechanism of *hsa-miR-146a-5p* in NSCLC cell survival and migration by suppressing its target gene, tumor necrosis factor receptor-associated factor 6. In BC, several studies indicated that *hsa-miR-146a* can function as oncomiR [42, 43,44,45]. Other studies have demonstrated *hsa-miR-146a* as a tumor suppressor miRNA [46,47]. The reports of Park et al. [48] and Kumaraswamy et al. [49] illustrated the tumor-suppressive effect of *hsa-miR-146a via* inhibiting epithelial-to-mesenchymal transition (EMT) and the metastatic potential in many cancer types, including BC. It has been proposed that hsa-miR-146a can sustain an epithelial phenotype in triple negative breast cancer (TNBC) *via* direct targeting of the TGFβ signaling activator SMAD3 [50]. A recent study reported that *hsa-miR-146a-5p* causes cell cycle arrest and stimulates apoptosis in gastric cancer cells and tissues by targeting cell division cycle 14 homolog A (CDC14A) gene in which *hsa-miR-146a-5p* binds to the 3'-UTR region of CDC14A mRNA resulting in downregulating both CDC14A mRNA and protein expressions [51].

To the best of our knowledge, the current study aimed to predict and validate the role of *FAM170B-AS1* for the first time in breast cancer using the bioinformatics tool and QPCR, respectively, to construct *FAM170B-AS1*/miRNA/mRNA network. Another goal of the present study is to explore the roles of both *hsa-miR-1202* and *hsa-miR-146a-5p* in BC.

2. Subjects and methods

2.1. Subjects

A total of 151 subjects were enrolled in the current study and divided into three groups: a control group ($n = 25$), patients with benign breast diseases ($n = 30$), and patients with primary breast cancer ($n = 96$). All individuals were chosen to be matched in age and the menopause stage. Patients with other malignancies or under tumor therapy were excluded.

Patients were chosen from those admitted to the National Cancer Institute, Cairo University, and Clinical Oncology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Full history and the pathological examinations of the breast mass from patients either with BBD or BC was obtained from their clinical sheets. The stages of BC, performed according to the tumor-node-metastasis classification system (TNM), were obtained from the clinical sheets. Furthermore, the expression of hormonal receptors (PR, ER, and HER-2) on the breast cancer tissue was examined using immunohistochemistry to identify the molecular subtype of BC.

The study was approved by the Scientific Research Ethics Committee, National Research Center, Dokki,

Giza, Egypt (ID: 15209). Informed consent for using the samples for research purposes was obtained from all participants before beginning the study. The research was carried out under the Declaration of Helsinki, printed in the British Medical Journal (18 July 1964).

2.2. Blood sample collection

Three milliliters blood samples were withdrawn from individuals in tubes with polymer gel and clot activator (Greiner bio-one, GmbH, Australia). The blood was left to clot at 37°C for 30 minutes, and all samples were centrifuged at 10,000× g for 10 min at 4°C (13-18KS, Sigma, Germany). The separated sera were aliquoted and stored at −80°C for further analysis.

2.3. Tumor markers assay

The serum venous levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 15.3 (CA 15.3) were estimated using commercial enzyme-linked immunosorbent assay kits provided by Immunospeccorporation, Netherlands.

2.4. Prediction of FAM170B-AS1 association with breast cancer

Predicting the association of *FAM170B-AS1* in different diseases was performed using miRWalk v.2 (http://mirwalk.umm.uni-heidelberg.de/), released in 2019.

2.5. Quantitative RT-PCR for hsa-miR-146a-5p and hsa-miR-1202 assays and FAM170B-AS1 validation

MiRNA was extracted from the sera of all the participants using an RNeasy mini kit (Qiagen, USA). The purity of the extracted RNA was detected at 260/280 nm using a nano-drop spectrophotometer (Quawell, Q-500, Scribner, USA). The extracted miRNA was reverse transcribed into cDNA using a MiScript II reverse transcription kit provided by Qiagen, USA. cDNA synthesis was performed using a thermal cycler (SureCycler 8800, Agilent, USA).

Real-time quantitative PCR was performed to analyse the expression of *hsa-miR-1202* (HS_*hsa-miR-1202* miScript Primer Assay, Cat no.MP00000259), *hsa-miR-146-5p* (HS_*hsa-miR-146-5p* miScript Primer Assay, Cat no.MS00003535), and *FAM170B-AS1* (RT^2 lncRNA qPCR Assay for Human, cat no. LPH11337A) using SYBR Green/ROX Master Mix (Qiagen, USA). Ready-made primers for the studied ncRNAs as well as their housekeeping genes [RNU6 for miRNA (HS_RNU6-2-11_ miScript Primer Assay, Cat no. MS00033740) and GAPDH for lncRNA (Hs_GPDH_1_SG QuantiTect Primer Assay, Cat no. QT00079247)] were provided from Qiagen, USA. The thermal reaction conditions were 95°C for 15 min followed by 40 cycles at 94°C for 15 s, 55°C for 30 s, and 70°C for 34 s using Max3005P QPCR system, Stratagene, Agilent biotechnology, USA. Expression for investigated miRNAs and lncRNA was assessed using the ΔCt method [52]. The cycle threshold (Ct) value is the number of qPCR cycles needed for the fluorescent signal to cross a definite threshold. ΔCt was calculated by subtracting the Ct values of RNU6–2 or GPDH from those of investigated miRNAs or lncRNA, respectively. ΔΔCt was calculated by subtracting the ΔCt of the control samples from the ΔCt of BBD and BC samples.

2.6. Targeted prediction and functional analysis

LncBase predicted v2 database (https://dianalab.ece.uth.gr/html/diana/web/index.php?r=lncbasev2%2Findex-predicted) was used to identify the target miRNAs of *FAM170B-AS1* and their binding sites where the threshold was adjusted at 0.5. In addition, four target prediction algorithms were used to predict the interactions between miRNAs and genes associated with breast cancer. The four corresponding prediction algorisms were miRWalk v.2 (http://mirwalk.umm.uni-heidelberg.de/) database released in 2022, miRDB (http://www.mirdb.org/), TargetScan 7.2 (https://www.targetscan.org/vert_72/), and miRTarBase (https://mirtarbase.cuhk.edu.cn/∼miRTarBase/miRTarBase_2022/php/index.php). The overlapped miRNAs were identified *via* Venn diagrams (https://bioinformatics.psb.ugent.be/webtools/Venn/). The target genes of the overlapped miRNAs from LncBase Predicted v.2, miRWalk v.2, miRDB, TargetScan 7.2, and miRTarBase were identified by Excel function to construct *FAM170B-AS1*/miRNA/mRNA network in breast cancer.

To further consider the potential functions, pathways, and networks of these target genes, the DAVID database (https://david.ncifcrf.gov/tools.jsp) was used for Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. GO was divided into three functional groups: biological process (BP), cellular component (CC), and molecular function (MF). Cytoscape (version 3.9.0, https://cytoscape.org/) was applied to construct the functional *FMA170B-AS1*/miRNA/mRNA network.

In addition, the human protein atlas (HPA) (https://www.proteinatlas.org/) was used to explore the protein expression of target genes in breast cancer and normal breast tissues. The online database STRING v.11.5 (https://string-db.org/) was used to build protein-protein interaction (PPI) networks using the overlapped target genes of proteins expressed in breast tissue. The minimum required interaction pairs were adjusted at $<$ 0.4.

2.7. Correlation to GEPIA and TCGA data

Gene expression profiling integrative analysis (GEPIA) was used to investigate the expression level of the selected target genes in normal breast tissue and BRCA tumors. Furthermore, GEPIA was utilized to analyse the correlation between the *FAM170B-AS1* gene and the target genes in the BRCA tumor. In addition, the genetic alteration of *FAM170B* in primary cancers was investigated based on TCGA with their overall survival analysis.

2.8. Statistical analysis

The power of the current study was calculated utilizing G*Power 3.1.9.7 software (Franz Faul, Universität Düsseldorf, Germany) (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The test family was adjusted at F test and the statistical test used was adjusted at ANCOVA: fixed effects, main effect, and interactions. Post-hoc: Compute achieved power is the type of power analysis used. The input parameters were effect size $F = 0.4$, α error probability $= 0.05$, total sample size $= 151$, numerator df $= 10$, number of groups $= 3$, and number of covariates $= 1$. The calculated power of the study equaled 93.2%.

The Statistical Package for Social Science version 26 for Windows (SPSS software package, Chicago, USA) was used to perform all statistical analyses. The data distribution was statistically determined using the Kolmogorov-Smirnov test with Lilliefors significance correction. The categorical variables were expressed as frequencies (percentages), and non-parametric data were expressed as median (interquartile range). The non-parametric variables were statistically analysed using the Kruskal-Wallis one-way ANOVA (all pairwise) test for multiple comparisons. χ^2 test was used to evaluate the differences in menopause, stage, grade, pathological types, molecular subtypes, and lymph node metastasis between the different studied groups.

Binary logistic regression analyses were used to investigate the strength of the association of the expression of *FAM170B-AS1, hsa-miR-1202*, and *hsa-miR-146a-5p* with the susceptibility to breast tumors between either benign breast lesion versus control group, malignant tumor versus control group, and malignant tumor versus benign breast lesion. The strength of associations was measured by the odds ratio (OR) with a 95% confidence interval (CI) adjusted for age and menopause. Multiple linear regression analysis was used to assess the association of the independent variables relative to the dependent variables after transforming non-parametric variables into the logistic scale. The regression models were constructed using the "enter" analysis.

The receiver operating characteristic curve (ROC) was performed using SPSS vs. 26 to detect the area under the curve (AUC), cutoff points, sensitivity, and specificity for the tested *miRNA-1202*, *miRNA-146a-5P*, and *FAM10B-AS1* between BC, BBD, and control groups, compared to the traditional tumor markers. All p-values were 2-sided, and a p-value < 0.05 was considered statistically significant. Significance had been adjusted by Bonferroni correction for multiple biomarkers analyses. For the association analyses, the statistical significance thresholds were set to $p < 0.017$ after the Bonferroni correction.

3. Results

The clinical characteristics of all individuals in the studied groups are listed in Table 1. Concerning patients with BBD, 36.7% were identified to have follicular hyperplasia, 26.7% had fibrocytic changes, and 36.7% had intraductal papillomatosis. Of women with BC, 38.5, 34.4, 35.4, and 47.9% were diagnosed to be at the early stage with low-grade, ductal carcinoma *in situ* (DCI) and no lymph node (LN) metastasis, respectively, $p < 0.0001$. On the other hand, 61.5, 65.6, 64.6, and 52.1% of BC patients were at the late stage with high-grade, invasive ductal carcinoma (IDC) and LN metastasis, respectively, $p < 0.0001$. The molecular classification of BC subtypes revealed that most BC patients (46.6%) were luminal B-like subtypes. 22.9, 19.8, 6.3, 2.1, and 2.1% of BC patients were classified as triple-negative, luminal B, luminal A (ER-positive), luminal A (PR positive), and luminal B (ER and HER-2 positive) subtypes, respectively.

Data represented in Supplementary file Table S1 predicts the association of *FAM170B-AS1* with different types of cancer in Homo Sapiens species. Us-

Table 1
Demographic characteristics of subjects in the different studied groups

	Control ($n = 25$)	BBD ($n = 30$)	BC ($n = 96$)	χ^2 value	P value
Age (years)	53 (13)	50 (19)	50 (18)		0.915
Menopause n (%)					
Yes	6 (24)	18 (60)	34 (35.40)	1.56	0.436
No	19 (76)	12 (40)	62 (64.60)		
Stage n (%)					
Early			37 (38.5)	151	$<$ 0.0001
Late			59 (61.5)		
Grade n (%)					
Low grade			33 (34.4)	151	$<$ 0.0001
High grade			63 (65.6)		
Pathological types n (%)					
Follicular hyperplasia		11 (36.7)		302	$<$ 0.0001
Fibrocytic changes		8 (26.7)			
Intraductal papillomatosis		11 (36.7)			
DCI			34 (35.4)		
IDC			62 (64.6)		
Molecular subtypes n (%)					
Luminal A (ER)			6 (6.3)	151	$<$ 0.0001
Luminal A (PR)			2 (2.1)		
Luminal B (ER, HER-2)			2 (2.1)		
Luminal B (PR, HER-2)			19 (19.8)		
Luminal B-like (ER, PR, HER-2)			45 (46.9)		
Basal-like (triple negative)			22 (22.9)		
LN metastasis n (%)					
No			46 (47.9)	151	$<$ 0.0001
Yes			50 (52.1)		

Data are expressed as Median (INTER QUARTILE RANGE) for non-parametric variables and frequencies (percentages) for categorical variables. BBD: benign breast diseases, BC: breast cancer, DCI: ductal carcinoma in situ, IDC: invasive ductal carcinoma, ER: estrogen receptor, PR: progesterone receptor, HER-2: human epidermal growth factor receptor-2, LN: lymph node. The mean difference is significant at $p < 0.05$.

