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Studying the Expression of miR-1, EVI1 and Calreticulin in Invasive Breast Carcinoma: Relationship with Clinicopathological Parameters

Hend E. Nasr [a], Omneya Y. Bassyoni [b], Omnia Y. Habashy [a], Lina A . Mohammed[a]

Abstract:

Background: Breast cancer is the most prevalent cause of cancer-related mortality among women, with significant rates of morbidity and mortality. Although breast cancer is conventionally treated using various methods including surgery, chemotherapy and endocrine therapy, recurrence and metastasis still remain a serious issue in advanced stage patients. So, new therapeutic strategies are awfully needed. **Objectives:** In this research we tended to determine the expression of miR-1, EVI1, and calreticulin- in breast cancer and to associate their expression with clinicopathological parameters. **Material & Methods:** This retrospective study focused on EVI1 and calreticulin was conducted using paraffin-embedded blocks from 100 cases of breast cancer, and miR-1 gene expression analysis was performed. **Results:** miR-1 was downregulated in cancer patients in contrast to controls. Otherwise EVI1 & calreticulin were over-expressed. miR-1 was inversely correlated with T stage (p=0.01), lymph node metastasis (p=0.003), TNM stage (p=0.01) and ki67 (p=0.01). miR-1 was higher in the hormone-positive group. EVI1 and calreticulin were directly correlated with T stage (p=0.01 & p <0.001), lymph node metastasis (p=0.000 & p=0.01), stage (p=0.000) ki67(p=0.003), and triple-negative breast cancer (p <0.01). miR-1 was negatively correlated with both EVI1 and calreticulin (p=0.02, p= 0.000). **Conclusion:** miR-1, EVI1, and calreticulin might make a substantial contribution to the onset and spread of breast cancer. Therefore, upregulating miR-1 and blocking EVI1 could be possible targets for therapy in breast cancer treatment. Additionally, there might be a link between miR-1, EVI1, and calreticulin and E-cadherin in induction of epithelial–mesenchymal transition.

Key words: Breast Cancer; miR-1; EVI1; Calreticulin.

[a] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine Benha University, Egypt.

[b] Pathology Department, Faculty of Medicine Benha University, Egypt.

Corresponding to:
Dr. Omneya Y. Bassyoni.
Pathology Department, Faculty of Medicine Benha University, Egypt.
Email: omneyayoussef2015@gmail.com

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Introduction

Breast cancer is the most prevalent cause of cancer-related mortality among women, with significant rates of morbidity and mortality [1]. It is the most common kind of cancer in women in Egypt, representing 32.4% of all cancers [2]. Conventional therapies like endocrine treatment, chemotherapy, and surgery- are employed to combat breast cancer. However, recurrence and metastasis continue to pose significant challenges for patients in advanced stages [3]. Consequently, there is a pressing need for innovative therapeutic approaches.

MicroRNAs, or miRNAs, consist of non-coding RNA sequences that typically measure between 19 and 25 nucleotides. They play crucial roles in controlling a variety of both normal and aberrant biological processes comprising cell division, differentiation, programmed cell death, and oncogenesis [4]. miR-1- a muscle-specific miRNA- is predominantly expressed in cardiac tissue [5]. It targets multiple proteins, such as actine related protein-3 (ACTR3), TPM3, and Calreticulin- which are significant in muscle-related functions and likely play crucial roles in vivo [6]. Beyond its association with cardiac diseases, miR-1 has emerged as a pivotal player in the beginning, growth, and spread of cancers in humans, acting as a tumor suppressor whose expression is downregulated in various cancers [7,8]. Nonetheless, its specific role in breast cancer remains underexplored.

Ecotropic viral integration site 1 (EVI1)- a nuclear transcription factor- is crucial in oncogenesis and is recognized as being activated and overexpressed in hematological malignancies, particularly acute myeloid leukemia [9]. EVI1 significantly influences several cellular processes during carcinogenesis, comprising; cell proliferation, transformation, differentiation, and survival, particularly in leukemic cells [10]. Its expression is also notable in many solid tumors, comprising prostate, breast, and hepatocellular carcinomas [11-13].

The interplay between EVI1 and miRNAs is increasingly recognized. Prior research has shown that miRNA-206/133b may promote cancer growth by augmenting EVI1 expression [14]. However, the mechanisms by which EVI1 and miR-1 regulate breast cancer oncogenesis are not fully comprehended. Venkov et al. [15] identified EVI1 as a master regulatory element in epithelial mesenchymal transition (EMT).

Calreticulin (CRT)- which miR-1 targets in vivo- is a versatile protein that binds calcium and is mainly present in the endoplasmic reticulum [16]. It significantly affects cell adhesion, metastasis, and tumor cell proliferation and is connected to resistance to apoptosis [17]. Increased levels of CRT expression have been noted in various malignant tumors, such as breast cancer, and have a worse prognosis linked to them [18]. Additionally, heightened CRT expression has been shown to diminish mRNA levels of EMT marker E-cadherin in gastric cancer cells, exhibiting a direct relationship between CRT and EMT processes [19].

Previous study [20] documented a pronounced association between the expression profiles of EVI1 and the epithelial-mesenchymal transition markers E-cadherin and N-cadherin in stage IV colon cancer. The potential influence of EVI1 on epithelial-mesenchymal transition mechanism in breast cancer via calreticulin remains an open question.

This research tended to determine the connections among the expressions of miR-1, EVI1, and Calreticulin, and their associations with different clinicopathological features in patients with breast cancer. The primary objective is to clarify their contributions to the advancement and prognostic outcomes of the disease, with the possibility of recognizing these markers as new targets for therapy.

Material and Methods

Study Groups:

This retrospective research analyzed from 100 cases of invasive breast carcinoma to evaluate miR-1 gene expression and immunohistochemical expression of EVI1 and Calreticulin. It was conducted using formalin-fixed, paraffin-embedded blocks processed from the same specimens. The procurement of specimens was executed through the collaborative efforts of the Pathology Department and the Early Cancer Detection Unit (ECDU) within the Faculty of Medicine at Benha University, encompassing the interval from July 2018 to November 2023. The Faculty of Medicine's Research Ethics Committee of Benha University in Egypt gave its approval to this work (NO: RC. 14-11-2023). *Each and every individual participant in the study gave their informed consent.*

The study subjects included selected 100 cases of breast carcinoma, all of which had previously been diagnosed by core biopsy. These patients underwent surgical resection, including lumpectomy with axillary clearance (45 cases) and modified radical mastectomy (55 cases). Additionally, 30 cases of normal breast tissue, obtained from reduction mammaplasty, served as controls.

Cases of breast carcinoma with a history of chemotherapy were excluded from the study.

Histopathological Analysis:

Formalin-fixed, paraffin-embedded tissue blocks were sectioned into 5 μm slices, followed by staining with hematoxylin and eosin. Subsequently, microscopic slides derived from all cases underwent independent evaluation by two pathologists.

Classification and Staging:

The breast carcinoma cases were graded into grades 1, 2, and 3 following previous study [21]. Patient staging was conducted concerning the 8th edition (2017) of AJCC Cancer Staging Manual[22] , categorizing individuals into early (I–II) and advanced (III–IV) stages for the purposes of statistical evaluation. The cases were categorized concerning the expression of hormonal receptors (ER, PR) and HER2/neu into four groups[23]:

Luminal A (ER positive, PR positive, HER2 negative, Ki67 proliferative index low)

Luminal B

A) HER2 negative (ER positive, PR negative / low, HER2 negative, Ki67 proliferative index high)
B) HER2 positive (ER positive, PR positive or negative, HER2 positive, Ki67 proliferative index varies)

Triple negative subtype (TNBC) (ER negative, PR negative, HER2 negative)

HER2 enriched (HER2 positive) ER negative, PR negative, HER2 / neu amplified or overexpressed.

Ki67 levels were classified into low (<14%) and high (>14%) following previous study [24].

Quantitative Real-Time PCR Analysis of miRNA-1 Gene Expression:

Breast tissue was promptly removed post-excision, rinsed in ice-cold saline, and stored in RNA protect Tissue Reagent (Cat. No./ID: 76104 Qiagen, Germany) at 10 μL per 1 mg of tissue, then kept at -80°C until RNA extraction.

For each stored biopsy, 25 mg of tissue was chopped, weighed, and homogenized utilizing a Mixer Mill MM400 (Retsch, Germany).

The TRIzol™ Plus RNA Purification Kit was utilized to extract total RNA from samples of breast tissue (Catalog No: 12183555; Invitrogen, Purification kit (cat. no. 12183555; Invitrogen; Thermo Fisher Scientific, Inc.) in compliance with manufacturer's instructions.