Table 2
Comparison of the levels of tumor markers as well as *FAM170B-AS1*, *hsa-miR-1202*, and *hsa-miR-146a-5p* expression in the different studied groups

	Control ($n = 25$)	BBD ($n = 30$)	BC ($n = 96$)
CEA (pg/ml)	8 (5)	14.2 (7.3)	12 (8.7)
P value		0.024[a]	0.014[a], 1.000[b]
Fold change		*1.78**	*1.5**/0.85*[#]
CA15.3 (pg/ml)	12.00 (3.00)	13.00 (9.00)	22.00 (s10.00)
P value		0.379[a]	$<$ 0.0001[a]/0.001[b]
Fold change		1.08*	1.83*/1.69[#]
FAM170B-AS1 expression	0.53 (0.22)	0.88 (0.27)	12.85 (7.25)
P value		0.208[a]	$<$ 0.0001[a,b]
Fold change		1.66*	24.25*/14.60[#]
hsa-miR-1202 expression	5.72 (1.44)	19.50 (28.83)	56.51 (82.29)
P value		0.005[a]	$<$ 0.0001[a,b]
Fold change		3.41*	9.88*/2.90[#]
miR-146a-5p expression	360 (184.50)	220 (180.00)	31 (41.00)
P value		0.403[a]	$<$ 0.0001[a,b]
Fold change		0.61*	0.09*/0.14[#]

Data are expressed as median (interquartile range). BBD: benign breast diseases, BC: breast cancer, CEA: carcinoembryonic antigen, CA 15.3: carbohydrate antigen 15.3. [a]: significance versus control group, [b]: significance versus benign breast diseases group. *: fold change versus control group, [#]: fold change versus benign breast diseases group. Significance had been adjusted by Bonferroni correction for multiple tests. The mean difference is significant at $p < 0.05$.

Table 3
Relationship between CEA and CA15.3 levels as well as ncRNAs expression in serum with clinicopathological parameters of patients with breast benign diseases

Clinical pathological criteria		CEA level (pg/ml)	CA15.3 level (pg/ml)	*FAM170B-AS1* expression	*hsa-miR-1202* expression	*hsa-miR-146a-5p* expression
Age	$<$ 50 years	15	13	0.88	10.10	220
	$>$ 50 years	13	12	0.88	21.60	150.00
p value		0.214	0.121	0.583	0.916	0.255
Menopause status	Yes	13.6	12	0.93	21.60	190.00
	No	15	13	0.88	14.8	220.00
p value		0.701	0.237	0.347	0.608	0.495
Pathological types	Follicular hyperplasia	12	12	0.88	35.51	220.00
	Fibrocytic changes	15.55	17	0.88	28.55	130.50
	Intraductal papillomatosis	13	13	0.88	10.10	287.00
p value		0.110	0.667	0.823	0.278	0.222

Data are expressed as a median. CEA: carcinoembryonic antigen, CA15.3: carbohydrate antigen 15.3. Significance had been adjusted by Bonferroni correction for multiple tests. $P < 0.05$ was considered significant.

Table 4
Relationship between CEA and CA15.3 levels in serum with clinicopathological parameters of patients with breast cancer

Clinical pathological criteria		CEA level (pg/ml)	p value	CA15.3 level (pg/ml)	p value
Age	$<$ 50 years	14	0.036	22	0.562
	$>$ 50 years	10.00		22	
Menopause status	Yes	10.50	0.063	22.00	0.706
	No	12		22	
Stage	Early	12.00	0.561	23.00	0.553
	Late	11.00		22.00	
Grade	Low	10.00	0.069	22.00	0.559
	High	12.00		22.00	
Pathological types	DCI	11.00	0.200	22.00	0.994
	IDC	12.00		22.00	
Molecular subtypes	ER	7.00	0.248	22.00	0.098
	PR	2.30		14.00	
	ER, HER-2	9.00		35.00	
	PR, HER-2	15.30		21.00	
	ER, PR, HER-2	11.00		22.00	
	Basal-like	12.00		23.00	
LN metastasis	No	11.00	0.597	18.00	0.133
	Yes	12.00		22.00	

Data are expressed as a median. DCI: ductal carcinoma in situ, IDC: invasive ductal carcinoma, ER: estrogen receptor-positive, PR: progesterone receptor positive, HER-2: human epidermal growth factor receptor-2 positive, LN: lymph node. Significance had been adjusted by Bonferroni correction for multiple tests. $P < 0.05$ was considered significant.

ing the lncRNA-disease association prediction (LDAP) method, *FAM170B-AS1* was anticipated to be associated with breast cancer with a 0.1828 score.

Table 2 shows the expression levels of *FAM170B-AS1*, *hsa-miR-1202*, and *hsa-miR-146a-5p* in the sera of all the studied groups, compared to the serum levels of CEA and CA15.3 (traditional tumor markers). There were significant elevations in the serum level of CEA in both BBD patients (1.78-fold change, $p = 0.024$) and BC patients (1.5-fold change, $p = 0.014$), compared to the control group, with no significant difference between BBD and BC groups ($p > 0.05$). The serum level of CA15.3 was significantly raised only in the BC patients (1.83-fold change, $p = 0.001$) compared to the control group. The expression of circulating *hsa-miR-1202* was significantly upregulated either in BBD (3.41-fold change, $p = 0.005$) or BC (9.88-fold change, $p < 0.0001$) patients, compared to the control group. When comparing BC with the BBD group, the *hsa-miR-1202* expression was higher in BC patients by a 2.9-fold change at $p < 0.0001$. On the contrary, *hsa-miR-146a-5p* showed low expression level in BC patients relative to both the control group (0.09-fold change, $p < 0.0001$) and the BBD group (0.14-fold change, $p < 0.0001$) with no significant difference between the BBD group and controls ($p > 0.05$). Regarding the ex-

Table 5
Relationship between *FAM170B-AS1, hsa-miR-1202*, and *hsa-miR-146a-5p* expression with clinicopathological parameters of patients with breast cancer

Clinical pathological criteria		*FAM170B-AS1* expression	p value	*hsa-miR-1202* expression	p value	*hsa-miR-146a-5p* expression	p value
Age	$<$ 50 years	12.85	0.046	52.4	0.991	49	$<$ 0.0001
	$>$ 50 years	12.85		81.5		20	
Menopause status	Yes	12.85	0.452	59.71	0.713	20	0.001
	No	12.85		53.3		42.00	
Stage	Early	12.85	0.501	36.80	$<$ 0.0001	20.00	0.01
	Late	12.85		91.30		44.00	
Grade	Low	12.85	0.666	33.13	$<$ 0.0001	19.00	$<$ 0.0001
	High	12.85		96.34		44.00	
Pathological types	DCI	12.85	0.143	33.13	$<$ 0.0001	19.50	0.001
	IDC	12.85		93.82		44.50	
Molecular subtypes	ER	12.85	0.084	24.10	$<$ 0.0001	19.50	0.038
	PR	9.27		15.30		32.00	
	ER, HER-2	4.70		52.40		40.00	
	PR, HER-2	12.85		38.05		32.00	
	ER, PR, HER-2	12.85		104.10		45.00	
	Basal-Like	12.85		41.05		19.50	
LN metastasis	No	12.85	0.378	33.80	$<$ 0.0001	44.50	0.005
	Yes	12.85		91.30		25.00	

Data are expressed as a median. DCI: ductal carcinoma in situ, IDC: invasive ductal carcinoma, ER: estrogen receptor-positive, PR: progesterone receptor positive, HER-2: human epidermal growth factor receptor-2 positive, LN: lymph node. Significance had been adjusted by Bonferroni correction for multiple tests. $P < 0.05$ was considered significant.

Table 6
Association of *FAM170B-AS1, hsa-miR-1202*, and *hsa-miR-146a-5p* expression with breast cancer risk, compared with the traditional tumor markers

	BBD vs. control		BC vs. control		BC vs. BBD	
	$^{\#}$Adjusted OR (95% CI)	P value	$^{\#}$Adjusted OR (95% CI)	P value	$^{\#}$Adjusted OR (95% CI)	P value
CEA	0.80 (0.69–0.93)	0.004	0.90 (0.85–0.98)	0.011	1.01 (0.941–1.08)	0.848
CA15.3	0.89 (0.79–1.00)	0.054	0.87 (0.81–0.94)	$<$ 0.0001	0.93 (0.88–0.98)	0.005
FAM170B-AS1	0.00 (0.00–0.03)	$<$ 0.0001	0.00 (0.00–0.00)	0.989	0.33 (0.19–0.58)	$<$ 0.0001
hsa-miR-1202	0.52 (0.33–0.80)	0.003	0.56 (0.35–0.88)	0.013	0.96 (0.95–0.98)	$<$ 0.0001
hsa-miR-146a-5p	1.01 (1.00–1.01)	0.008	1.08 (1.02–1.14)	0.008	1.05 (1.03–1.07)	$<$ 0.0001

BC: breast cancer, BBD: benign breast diseases, OR: odds ratio, 95% CI: 95% confidence interval. $^{\#}$: Adjusted for age and menopause. $P < 0.010$ was considered significant after the Bonferroni correction.

pression of *FAM170B-AS1*, no significant change was recorded in BBD patients ($p > 0.05$) compared to the control group. However, it was significantly upregulated in BC patients when compared to both the control (24.24-fold change) and BBD (14.6-fold change) groups at $p < 0.0001$.

Table 3 shows no significant difference ($p > 0.05$) in the serum levels of CEA and CA15.3 among women with BBD regarding age, menopause status, and pathological types. Also, there was no significant difference ($p > 0.05$) in the expression levels of *FAM170B-AS1*, *hsa-miR-1202*, and *hsa-miR-146a-5p* among BBD patients concerning age, menopause status, and pathological types.

Both the serum levels of CEA and CA15.3 were found to be similar ($p > 0.05$) among BC patients concerning menopause status, stage, grade, pathological types, and molecular subtypes (Table 4). However, only the serum level of CEA was significantly higher ($p = 0.036$) in BC patients $<$ 50 years old.

Data in Table 5 illustrate the difference between the expression of the studied ncRNAs among BC patients with their age, menopause status, stage, grade, molecular subtypes, and LN metastasis. No significant difference was recorded in the expression of *hsa-miR-1202* in BC patients with $<$ or $>$ 50 years old, pre- or postmenopausal status. On the other hand, high expression levels were recorded in *hsa-miR-1202* in BC patients at late stage, with high-grade, IDC, and LN metastasis ($p < 0.0001$). The significantly highest expression level of *hsa-miR-1202* ($p < 0.0001$) was observed in BC patients with luminal B-like subtype followed by luminal B (ER, HER-2 positive), triple negative, luminal B (PR, HER-2 positive), ER-positive, and PR positive.