A Nano Drop One spectrophotometer was utilized to determine the absorbance at 260 and 280 nm to ascertain the RNA's content and purity. (Thermo Fisher Scientific, USA). Pure RNA has an A260/A280 ratio of 1.8–2.2 [25].

Human hsa-mir-1 Real-time RT-PCR Detection and U6 Calibration kit (cat. no. MBS825234; MyBioSource, Inc.) was

employed to detect and measure mir-1. Using the Step One Plus Real-Time PCR System, RT was carried out in accordance with the Standard RT Reaction Program (30 min at 25˚C, 30 min at 42˚C, and 5 min at 85˚C). This was then a PCR reaction (95˚C for 3 min hold, 40 cycles of 95˚C, 12 sec; 62˚C, 40 sec) (Thermo Fisher Scientific, Inc.).

Livak and Schmittgen's $2^{-\Delta\Delta Ct}$ method was utilized to determine the relative expression [26]. Fold-change in relation to the control is how the results are presented.

Immunohistochemical Procedure:

For immunohistochemical analysis, the streptavidin-biotin method was utilized concerning instructions supplied by manufacturer (Neomarker, LABVISION, USA, CA 94538-7310). Data concerning primary antibodies, **antigen retrieval** and positive controls were shown in **Table (1)** Tissue sections were stained for EVI1 and Calreticulin using a 0.02% diaminobenzidine (DAB) solution as the chromogen. After staining, sections were dehydrated, mounted, and counterstained with hematoxylin. Negative controls were executed by omitting the primary antibody.

To evaluate EVI1 and CRT staining, light yellow to brown nuclear expression of EVI1 and brown cytoplasmic staining of CRT- were considered positive indicators. A semi-quantitative scoring system was utilized to ascertain the overall staining score, considering both percentage of cells that are positively stained and the tissue staining intensity, as delineated by the methodology outlined by previous study [27].

Statistical methods:

Utilizing Statistical Package for the Social Sciences (SPSS) version 25.0 for Windows, the gathered data was methodically recorded, displayed, and statistically examined (SPSS Inc., Chicago, IL, USA). Analysis was conducted as follows: The gathered data were synthesized into numerical counts and percentages for qualitative variables. Comparative analyses between the various study groups were executed employing the Chi-square test. Utilizing Shapiro-Wilks test, which assumes normality at P>0.05, the normality of distribution for quantitative data was examined. Median and Inter Quartile Range (IQR), as suitable for nonparametric data, were utilized to summarize the gathered information. Mann-Whitney test- which is suitable for nonparametric data- was utilized to determine the statistical significance of variation between the two groups. Kruskall Wallis test and Mann-Whitney test- which are suitable for nonparametric data- were utilized to ascertain statistical significance of the variation between more than two groups. All tests were two-sided. The accepted level of significance in this research was created at ($p < 0.05$).

Results

Clinicopathological features of the research cases

This study encompassed 100 cases of invasive breast carcinoma. Patients were between ages of 30 and 80, with a mean age of 41 ± 11.7 years. The clinicopathological variables are detailed in **Table (2).**

Figure (1) shows the MiR-1 expression fold change.

Immunohistochemical expression of miR-1, EVI1 and Calreticulin in breast cancer cases and control group:

miR-1 was downregulated in breast cancer cases in relation to control with statistically highly significant correlation (p= 0.000). In contrast, EVI1 and calreticulin were overexpressed in cancer in comparison to control with a significant connection (p=0.01 & p=0.03, respectively) as stated in **Table (2).**

Correlation of miR-1, EVI1, and Calreticulin with clinicopathological features in the studied breast cancer cases

miR-1 was negatively expressed among patients with higher grade (p =0.003.), higher T stage (p=0.01), positive lymph

node metastasis (p=0.003), advanced TNM stage (*p*=0.01) and higher ki67 (*p*=0.01) with statistically significant correlation. miR-1 was inversely correlated with molecular subtypes with positive expression in hormonal positive group (luminal A & luminal B) and negatively expressed in Tripple negative breast cancers (*p*=0.001). No statistically significant correlations between miR-1 and histopathological type, illustrated in **Table (3).**

As shown in **Table (3),** overexpression of EVI1 in the nuclei of tumor cells as shown in **Figure 2 (a & b)**- was closely related to higher T stage (*p*=0.01), advanced stage (*p*=0.000), and positive lymph node metastasis (*p*=0.000.), higher ki67(*p*=0.003)- with a statistically significant positive correlation.