Table 7
Multiple linear regression analysis for the association between *FAM170B-AS1* and the studied miRNAs and the association of the tumor markers as well as the studied genes with the stage, grade, and LN metastasis

Dependent variable	Predictors	R	r^2	F	P value*	β	t	P value	VIF
FAM170B-AS1 expression	*hsa-miR-1202* expression	0.67	0.44	118.00	< 0.0001	0.67	10.86	< 0.0001	1.00
	hsa-miR-146a-5p expression	0.80	0.64	288.66	< 0.0001	-0.80	-16.39	< 0.0001	1.00
Stage	CEA	0.87	0.76	89.52	< 0.0001	-0.03	-0.78	0.44	1.09
	CA 15.3					-0.002	-0.05	0.957	1.16
	FAM170B-AS1 expression					0.66	7.86	< 0.0001	4.14
	hsa-miR-1202 expression					0.27	4.79	< 0.0001	1.92
	hsa-miR-146a-5p expression					-0.01	-0.19	0.851	3.15
Grade	CEA	0.88	0.78	103.40	< 0.0001	-0.02	-0.39	0.700	1.09
	CA 15.3					0.01	0.20	0.839	1.16
	FAM170B-AS1 expression					0.67	8.40	< 0.0001	4.14
	hsa-miR-1202 expression					0.33	6.20	< 0.0001	1.92
	hsa-miR-146a-5p expression					0.05	0.75	0.457	3.15
LN metastasis	CEA	0.86	0.73	79.54	< 0.0001	-0.05	-1.18	0.242	1.09
	CA 15.3					0.06	1.35	0.179	1.16
	FAM170B-AS1 expression					0.66	7.59	< 0.0001	4.14
	hsa-miR-1202 expression					0.28	4.70	< 0.0001	1.92
	hsa-miR-146a-5p expression					0.04	0.55	0.580	3.15

β: standardized coefficient, VIF: variance inflation factor, LN: lymph node. *: P-value obtained from ANOVA table. $P < 0.05$ was considered significant.

hsa-miR-146a-5p was significantly downregulated in BC patients aged > 50 years old ($p < 0.0001$) and at post-menopause status ($p = 0.001$), late stage ($p = 0.01$), with high grade ($p = 0.001$), IDC ($p = 0.01$), and LN metastasis ($p = 0.005$). BC patients with ER-positive and triple-negative molecular subtypes showed a more significant down-regulation in *hsa-miR-146a-5p* expression level ($p = 0.038$) compared to the other subtypes. On the other hand, no statistical difference was recorded in the expression level of *FAM170B-AS1* among BC patients based on age, menopause status, stage, grade, pathological subtypes, molecular subtypes, and LN metastasis ($p > 0.05$).

The associations of the tumor markers and the studied ncRNAs with BBD and BC are illustrated in Table 6 using binary logistic regression analysis. The serum level of CA15.3 and the expression level of hsa-miR-146a-5p were not significantly associated with BBD ($p > 0.05$) compared to the control group. The serum levels of CEA (OR $= 0.69$, $p = 0.004$) and the expression level of *hsa-miR-1202* (OR $= 0.52$, $p = 0.003$) were significantly associated with BBD, compared to the control group. On the other hand, CEA was not significantly associated with BC ($p > 0.05$) compared to the controls. CA15.3 was significantly associated with BC (OR $= 0.93$, $p = 0.005$) compared to the controls. Regarding *hsa-miR-1202* and *hsa-miR-146a-5p*, they were significantly associated with BC when compared to both the control group (OR $= 0.56$ and 1.08, respectively, at $p = 0.013$ and 0.008) and BBD group (OR $=$ 0.96 and 1.05, respectively, at $p < 0.0001$) after adjustment for age and menopause. On the contrary, no significant associations were observed between *FAM170B-AS1* and both BBD and BC relative to the control group. However, when comparing BC with the BBD group, *FAM170B-AS1* was significantly associated with BC (OR $= 0.33$ at $p < 0.0001$).

Multiple linear regression analysis (Table 7) reported a significantly positive correlation between *FAM170B-AS1* expression and the expression of *hsa-miR-1202* ($\beta = 0.67$, $t = 10.86$, VIF $= 1$, $p < 0.0001$) while *FAM170B-AS1* expression was found to be negatively correlated with the expression of *hsa-miR-146a-5p* ($\beta = -0.80$, $t = -16.39$, VIF $= 1$, $p < 0.0001$). CEA, CA15.3, and *hsa-miR-146a-5p* did not correlate with stage, grade, and LN metastasis of BC ($p > 0.05$). On the other hand, both *hsa-miR-1202* and *FAM170B-AS1* were significantly associated with stage, grade, and LN metastasis ($p < 0.0001$).

Table 8 and Fig. 1 demonstrate the results of the ROC curve of the traditional tumor markers and the studied ncRNAs when comparing BC patients with non-BC patients (Control group + BBD group). CEA yields an AUC of 0.571 at $p > 0.05$ (sensitivity of 63.6% with a specificity of 65.6%), CA15.3 yields an AUC of 0.765 at $p < 0.0001$ (sensitivity of 63.6% with a specificity of 100%), *hsa-miR-1202* yields an AUC of 0.893 at $p < 0.0001$ (sensitivity of 64.9% with a specificity of 100%). *hsa-miR-146a-5p* also yields an AUC of 0.967 (sensitivity of 88.9% with a specificity of 100%). *FAM170B-AS1* also yields an AUC of 0.992 (sensitivity of 97% with a specificity of 100%).

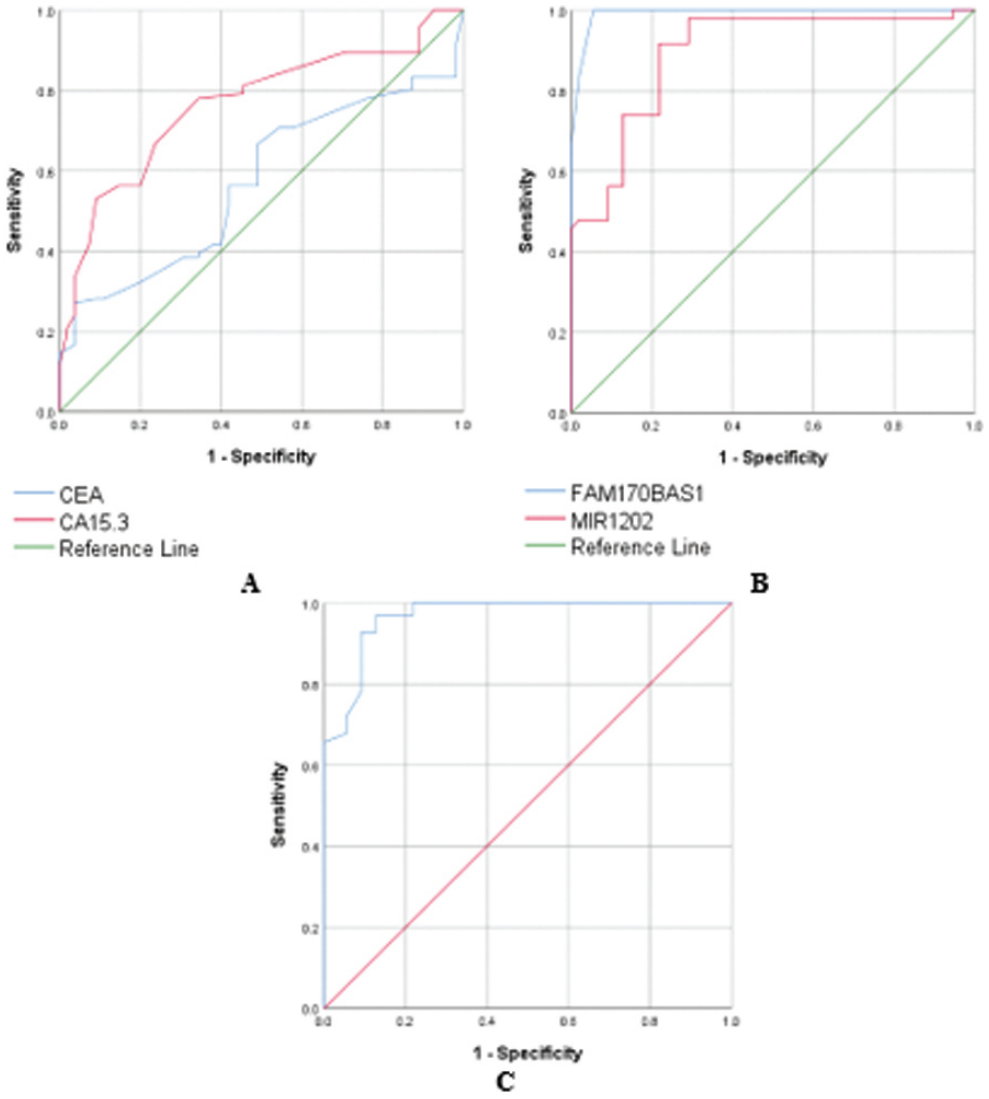

Fig. 1. The receiver operating characteristic curve of all tested markers in BC group compared to the groups of control + benign breast diseases. A: The traditional tumor markers (CEA, CA15.3) B: *FAM170B-AS1* and *hsa-miR-1202*, and C: *hsa-miR-146a-5p*.

Table 8
The ROC curve plot of CEA, CA15.3, *FAM170B-AS1*, *hsa-miR-1202*, and *hsa-miR-146a-5P* in BC group, compared to non-BC individuals (control group + BBD group)

C	AUC	*P* value	Specificity (%)	Sensitivity (%)
CEA	0.571	0.147	65.6	63.6
CA15.3	0.765	< 0.0001	100	65.3
FAM170B-AS1	0.992	< 0.0001	100	97
hsa-miR-1202	0.893	< 0.0001	100	64.9
hsa-miR-146a-5p	0.967	< 0.0001	100	88.9

AUC: area under the curve, CEA: carcinoembryonic antigen, CA 15.3: carbohydrate antigen 15.3. $P < 0.05$ was considered significant.

Supplementary file Table S2 illustrates the prediction of the interaction between *FAM170B-AS1* and 710 miRNAs with the scores of predictions and binding besides the binding sites of miRNAs on *FAM170B-AS1* lncRNA. Among these miRNAs, *hsa-miR-1202* and *hsa-miR-146a-5p* were predicted to interact with *FAM170B-AS1* with prediction scores of 0.611 and 0.539, respectively. The binding scores of *hsa-miR-1202* and *hsa-miR-146a-5p* with *FAM170B-AS1* were 0.018 and 0.007, respectively. One of the miRNAs predicted to interact with FAM170B-AS1 is hsa-miR-203a-3p with a prediction score of 0.555 and a binding score of 0.007. This miRNA is predicted by TargetScan 7.2 to interact with FAM170B gene (ENST00000311787.5)

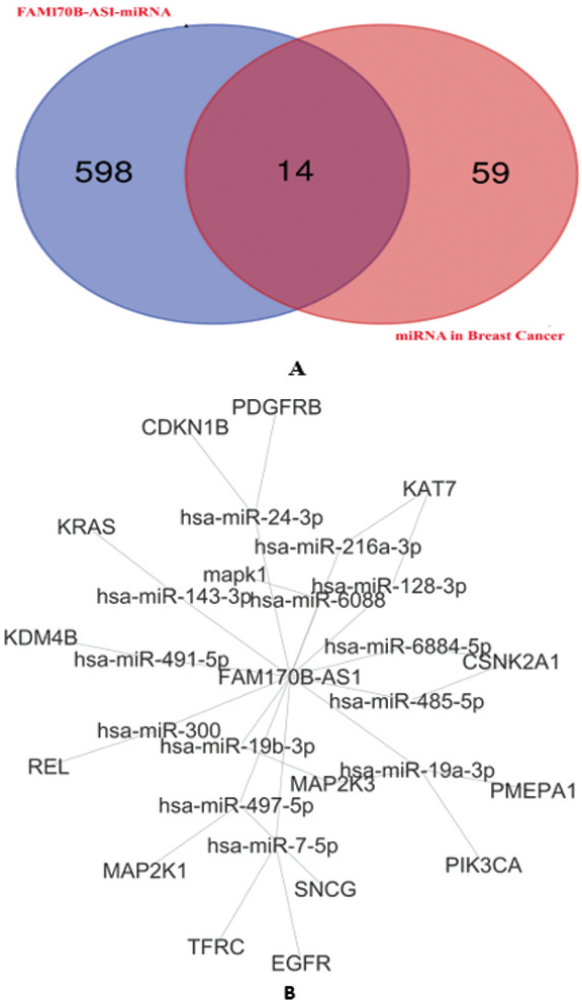

Fig. 2. A: Venn diagram identifies the overlapped miRNAs targeted by *FAM170B-AS1* and associated with breast cancer identified. B: *FAM170B-AS1*-overlapped miRNA-mRNA network in breast cancer and constructed by Cytoscape.

with 8mer which needs further practical validation. FAM170B is located on chromosome 10: 49, 131, 154-49, 134, 021 reverse strand. This gene codes for a protein with 283 amino acids.

In the current study, four miRNA target prediction algorithms were used to predict the potential target genes of miRNAs in breast cancer (Supplementary file Table S3). Several miRNA interactions with genes (259 interactions) were anticipated. The binding scores of miRNAs with genes were $\geqslant 0.96$. Venn diagrams (Fig. 2A) were used to deduce the overlapped miRNAs between *FAM170B-AS1*/miRNAs and miRNAs/mRNA in breast cancer disease. Only 612 and 73 miRNAs, respectively, were unique. Among these miRNAs, only 14 miRNAs were found to be overlapped. These miRNAs were *hsa-miR-373-3p, hsa-miR-6884-5p, hsa-miR-216a-3p, hsa-miR-497-5p, hsa-miR-24-3p, hsa-miR-6088, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-491-5p, hsa-miR-143-3p, hsa-miR-128-3p, hsa-miR-7-5p, hsa-miR-485-5p, and hsa-miR-300.* A network between *FAM170B-AS1*, miRNAs, and target genes was constructed *via* Cytoscape (Fig. 2B). 16 genes were predicted to be targeted by *FAM170B-AS1*. These genes were *PMEPA1* (ENSG00000124225), *SNCG* (ENSG00000173267), *CSNK2A1* (ENSG00000101266), *KAT7* (ENSG00000136504), *MAP2K3* (ENSG00000034152), *KDM4B* (ENSG00000127663), *REL* (ENSG00000162924), *TERC* (ENSG00000072274), *PDGFRB* (ENSG00000113721), *CDKN1B* (ENSG00000111276), *MAP2K1* (ENSG00000169032), *PIK3CA* (ENSG00000121879), *KRAS* (ENSG00000133703), *MAPK1* (ENSG00000100030), *EGFR* (ENSG00000146648), and *ESR1* (ENSG00000091831).

The 16 genes were underwent for GO and pathways analyses. The GO analysis (Supplementary file Table S4) indicated that the target genes were involved in complex cellular pathways (BP), such as signal transduction, positive regulation of transcription, and cell proliferation. In addition, the target genes were located (CC) in the cytosol, nucleus, and plasma membrane. Furthermore, The GO molecular function of the target genes showed their involvement in numerous MFs, such as protein binding, ATP binding, protein kinase binding, and protein tyrosine kinase activity. The KEGG pathway analysis (Supplementary file Table S5) revealed that PD-L1 expression and PD-1 checkpoint pathway in cancer, PI3K-Akt signaling pathway, Ras signaling pathway, Rap1 signaling pathway, EGFR tyrosine kinase inhibitor resistance, ErbB signaling pathway, Estrogen signaling pathway, and MAPK signaling pathway are associated with the regulation of the 14 overlapped miRNAs.

In addition, the genes associated with PD-L1 expression and PD-1 checkpoint pathway in cancer, PI3K-Akt signaling pathway, Ras signaling pathway, Rap1 signaling pathway, EGFR tyrosine kinase inhibitor resistance, ErbB signaling pathway, Estrogen signaling pathway, and MAPK signaling pathway were selected based on the KEGG pathway analysis. Four genes (*MAPK1, EGFR, KRAS, and MAP2K1*) overlapped based on the Venn diagrams. Next, the current study investigated the preliminary expression level of the four genes based on GEPIA. Thus, *MAPK1* and *KRAS* were upregulated, whereas *MAP2K1* and *EGFR* were downregulated in BRCA tumors compared to normal BRCA tissues. Furthermore, positive correlations were found between the expression of *FAM170B-AS1* and the genes expression

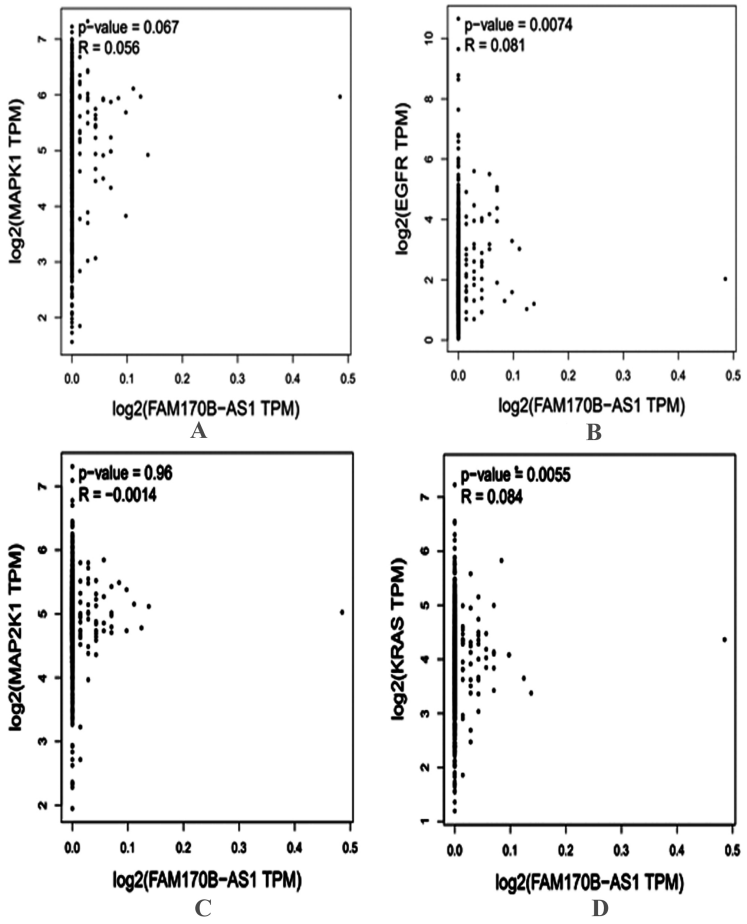

Fig. 3. Correlation between the gene expression of *FAM170B-AS1* with *MAPK1* (A), *EGFR* (B), *MAP2K1* (C), and *KRAS* (D) in BRCA tumor based on the GEPIA database.

of *MAPK1* ($r = 0.056$, $p > 0.05$, Fig. 3A), *EGFR* ($r = 0.081$, $p = 0.0074$, Fig. 3B), and *KRAS* ($r = 0.084$, $p = 0.0055$, Fig. 3D) in BRCA tumors, whereas a negative correlation was found between *FAM170B-AS1* and *MAP2K1* ($r = -0.0014$, $p > 0.05$, Fig. 3C).

The String database was applied to construct the PPI network, and 66 PPI pairs with a combined score of $<$ 0.4 were selected (Fig. 4). The highest degree of interaction in the PPI network was ESR1 & EGFR (degree 9), MAPK1 & KRAS (degree 8), PIK3CA (degree 7), and MAP2K1 & CDKN1B (degree 6). The number of nodes was 16, accounting for 100% of all the target genes. The clustering coefficient of the PPI network was 0.697, which indicates that the PPI network had high cluster properties.

Moreover, based on the HPA database, strong staining in breast duct carcinoma was observed for MAPK1 (Fig. 5A-2), whereas moderate staining was observed for MAP2K1 (Fig. 5C-2) and KRAS (Fig. 5D-2) and negatively staining was observed for EGFR (Fig. 5B-2). Negative staining in normal breast tissues was observed for KRAS (Fig. 5D-1), while moderate staining was observed for MAPK1 (Fig. 5A-1) and EGFR (Fig. 5B-1), and weak staining was observed for MAP2K1 (Fig. 5C-1). Based on these results, EGFR and KRAS were selected. Using bioinformatics tool, it has been hypothesized that *FAM170B-AS1* may dysregulate the expression of *KRAS* and *EGFR* genes in the PD-L1 expression and PD-1 checkpoint pathway in cancer, PI3K-Akt signaling pathway, Ras signaling pathway, Rap1 signaling pathway, EGFR tyrosine kinase inhibitor resistance, ErbB signaling pathway, Estrogen signaling pathway, and MAPK signaling pathway to participate in the different biological processes of BC.

Data collected from TCGA report 28 cases with ten types of primary cancers with somatic mutations in the *FAM170B* gene with different severity (six patients with the hematopoietic and reticuloendothelial system, five

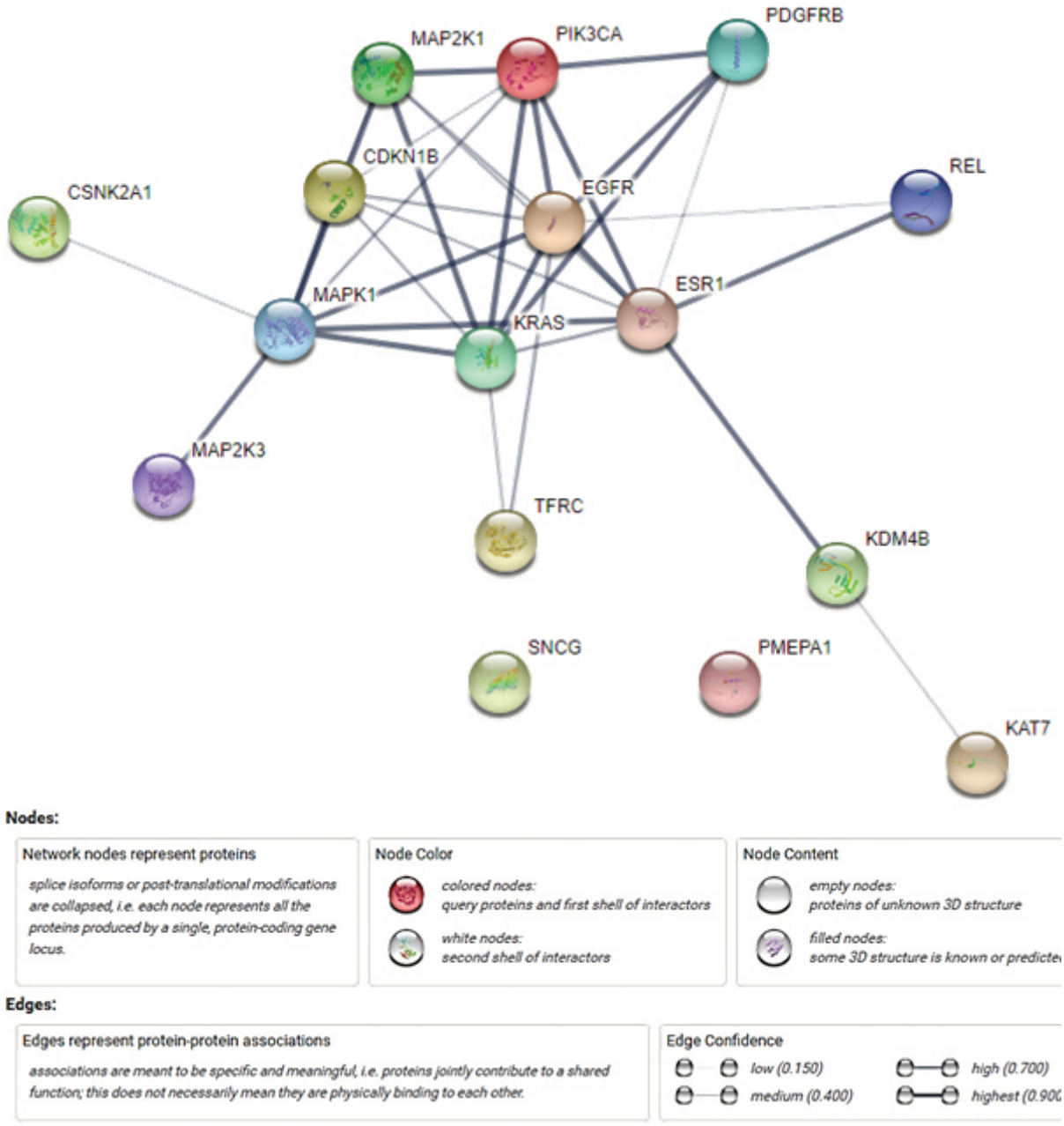

Fig. 4. The PPI network of the target genes.

individuals with colon, four cases with bronchus and lung, three subjects with skin, two patients with breast, two individuals with other and ill-defined sites, two samples with the pancreas, and one sample for each of kidney, rectum, small intestine, and uterus). The types of mutations are listed in the Supplementary file Table S6. The high severity of mutations in *FAM170B* was observed in ductal & lobular neoplasm of breast cancer *via* deletion causing frameshift mutation and in ductal & lobular neoplasm of pancreatic cancer *via* substitution causing stop gained mutation. TCGA demonstrated that cancer patients with mutations in *FAM170B* show improved overall survival (Fig. 6).

4. Discussion

Since the most diagnosed cancer in women is BC,

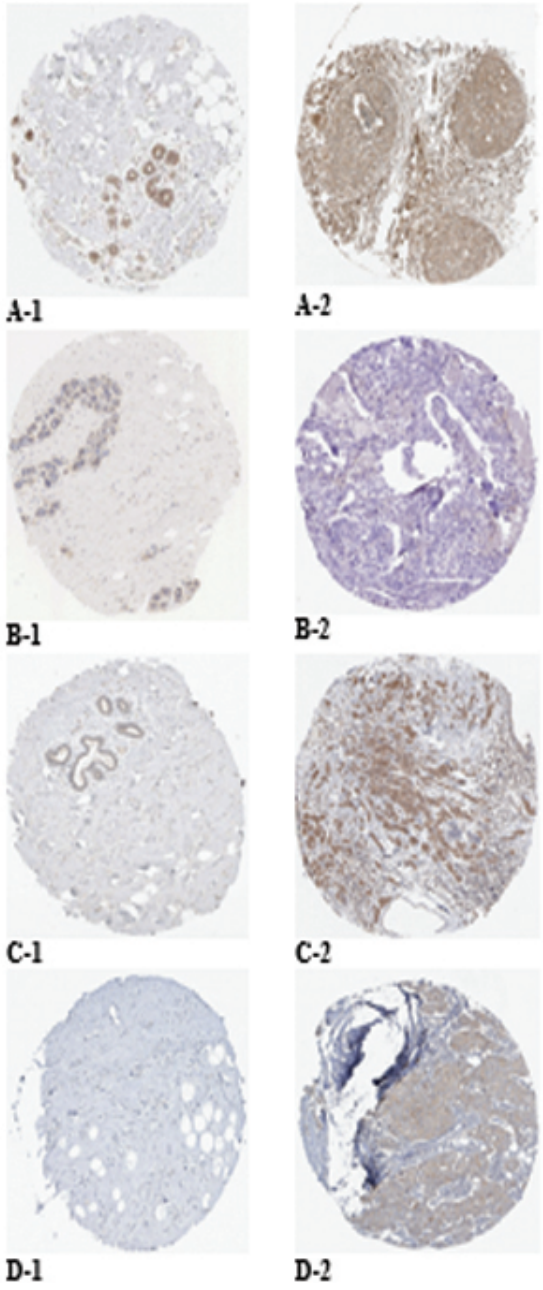

Fig. 5. Protein expression of the targeted genes in both normal breast and breast cancer tissues based on the HPA database. MAPK1 was moderately stained in glandular and myoepithelial cells of the normal breast (A-1) and was strongly stained in breast duct carcinoma (A-2). EGFR was moderately stained in glandular and myoepithelial cells of the normal breast (B-1) and was negatively stained in breast duct carcinoma (B-2). MAP2K1 was weakly stained in glandular cells of the normal breast (C-1) and was moderately stained in breast duct carcinoma (C-2). KRAS was negatively stained in glandular and myoepithelial cells of the normal breast (D-1) and was moderately stained in breast duct carcinoma (D-2).

more efficient diagnostic and therapeutic methods for BC are required. In the last decades, discovering specific and sensitive molecular biomarkers with statistically significant association with BC has attracted important attention for prediction, prognosis, and diagnosis [53,54]. Among molecular biomarkers, the lncRNAs and miRNAs circulating in the blood are of great importance because of their stability [47,48]. lncRNAs are proven to be associated with numerous types of cancer *via* many pathways, including cell proliferation, invasion, and metastasis [57,58,59]. lncRNAs can control these pathways through lncRNAs/miRNAs/mRNA interaction networks either by acting as oncogenic or tumor suppressor lncRNAs directly or indirectly [60, 61,62]. Therefore, bioinformatic prediction of lncRNAs followed by experimental validation becomes one of the most important research fields in cancer [63].

Discovering novel biomarkers that are more specific and sensitive to BC diagnosis is still mandatory because of the limited sensitivity and specificity of CEA and CA15.3 for BC, especially at the early stage [64,65]. The recent study by Jintao et al. [66] reported 85.71% sensitivity, 63.49% specificity of CEA, 64.29% sensitivity, and 80.95% specificity of CA15.3 for BC. In addition, measuring the levels of CEA and CA15.3 in sera of BC patients could not significantly predict the stage, grade, and LN metastasis of BC [67]. As far as we know, the current study bioinformatically anticipated and practically validated the involvement of *FAM170B-AS1* in breast tumors for the first time. The upregulation of *FAM170B-AS1* gene expression in the serum of BC patients and its significant associations with BC could make *FAM170B-AS1* a potential predictive marker for BC among BBD patients. In addition, the present study revealed the probable diagnostic role of *FAM170B-AS1* in BC by giving higher specificity (100%) and sensitivity (97%) than that of CEA and CA15.3. Multiple linear regression analysis performed in the present study reports that serum *FAM170B-AS1* expression was incorporated as an independent predictor of stage, grade, and LN metastasis of BC, while CEA and CA15.3 did not. Previous studies demonstrated that increased CEA and CA15.3 levels in the serum of BC patients at diagnosis are associated only with higher stage, tumor size, and positive axillary lymph nodes [68,69,70,71,72,73]. All of these make serum *FAM170B-AS1* a promising biomarker for BC.

MiRNAs function as regulators for multiple genes by binding to their 3'-UTRs and, therefore, up or down-regulate their expression [74]. Concerning *hsa-miR-1202*, various reports suggest its valuable role in pre-

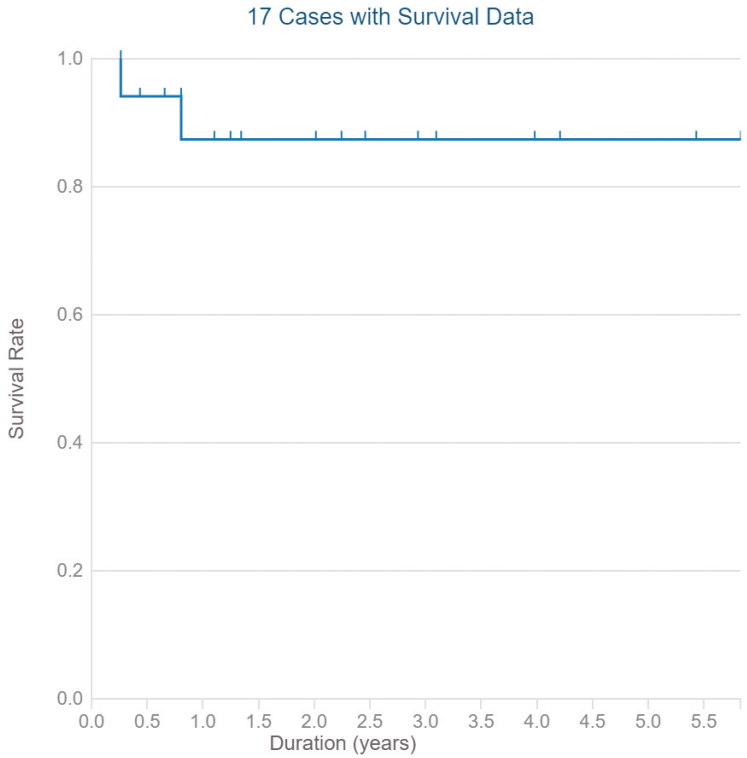

Fig. 6. Overall survival of *FAM170B* genetic alterations in different types of primary cancer extracted from TCGA.

dicting the prognosis of cancers [35,75]. Chen et al. found that silenced *hsa-miR-1202* in endometrial cancer could enhance the apoptotic level of tumor cells, arrest tumor cells in the G1 phase, and decline the migratory and invasive abilities of the tumor cells [76]. Wang et al. illustrated the association of the upregulated *hsa-miR-1202* with lymph nodes in papillary thyroid carcinoma [75]. Quan et al. demonstrated that *hsa-miR-1202* inhibits tumor cell proliferation by targeting *Rab1A* in glioma cells [37]. Du et al. indicated that *hsa-miR-1202* is down-regulated in HCC, which was associated with metastasis, clinicopathologic features, and worse prognosis of HCC *via* direct binding to the *CDK14* gene to exert its anti-tumor effects [38]. These reports suggested that *hsa-miR-1202* may play an oncogene or tumor suppressor role in cancers by controlling cell proliferation, apoptosis, migration, and invasion.

The current study documented an upregulation in *hsa-miR-1202* gene in both BBD and BC patients. This upregulation varied among BC patients with stage, grade, pathological types, molecular subtypes, and LN metastasis of BC. Also, *hsa-miR-1202* showed significant associations with both BBD and BC, compared to the control group, making *hsa-miR-1202* a probable risk factor for both BBD and BC. The multiple linear regression analysis performed in the present study reveals that the upregulation of *hsa-miR-1202* in sera is incorporated as an independent predictor of stage, grade, and LN metastasis of BC, compared to the traditional tumor markers (CEA and CA15.3). Measuring the expression level of *hsa-miR-1202* in sera gave high specificity but low sensitivity towards BC. It has been previously shown that *hsa-miR-1202* is an oncomiR in BC [34]. The study of Hamam et al. [34] showed an upregulation of *hsa-miR-1202* in BC patients with stages I, II, and III, compared to stage IV, with potential utilization as a tumor biomarker for early detection. Furthermore, its expression was slightly higher in the HER2 and TN compared to patients with luminal subtypes.

Regarding *hsa-miR-146a* role in BC, a controversial role has been illustrated. Particularly, *hsa-miR-146a* has been revealed to downregulate *BRCA1* directly [77], where another study showed a binding between BRCA1 and the promoter of *MIR146A*, which activates the transcription of *MIR146A* leading to attenuation of EGFR expression in a *hsa-miR-146a*-dependent manner [49]. Interestingly, a negative association between *hsa-miR-146a* and *EGFR, NOTCH2*, and *SOX2* genes was practically reported and data extracted from TCGA dataset confirmed the reported negative association of *hsa-miR-*

146a with *EGFR* gene which is upregulated in TNBC and its protein product enhances tumorigenesis and immune escape *via* glycolysis stimulation [78] and controls TNBC aggressiveness and stemness through the management of E2/Eβ axis [79], leading to poor prognosis.

In the present study, *hsa-miR-146a-5p* was downregulated in BC patients only. Their downregulation varied among BC patients with menopause status, stage, grade, pathological types, molecular subtypes, and LN metastasis of BC. Also, *hsa-miR-146a-5p* showed significant association with BC, compared to the control and BBD groups, making *hsa-miR-146a-5p a* probable risk factor for BC. The multiple linear regression analysis performed in the present study reveals that the down regulation of *hsa-miR-146a-5p* in sera is not incorporated as an independent predictor of stage, grade, and LN metastasis of BC. Measuring the expression level of *hsa-miR-146a-5p* in sera gave high specificity and sensitivity towards BC. Iacona and Lutz explained the repression in *hsa-miR-146a* is mainly due to hypermethylation of promoter, methylation, and deacetylation of the histone [80]. Recently, repression of *hsa-miR-146a* in TNBC cells was reported, which could drive EGFR abundance and signaling, leading to tumor progression and poor disease outcome [81].

One of the functions of lncRNAs is their counteract with miRNAs, making miRNA sponges [82,83]. Multiple linear regression analyses carried out in the present research display significant correlations of the expressions of *FAM170B-AS1* with both *hsa-miR-1202* and *hsa-miR-146a-5p*. This statistical evidence confirmed the bioinformatic prediction of *FAM170B-AS1* interaction with both miRNAs using LncBase Predicted v2 database. Also, the current study revealed that *FAM170B-AS1* and *hsa-miR-146a-5p*, other than *hsa-miR-1202*, may be considered excellent diagnostic markers because of their higher specificity, sensitivity, and AUC.

Cross-talks between LncRNAs/miRNAs form complex regulatory networks of post-transcriptional gene regulation. Relying on the specific lncRNA/miRNA interaction, the lncRNA/miRNA axis can have tumor suppressor or oncogenic effects [84]. The present study used the bioinformatic analysis tool to construct *FAM170B-AS1*/miRNA interaction network in BC for the first time, followed by identifying their target mRNAs. According to GO analysis, the target genes are involved in complex cellular pathways, such as signal transduction, transcription, and cell proliferation. The KEGG pathway analysis revealed that the PD-L1 expression and PD-1 checkpoint pathway in cancer, PI3K-Akt signaling pathway, Ras signaling pathway, Rap1 signaling pathway, EGFR tyrosine kinase inhibitor resistance, ErbB signaling pathway, Estrogen signaling pathway, and MAPK signaling pathway are potentially correlated with the regulation of the 14 predicted miRNAs. As reported, these pathways are associated with the escape of tumor cells from immune surveillance [85], resistance to endocrine therapy [86], proliferation, apoptosis, differentiation, and survival [86,87,88,89].

The detailed roles for *FAM170B-AS1* in BC need more explanation. The current research work combined *FAM170B-AS1* analysis with TCGA and GEPIA databases to explore the potential biological functions of *FAM170B-AS1* in BC. Data collected from the TCGA database display that having mutations in *FAM170B* showed improved OS of patients with different types of cancer, including breast cancer. The present study also bioinformatically predicted the genes from the KEGG signaling pathways and the hub genes from PPI and hypothesized that *FAM170B-AS1* may affect both *KRAS* expression through interaction with *hsa-miR-143-3p* and *EGFR* expression by interaction with *hsa-miR-7-5p* to contribute to different biological processes of BC.

KRAS is involved in the tumorigenesis and progression of breast cancers *via* activating AKT/MEK/ERK pathways signaling [90]. In addition, overactivation of the KRAS pathway occurs in TNBCs, leading to chemoresistance [91]. Studies targeting the *KRAS* gene instead of the KRAS protein have attracted increasing attention in recent decades. miRNAs targeting the UTR of *KRAS* were reported to reduce the level of KRAS and thus inhibit the progression of TNBCs [92]. It was reported that *hsa-miR-143-3p* is downregulated in TNBC patients, which is associated with EMT, prognosis, and resistance to cancer therapy [93,94]. This therapeutic resistance could be due to cytokine-induced apoptosis inhibitor-1 protein activation, which may repress *hsa-miR-143-3p* expression [95]. Hence, *FAM170B-AS1* may affect *KRAS* expression through *hsa-miR-143-3p* according to BC subtype, which needs further investigation.

The epidermal growth factor receptor (EGFR), a member of the erbB receptor family, controls proliferation, differentiation, and development in several human tissues, including the breast [96]. The upregulation of EGFR is observed in several solid tumors causing cancer progression, poor prognosis, and resistance to chemotherapy & radiation therapy [97]. The expression of EGFR mRNA varies among different BC sub-

types, either by upregulation or downregulation. Consequently, understanding the way by which EGFR is dysregulated attracted great attention [98]. *hsa-miR-7* has a tumor suppressor effect in breast cancers *via* suppressing proliferation and enhancing apoptosis [99,100,101] as well as inhibiting invasion & metastasis and mediating cytotoxic T-lymphocyte-mediated lysis of breast cancer cells [102,103]. It was reported that *hsa-miR-7* suppresses *EGFR* mRNA in lung, breast, and glioblastoma by binding to sites in its 3'-UTR [104]. Thus, *FAM170B-AS1* may affect *EGFR* expression through *hsa-miR-7-5p* according to BC subtype, which needs further investigation.

5. Conclusions and future perspectives

The current study made a spot for the first time on the involvement of *FAM170B-AS1* in BC. The study experimentally validated the bioinformatic prediction of *FAM170B-AS1* association with BC. The associations of *FAM170B-AS1, hsa-miR-1202, and hsa-miR-146a-5p* had been reported in BC, making them probable risk factors for BC. The statistically significant correlations of *FAM170B-AS1* with *hsa-miR-1202* and *hsa-miR-146a-5p* verified the bioinformatic prediction of their interactions. *FAM170B-AS1* and *hsa-miR-1202* might be considered predictors of BC stage, grade, and LN metastasis. In addition, *FAM170B-AS1* and *hsa-miR-146a-5p* might be appraised as excellent diagnostic markers for BC. The current study could bioinformatically predict *FAM170B-AS1/hsa-miR-143-3p/KRAS* and *FAM170B-AS1/hsa-miR-7-5p/EGFR* network in BC. This network needs further practical study in breast cancer cells to be confirmed. The mechanism by which *FAM170B-AS1* is upregulated in breast tumors needs to be elucidated by studying the *FAM170B-AS1* promoter region and histone. The present work opened a new era to design a therapeutic approach against BC by targeting *FAM170B-AS1* that might lead to novel therapeutic strategies. In addition, The link between FAM170B-AS1 and FAM170B in BC needs to be practically explored.

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Declaration

The authors declare that there is no conflict of interest.

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Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Authors contribution

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Supplementary data

The supplementary files are available to download from http://dx.doi.org/10.3233/CBM-230396.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre and A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J Clin* **68** (2018), 394–424.
[2] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J Clin* **71** (2021), 209–249.
[3] C.E. DeSantis, J. Ma, M.M. Gaudet, L.A. Newman, K.D. Miller, A. Goding Sauer, A. Jemal and R.L. Siegel, Breast cancer statistics, 2019, *CA Cancer J Clin* **69** (2019), 438–451.

[4] B. Orr and J.L. Kelley 3rd, Benign breast diseases: Evaluation and management, *Clin Obstet Gynecol* **59** (2016), 710–726.
[5] F.C. Verdial, R. Etzioni, C. Duggan and B.O. Anderson, Demographic changes in breast cancer incidence, stage at diagnosis and age associated with population-based mammographic screening, *J Surg Oncol* **115** (2017), 517–522.
[6] A. Stachs, J. Stubert, T. Reimer and S. Hartmann, Benign breast disease in women, *Dtsch Arztebl Int* **116** (2019), 565–574.
[7] Q. Wang, M. Xu, Y. Sun, J. Chen, C. Chen, C. Qian, Y. Chen, L. Cao, Q. Xu, X. Du and W. Yang, Gene expression profiling for diagnosis of triple-negative breast cancer: A multicenter, retrospective cohort study, *Front Oncol* **9** (2019), 354.
[8] P. Kumar and R. Aggarwal, An overview of triple-negative breast cancer, *Arch Gynecol Obstet* **293** (2016), 247–269.
[9] N.D. Arvold, A.G. Taghian, A. Niemierko, R.F. Abi Raad, M. Sreedhara, P.L. Nguyen, J.R. Bellon, J.S. Wong, B.L. Smith and J.R. Harris, Age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy, *J Clin Oncol Off J Am Soc Clin Oncol* **29** (2011), 3885–3891.
[10] M.J. Engstrøm, S. Opdahl, A.I. Hagen, P.R. Romundstad, L.A. Akslen, O.A. Haugen, L.J. Vatten and A.M. Bofin, Molecular subtypes, histopathological grade and survival in a historic cohort of breast cancer patients, *Breast Cancer Res Treat* **140** (2013), 463–473.
[11] S. Fayaz, G.A. Demian, M. El-Sherify, H. Eissa, M. Aziz and S. Abuzallouf, Triple negative breast cancer: 10-year survival update of the applied treatment strategy in kuwait, *Gulf J Oncolog* **1** (2019), 53–59.
[12] C. Liedtke, A. Rody, O. Gluz, K. Baumann, D. Beyer, E.-B. Kohls, K. Lausen, L. Hanker, U. Holtrich, S. Becker and T. Karn, The prognostic impact of age in different molecular subtypes of breast cancer, *Breast Cancer Res Treat* **152** (2015), 667–673.
[13] E. Senkus, S. Kyriakides, S. Ohno, F. Penault-Llorca, P. Poortmans, E. Rutgers, S. Zackrisson and F. Cardoso, Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, *Ann Oncol Off J Eur Soc Med Oncol* **26 Suppl 5** (2015), v8-30.
[14] M. Hamann, S. Grill, J. Struck, A. Bergmann, O. Hartmann, M. Pölcher and M. Kiechle, Detection of early breast cancer beyond mammographic screening: A promising biomarker panel, *Biomark Med* **13** (2019), 1107–1117.
[15] S. Mayor, Screening for early breast cancer reduces invasive cancer, study finds, *BMJ* **351** (2015), h6576.
[16] K. Zhang, Z. Luo, Y. Zhang, L. Zhang, L. Wu, L. Liu, J. Yang, X. Song and J. Liu, Circulating lncRNA H19 in plasma as a novel biomarker for breast cancer, *Cancer Biomark* **17** (2016), 187–194.
[17] M.J. Delás and G.J. Hannon, lncRNAs in development and disease: From functions to mechanisms, *Open Biol* **7** (2017).
[18] A.M. Schmitt and H.Y. Chang, Long noncoding RNAs in cancer pathways, *Cancer Cell* **29** (2016), 452–463.
[19] Y. Du, Y. Chen, T. Wu, X. Fan, W. Lin and Z. Jiang, miR-2682-3p antagonizes its host lncRNA-MIR137HG by interacting with the same target FUS to regulate the progression of gastric cancer, *BMC Cancer* **22** (2022), 689.
[20] J. Matuszyk, MALAT1-miRNAs network regulate thymidylate synthase and affect 5FU-based chemotherapy, *Mol Med* **28** (2022), 89.
[21] J. Ramírez-Moya, L. Wert-Lamas, A. Acuña-Ruíz, A. Fletcher, C. Wert-Carvajal, C.J. McCabe, P. Santisteban and G. Riesco-Eizaguirre, Identification of an interactome network between lncRNAs and miRNAs in thyroid cancer reveals SPTY2D1-AS1 as a new tumor suppressor, *Sci Rep* **12** (2022), 7706.
[22] D.P. Bartel, MicroRNAs: Target recognition and regulatory functions, *Cell* **136** (2009), 215–233.
[23] J.-H. Yoon, K. Abdelmohsen and M. Gorospe, Functional interactions among microRNAs and long noncoding RNAs, *Semin Cell Dev Biol* **34** (2014), 9–14.
[24] M.-C.D. Wasson, J.M. Brown, J. Venkatesh, W. Fernando and P. Marcato, Datasets exploring putative lncRNA-miRNA-mRNA axes in breast cancer cell lines, *Data Br* **37** (2021), 107241.
[25] Y. Shen, X. Peng and C. Shen, Identification and validation of immune-related lncRNA prognostic signature for breast cancer, *Genomics* **112** (2020), 2640–2646.
[26] Y. Yamada, Y. Yasukochi, K. Kato, M. Oguri, H. Horibe, T. Fujimaki, I. Takeuchi and J. Sakuma, Identification of 26 novel loci that confer susceptibility to early-onset coronary artery disease in a Japanese population, *Biomed Reports* **9** (2018), 383–404.
[27] J. Ke, X. Hu, C. Wang and Y. Zhang, Identification of the hub susceptibility genes and related common transcription factors in the skeletal muscle of type 2 diabetes mellitus, *BMC Endocr Disord* **22** (2022), 276.
[28] Y. Zhou, X. Chen, Y. Zheng, R. Shen, S. Sun, F. Yang, J. Min, L. Bao, Y. Zhang, X. Zhao, J. Wang and Q. Wang, Long noncoding RNAs and mRNAs expression profiles of monocyte-derived dendritic cells from PBMCs in AR, *Front Cell Dev Biol* **9** (2021), 636477.
[29] W. Hu, P. Li, N. Zeng and S. Tan, Exploring the hub mechanisms of ischemic stroke based on protein-protein interaction networks related to ischemic stroke and inflammatory bowel disease, *Sci Rep* **13** (2023), 1741.
[30] T. Falcon, M. Freitas, A.C. Mello, L. Coutinho, M.R. Alvares-da-Silva and U. Matte, Analysis of the cancer genome atlas data reveals novel putative ncRNAs targets in hepatocellular carcinoma, *Biomed Res Int* **2018** (2018), 1–9.
[31] Y. Zhang, T. Jin, H. Shen, J. Yan, M. Guan and X. Jin, Identification of Long Non-Coding RNA Expression Profiles and Co-Expression Genes in Thyroid Carcinoma Based on The Cancer Genome Atlas (TCGA) Database, *Med Sci Monit* **25** (2019), 9752–9769.
[32] S. Ramalingam, Cancer Genes, Bentham Science Publishers, 2023.
[33] X. Liu, D. Zhang, J. Hu, S. Xu, C. Xu and Y. Shen, Allograft inflammatory factor 1 is a potential diagnostic, immunological, and prognostic biomarker in pan-cancer, *Aging (Albany NY)* **15** (2023), 2582–2609.
[34] R. Hamam, A.M. Ali, K.A. Alsaleh, M. Kassem, M. Alfayez, A. Aldahmash and N.M. Alajez, microRNA expression profiling on individual breast cancer patients identifies novel panel of circulating microRNA for early detection, *Sci Rep* **6** (2016).
[35] H. Chen, Y. Fan, W. Xu, J. Chen, Y. Meng, D. Fang and J. Wang, Exploration of miR-1202 and miR-196a in human endometrial cancer based on high throughout gene screening analysis, *Oncol Rep* **37** (2017), 3493–3501.
[36] A. Yan, X. Song, B. Liu and K. Zhu, IGF2BP3 Worsens Lung Cancer through Modifying Long Non-coding RNA CERS6-AS1/microRNA-1202 Axis, *Curr Med Chem* **30** (2023), 878–891.
[37] Y. Quan, Q. Song, J. Wang, L. Zhao, J. Lv and S. Gong, MiR-1202 functions as a tumor suppressor in glioma cells by targeting Rab1A, *Tumour Biol J Int Soc Oncodevelopmental Biol Med* **39** (2017), 1010428317697565.

[38] B. Du, P. Zhang, Z. Tan and J. Xu, MiR-1202 suppresses hepatocellular carcinoma cells migration and invasion by targeting cyclin dependent kinase 14, *Biomed Pharmacother* **96** (2017), 1246–1252.

[39] C. Wu, Y. Cao, Z. He, J. He, C. Hu, H. Duan and J. Jiang, Serum levels of miR-19b and miR-146a as prognostic biomarkers for non-small cell lung cancer, *Tohoku J Exp Med* **232** (2014), 85–95.

[40] R.-J. Wang, Y.-H. Zheng, P. Wang and J.-Z. Zhang, Serum miR-125a-5p, miR-145 and miR-146a as diagnostic biomarkers in non-small cell lung cancer, *Int J Clin Exp Pathol* **8** (2015), 765–771.

[41] X. Liu, B. Liu, R. Li, F. Wang, N. Wang, M. Zhang, Y. Bai, J. Wu, L. Liu, D. Han, Z. Li, B. Feng, G. Zhou, S. Wang, L. Zeng, J. Miao, Y. Yao, B. Liang, L. Huang, Q. Wang and Y. Wu, miR-146a-5p Plays an Oncogenic Role in NSCLC via Suppression of TRAF6, *Front Cell Dev Biol* **8** (2020), 847.

[42] H. Liang, W. Huang, Y. Wang, L. Ding and L. Zeng, Overexpression of MiR-146a-5p Upregulates lncRNA HOTAIR in Triple-Negative Breast Cancer Cells and Predicts Poor Prognosis, *Technol Cancer Res Treat* **18** (2019), 1533033819882949.

[43] H. Tashkandi, N. Shah, Y. Patel and H. Chen, Identification of new miRNA biomarkers associated with HER2-positive breast cancers, *Oncoscience* **2** (2015), 924–929.

[44] R. Elango, K.A. Alsaleh, R. Vishnubalaji, M. Manikandan, A.M. Ali, N. Abd El-Aziz, A. Altheyab, A. Al-Rikabi, M. Alfayez, A. Aldahmash and N.M. Alajez, MicroRNA Expression Profiling on Paired Primary and Lymph Node Metastatic Breast Cancer Revealed Distinct microRNA Profile Associated With LNM, *Front Oncol* **10** (2020), 756.

[45] P. Cabello, S. Torres-Ruiz, A. Adam-Artigues, J. Forés-Martos, M.T. Martínez, C. Hernando, S. Zazo, J. Madoz-Gúrpide, A. Rovira, O. Burgués, F. Rojo, J. Albanell, A. Lluch, B. Bermejo, J.M. Cejalvo and P. Eroles, miR-146a-5p Promotes Angiogenesis and Confers Trastuzumab Resistance in HER2+ Breast Cancer, *Cancers (Basel)* **15** (2023).

[46] D. Escuin, L. López-Vilaró, J. Mora, O. Bell, A. Moral, I. Pérez, C. Arqueros, B. García-Valdecasas, T. Ramón, Y. Cajal, E. Lerma and A. Barnadas, Circulating microRNAs in early breast cancer patients and its association with lymph node metastases, *Front Oncol* **11** (2021), 627811.

[47] J.P. Long, L.F. Dong, F.F. Chen and Y.F. Fan, Mir-146a-5p targets interleukin-1 receptor-associated kinase 1 to inhibit the growth, migration, and invasion of breast cancer cells, *Oncol Lett* **17** (2019), 1573–1580.

[48] D.H. Park, H.S. Jeon, S.Y. Lee, Y.Y. Choi, H.W. Lee, S. Yoon, J.C. Lee, Y.S. Yoon, D.S. Kim, M.J. Na, S.J. Kwon, D.S. Kim, J. Kang, J.Y. Park and J.W. Son, MicroRNA-146a inhibits epithelial mesenchymal transition in non-small cell lung cancer by targeting insulin receptor substrate 2, *Int J Oncol* **47** (2015), 1545–1553.

[49] E. Kumaraswamy, K.L. Wendt, L.A. Augustine, S.R. Stecklein, E.C. Sibala, D. Li, S. Gunewardena and R.A. Jensen, BRCA1 regulation of epidermal growth factor receptor (EGFR) expression in human breast cancer cells involves microRNA-146a and is critical for its tumor suppressor function, *Oncogene* **34** (2015), 4333–4346.

[50] S. Khawaled, G. Nigita, R. Distefano, S. Oster, S.-S. Suh, Y. Smith, A. Khalaileh, Y. Peng, C.M. Croce, T. Geiger, V.L. Seewaldt and R.I. Aqeilan, Pleiotropic tumor suppressor functions of WWOX antagonize metastasis, *Signal Transduct Target Ther* **5** (2020), 43.

[51] P. Jiang, B. Liang, Z. Zhang, B. Fan, L. Zeng, Z. Zhou, Z. Mao, Q. Lin, W. Yao and Q. Shen, MicroRNA-146a-5p induces cell cycle arrest and enhances apoptosis in gastric cancer via targeting CDC14A, *Front Cell Dev Biol* **11** (2023), 1181628.

[52] K.J. Livak and T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, *Methods* **25** (2001), 402–408.

[53] J.D. Cohen, L. Li, Y. Wang, C. Thoburn, B. Afsari, L. Danilova, C. Douville, A.A. Javed, F. Wong, A. Mattox, R.H. Hruban, C.L. Wolfgang, M.G. Goggins, M. Dal Molin, T.-L. Wang, R. Roden, A.P. Klein, J. Ptak, L. Dobbyn, J. Schaefer, N. Silliman, M. Popoli, J.T. Vogelstein, J.D. Browne, R.E. Schoen, R.E. Brand, J. Tie, P. Gibbs, H.-L. Wong, A.S. Mansfield, J. Jen, S.M. Hanash, M. Falconi, P.J. Allen, S. Zhou, C. Bettegowda, L.A.J. Diaz, C. Tomasetti, K.W. Kinzler, B. Vogelstein, A.M. Lennon and N. Papadopoulos, Detection and localization of surgically resectable cancers with a multi-analyte blood test, *Science* **359** (2018), 926–930.

[54] J. Shen, N.W. Todd, H. Zhang, L. Yu, X. Lingxiao, Y. Mei, M. Guarnera, J. Liao, A. Chou, C.L. Lu, Z. Jiang, H. Fang, R.L. Katz and F. Jiang, Plasma microRNAs as potential biomarkers for non-small-cell lung cancer, *Lab Invest* **91** (2011), 579–587.

[55] M.G. Schrauder, R. Strick, R. Schulz-Wendtland, P.L. Strissel, L. Kahmann, C.R. Loehberg, M.P. Lux, S.M. Jud, A. Hartmann, A. Hein, C.M. Bayer, M.R. Bani, S. Richter, B.R. Adamietz, E. Wenkel, C. Rauh, M.W. Beckmann and P.A. Fasching, Circulating micro-RNAs as potential blood-based markers for early stage breast cancer detection, *PLoS One* **7** (2012), e29770.

[56] D.B. Martin, P.S. Nelson, B.S. Knudsen, R.K. Parkin, J. Noteboom, E.M. Kroh, K.C. O'Briant, C.W. Drescher, R.L. Vessella, R. Gentleman, B.R. Fritz, N. Urban, A. Peterson, M. Tewari, E.L. Pogosova-Agadjanyan, S.K. Wyman, A. Allen, D.L. Stirewalt, P.S. Mitchell and D.W. Lin, Circulating microRNAs as stable blood-based markers for cancer detection, *Proc Natl Acad Sci* **105** (2008), 10513–10518.

[57] R. Gao, R. Zhang, C. Zhang, L. Zhao and Y. Zhang, Long noncoding RNA CCAT1 promotes cell proliferation and metastasis in human medulloblastoma via MAPK pathway, *Tumori* **104** (2018), 43–50.

[58] L. Jin, H. Fu, J. Quan, X. Pan, T. He, J. Hu, Y. Li, H. Li, Y. Yang, J. Ye, F. Zhang, L. Ni, S. Yang and Y. Lai, Overexpression of long non-coding RNA differentiation antagonizing non-protein coding RNA inhibits the proliferation, migration and invasion and promotes apoptosis of renal cell carcinoma, *Mol Med Rep* **16** (2017), 4463–4468.

[59] Z. Zhang, T. Liu, K. Wang, X. Qu, Z. Pang, S. Liu, Q. Liu and J. Du, Down-regulation of long non-coding RNA MEG3 indicates an unfavorable prognosis in non-small cell lung cancer: Evidence from the GEO database, *Gene* **630** (2017), 49–58.

[60] G. Arun, S.D. Diermeier and D.L. Spector, Therapeutic Targeting of Long Non-Coding RNAs in Cancer, *Trends Mol Med* **24** (2018), 257–277.

[61] D. Barsyte-Lovejoy, S.K. Lau, P.C. Boutros, F. Khosravi, I. Jurisica, I.L. Andrulis, M.S. Tsao and L.Z. Penn, The c-Myc oncogene directly induces the H19 noncoding RNA by allele-specific binding to potentiate tumorigenesis, *Cancer Res* **66** (2006), 5330–5337.

[62] M. Huarte, M. Guttman, D. Feldser, M. Garber, M.J. Koziol, D. Kenzelmann-Broz, A.M. Khalil, O. Zuk, I. Amit, M. Rabani, L.D. Attardi, A. Regev, E.S. Lander, T. Jacks and J.L. Rinn, A large intergenic noncoding RNA induced by p53

mediates global gene repression in the p53 response, *Cell* **142** (2010), 409–419.
[63] A. Rahimi, R. Sedighi, M. Emadi-Baygi, M.-A. Honardoost, S.-J. Mowla, H. Khanahmad and P. Nikpour, Bioinformatics prediction and experimental validation of a novel microRNA: hsa-miR-B43 within human CDH4 gene with a potential metastasis-related function in breast cancer, *J Cell Biochem* **121** (2020), 1307–1316.
[64] M.J. Duffy, Serum tumor markers in breast cancer: Are they of clinical value, *Clin Chem* **52** (2006), 345–351.
[65] A. Perrier, P.-Y. Boelle, Y. Chrétien, J. Gligorov, J.-P. Lotz, D. Brault, E. Comperat, G. Lefèvre and M. Boissan, An updated evaluation of serum sHER2, CA15.3, and CEA levels as biomarkers for the response of patients with metastatic breast cancer to trastuzumab-based therapies, *PLoS One* **15** (2020), e0227356.
[66] J. Mi, H. Zhang, W. Cao and C. Yuan, FTO, PIK3CB serve as potential markers to complement CEA and CA15-3 for the diagnosis of breast cancer, *Medicine (Baltimore)* **102** (2023), e35361.
[67] K.N. Seale and K.H.R. Tkaczuk, Circulating biomarkers in breast cancer, *Clin Breast Cancer* **22** (2022), e319–e331.
[68] P. Gaglia, B. Caldarola, R. Bussone, F. Potente, D. Lauro, A. Jayme and L. Caldarola, Prognostic value of CEA and ferritin assay in breast cancer: A multivariate analysis, *Eur J Cancer Clin Oncol* **24** (1988), 1151–1155.
[69] R. Molina, J. Jo, X. Filella, G. Zanon, J. Pahisa, M. Mu noz, B. Farrus, M.L. Latre, C. Escriche, J. Estape and A.M. Ballesta, c-erbB-2 oncoprotein, CEA, and CA 15.3 in patients with breast cancer: Prognostic value, *Breast Cancer Res Treat* **51** (1998), 109–119.
[70] S. Wu, Z. He, J. Zhou, J. Sun, F. Li, Q. Lin, L. Guo and H. Lin, Serum levels of CEA and CA15-3 in different molecular subtypes and prognostic value in Chinese breast cancer, *Breast* **23** (2014), 88–93.
[71] Y. Shao, X. Sun, Y. He, C. Liu and H. Liu, Elevated Levels of Serum Tumor Markers CEA and CA15-3 Are Prognostic Parameters for Different Molecular Subtypes of Breast Cancer, *PLoS One* **10** (2015), e0133830.
[72] Y. Fu and H. Li, Assessing Clinical Significance of Serum CA15-3 and Carcinoembryonic Antigen (CEA) Levels in Breast Cancer Patients: A Meta-Analysis, *Med Sci Monit Int Med J Exp Clin Res* **22** (2016), 3154–3162.
[73] D. Di Gioia, M. Dresse, D. Mayr, D. Nagel, V. Heinemann and P. Stieber, Serum HER2 in combination with CA 15-3 as a parameter for prognosis in patients with early breast cancer, *Clin Chim Acta* **440** (2015), 16–22.
[74] M. Long, M. Zhan, S. Xu, R. Yang, W. Chen, S. Zhang, Y. Shi, Q. He, M. Mohan, Q. Liu and J. Wang, miR-92b-3p acts as a tumor suppressor by targeting Gabra3 in pancreatic cancer, *Mol Cancer* **16** (2017), 167.
[75] Z. Wang, H. Zhang, P. Zhang, J. Li, Z. Shan and W. Teng, Upregulation of miR-2861 and miR-451 expression in papillary thyroid carcinoma with lymph node metastasis, *Med Oncol* **30** (2013).
[76] M. Allaire and J.-C. Nault, Advances in management of hepatocellular carcinoma, *Curr Opin Oncol* **29** (2017), 288–295.
[77] A.I. Garcia, M. Buisson, P. Bertrand, R. Rimokh, E. Rouleau, B.S. Lopez, R. Lidereau, I. Mikaélian and S. Mazoyer, Down-regulation of BRCA1 expression by miR-146a and miR-146b-5p in triple negative sporadic breast cancers, *EMBO Mol Med* **3** (2011), 279–290.
[78] S.-O. Lim, C.-W. Li, W. Xia, H.-H. Lee, S.-S. Chang, J. Shen, J.L. Hsu, D. Raftery, D. Djukovic, H. Gu, W.-C. Chang, H.-L. Wang, M.-L. Chen, L. Huo, C.-H. Chen, Y. Wu, A. Sahin, S.M. Hanash, G.N. Hortobagyi and M.-C. Hung, EGFR signaling enhances aerobic glycolysis in triple-negative breast cancer cells to promote tumor growth and immune escape, *Cancer Res* **76** (2016), 1284–1296.
[79] H. Zhang, Y. Zhang, W. Yan, W. Wang, X. Zhao, X. Ma, X. Gao and S. Zhang, Association between three functional microRNA polymorphisms (miR-499 rs3746444, miR-196a rs11614913 and miR-146a rs2910164) and breast cancer risk: a meta-analysis, *Oncotarget* **8** (2017), 393–407.
[80] J.R. Iacona and C.S. Lutz, miR-146a-5p: Expression, regulation, and functions in cancer, *Wiley Interdiscip Rev RNA* **10** (2019), e1533.
[81] K. Panoutsopoulou, Y. Liu, M. Avgeris, T. Dreyer, J. Dorn, V. Magdolen and A. Scorilas, Repression of miR-146a in predicting poor treatment outcome in triple-negative breast cancer, *Clin Biochem* **114** (2023), 43–51.
[82] H. Ling, M. Fabbri and G.A. Calin, MicroRNAs and other non-coding RNAs as targets for anticancer drug development, *Nat Rev Drug Discov* **12** (2013), 847–865.
[83] K. V Morris and J.S. Mattick, The rise of regulatory RNA, *Nat Rev Genet* **15** (2014), 423–437.
[84] J. Venkatesh, M.-C.D. Wasson, J.M. Brown, W. Fernando and P. Marcato, LncRNA-miRNA axes in breast cancer: Novel points of interaction for strategic attack, *Cancer Lett* **509** (2021), 81–88.
[85] R.K. Beckers, C.I. Selinger, R. Vilain, J. Madore, J.S. Wilmott, K. Harvey, A. Holliday, C.L. Cooper, E. Robbins, D. Gillett, C.W. Kennedy, L. Gluch, H. Carmalt, C. Mak, S. Warrier, H.E. Gee, C. Chan, A. McLean, E. Walker, C.M. McNeil, J.M. Beith, A. Swarbrick, R.A. Scolyer and S.A. O'Toole, Programmed death ligand 1 expression in triple-negative breast cancer is associated with tumour-infiltrating lymphocytes and improved outcome, *Histopathology* **69** (2016), 25–34.
[86] E. Paplomata and R. O'Regan, New and emerging treatments for estrogen receptor-positive breast cancer: Focus on everolimus, *Ther Clin Risk Manag* **9** (2013), 27–36.
[87] N. Normanno, C. Bianco, A. De Luca, M.R. Maiello and D.S. Salomon, Target-based agents against ErbB receptors and their ligands: A novel approach to cancer treatment, *Endocr Relat Cancer* **10** (2003), 1–21.
[88] L. Lu, J. Wang, Y. Wu, P. Wan and G. Yang, Rap1A promotes ovarian cancer metastasis via activation of ERK/p38 and notch signaling, *Cancer Med* **5** (2016), 3544–3554.
[89] A.S. Dhillon, S. Hagan, O. Rath and W. Kolch, MAP kinase signalling pathways in cancer, *Oncogene* **26** (2007), 3279–3290.
[90] M.S. Kumar and C. Swanton, KRAS 3'-UTR variants and stratification of breast-cancer risk, *Lancet Oncol* **12** (2011), 318–319.
[91] S. Loibl, D. Treue, J. Budczies, K. Weber, A. Stenzinger, W.D. Schmitt, W. Weichert, P. Jank, J. Furlanetto, F. Klauschen, T. Karn, N. Pfarr, G. von Minckwitz, M. Möbs, C. Jackisch, C. Sers, A. Schneeweiss, P.A. Fasching, C. Schem, M. Hummel, M. van Mackelenbergh, V. Nekljudova, M. Untch and C. Denkert, Mutational diversity and therapy response in breast cancer: A Sequencing Analysis in the Neoadjuvant GeparSepto Trial, *Clin Cancer Res an Off J Am Assoc Cancer Res* **25** (2019), 3986–3995.
[92] H.A. Mokhlis, R. Bayraktar, N.N. Kabil, A. Caner, N. Kahraman, C. Rodriguez-Aguayo, E.P. Zambalde, J. Sheng, K. Karagoz, P. Kanlikilicer, A.A.H. Abdel Aziz, T.M. Abdelghany, A.A. Ashour, S. Wong, M.L. Gatza, G.A. Calin, G.

Lopez-Berestein and B. Ozpolat, The Modulatory Role of MicroRNA-873 in the Progression of KRAS-Driven Cancers, *Mol Ther Nucleic Acids* **14** (2019), 301–317.

[93] M. Koleckova, J. Ehrmann, J. Bouchal, M. Janikova, A. Brisudova, J. Srovnal, K. Staffova, M. Svoboda, O. Slaby, L. Radova, K. Vomackova, B. Melichar, L. Veverkova and Z. Kolar, Epithelial to mesenchymal transition and microRNA expression are associated with spindle and apocrine cell morphology in triple-negative breast cancer, *Sci Rep* **11** (2021), 5145.

[94] J. Feng, L. Wang, K. Zhang, S. Ni, B. Li, J. Liu and D. Wang, Identification and panoramic analysis of drug response-related genes in triple negative breast cancer using as an example NVP-BEZ235, *Sci Rep* **13** (2023), 5984.

[95] Y.W. Deng, W.J. Hao, Y.W. Li, Y.X. Li, B.C. Zhao and D. Lu, Hsa-miRNA-143-3p reverses multidrug resistance of triple-negative breast cancer by inhibiting the expression of its target protein cytokine-induced apoptosis inhibitor 1 *in vivo*, *J Breast Cancer* **21** (2018), 251–258.

[96] S. Yano, K. Kondo, M. Yamaguchi, G. Richmond, M. Hutchison, A. Wakeling, S. Averbuch and P. Wadsworth, Distribution and function of EGFR in human tissue and the effect of EGFR tyrosine kinase inhibition, *Anticancer Res* **23** (2003), 3639–3650.

[97] C.L. Arteaga, The epidermal growth factor receptor: From mutant oncogene in nonhuman cancers to therapeutic target in human neoplasia, *J Clin Oncol Off J Am Soc Clin Oncol* **19** (2001), 32S–40S.

[98] R. Bianco, T. Troiani, G. Tortora and F. Ciardiello, Intrinsic and acquired resistance to EGFR inhibitors in human cancer therapy, *Endocr Relat Cancer* **12 Suppl 1** (2005), S159-71.

[99] Y. Shi, X. Luo, P. Li, J. Tan, X. Wang, T. Xiang and G. Ren, miR-7-5p suppresses cell proliferation and induces apoptosis of breast cancer cells mainly by targeting REGγ, *Cancer Lett* **358** (2015), 27–36.

[100] J.L. Horsham, F.C. Kalinowski, M.R. Epis, C. Ganda, R.A.M. Brown and P.J. Leedman, Clinical Potential of microRNA-7 in Cancer, *J Clin Med* **4** (2015), 1668–1687.

[101] T.B. Hansen, J. Kjems and C.K. Damgaard, Circular RNA and miR-7 in cancer, *Cancer Res* **73** (2013), 5609–5612.

[102] E. van Schooneveld, H. Wildiers, I. Vergote, P.B. Vermeulen, L.Y. Dirix and S.J. Van Laere, Dysregulation of microRNAs in breast cancer and their potential role as prognostic and predictive biomarkers in patient management, *Breast Cancer Res* **17** (2015), 21.

[103] H. Zhang, K. Cai, J. Wang, X. Wang, K. Cheng, F. Shi, L. Jiang, Y. Zhang and J. Dou, MiR-7, inhibited indirectly by lincRNA HOTAIR, directly inhibits SETDB1 and reverses the EMT of breast cancer stem cells by downregulating the STAT3 pathway, *Stem Cells* **32** (2014), 2858–2868.

[104] R.J. Webster, K.M. Giles, K.J. Price, P.M. Zhang, J.S. Mattick and P.J. Leedman, Regulation of epidermal growth factor receptor signaling in human cancer cells by microRNA-7, *J Biol Chem* **284** (2009), 5731–5741.