Calreticulin was detected by brown cytoplasmic staining of the tumor cells as shown in **Figure 2 (c & d)**. Tumors with higher T stage (p <0.001), advanced stage (*p*=0.000), positive lymph deposits (*p*=0.01) & higher ki76 proliferative index (*p*=0.003) had a relatively higher expression of CRT with significant positive correlations, as shown in **Table (3).**

Both EVI1, Calreticulin were positively correlated with molecular subtypes as they both showed lower expression in hormonal positive group (luminal A & luminal B) and higher expression in Tripple negative breast cancers with a highly significant positive correlation **(***p* <.001).

Correlation between miR1, EVI1 and Calreticulin in the studied breast cancer cases

A statistically significant negative correlation was existed between miR-1 and both EVI1and Calreticulin (*p*=0.02, *p*= 0.000, respectively). While EVI has a direct positive correlation with calreticulin in the studied cases of invasive breast carcinomas (*p*=0.000) as shown in **Table (4).**

Table 1: Data for using of EVI1, Calrecticulin antibodies.

Antibody	Type	Cat.No	Dilution	Positive control	incubation	Antigen retrieval
EVI1	Rabbit Polyclonal	Thermo Fisher Scientific, Catalog # PA5-115561	1:50	Spleen	90 minutes at 22C	Citrate buffer, pH 7.2
CRT	Rabbit Monoclonal	Thermo Fisher Scientific, Catalog # MA5-32131	1:50	Lung cancer	30 minutes	Citrate buffer, pH 7.2

EVI1: Ecotropic viral integration site 1, CRT: Calreticulin.

Table 2: Comparison of miR-1, EVI1, and e between the breast cancer cases and control group

		Breast cancer Cases (N.=100)	Control cases (N.=100)	P value
miR1 median (IQR)		0.49 (0.45-0.54)	0.91 (0.88-0.95)	**.000****
EV1	**Low (137)**	60 60%	77 77%	**.01***
	High (63)	40 40%	23 23%	
CRT	**Low (111)**	48 48%	63 63%	**.033***
	High (89)	52 52%	37 37%	

EVI1: Ecotropic viral integration site 1, CRT: Calreticulin, * significant, **highly significant

Table 3: Relation between miR1, EVI1, Calrecticulin and clinicopathological parameters of studied cases

Pathological parameters	NO %		miR1 median (IQR)		EVI1		P Value	CRT		P value
					Low N.=60 N. %	High N.=40 N. %		Low N.=48 N. %	High N.=52 N. %	
Age	**<50 (50)**		0.49 (0.45-0.56)	0.16	33 66.0%	17 34%	0.22	25 50%	25 50%	0.68
	≥50 (50)		0.48 (0.43-0.53)		27 54 %	23 46 %		23 46%	27 54%	
Histological type	**Invasive ductal carcinoma (77)**		0.49 (0.43-0.55)	0.71	42 54.5%	35 45.5 %	.05	37 48.1%	40 52.9%	0.98
	Non ductal carcinoma (23)		0.49 (0.45-0.52)		18 78.3 %	5 21.7 %		11 47.8%	12 52.2%	
Grade	**G1+G2 (69)**		0.49(0.45-0.56)	**.003****	39 56.5%	30 43.5%	0.28	30 43.5%	39 56.5%	.17
	G3 (31)		0.46(0.41-0.51)		21 67.7%	10 32.3%		18 58.1%	13 41.9%	
T stage	**T1 (28)**		0.52 (0.49-0.59)	**.01***	14 93.3%	1 6.7 %	**.01***	13 86.7%	2 13.3%	**<.001****
	T2 (53)		* 0.46 (0.41-0.53)		17 58.6%	12 41.4%		9 31%	20 69%	
	T3(19)		*0.46 (0.41-0.49)		4 40 %	6 60 %		1 10%	9 90%	
N stage	**N0 (54)**		0.51 (0.46-0.56)	**.003****	26 89.7%	3 10.3 %	**0.000***	17 58.6%	12 41.4 %	**.01***
	N1+N2 (46)		0.45 (0.41-0.49)		9 36 %	16 64%		6 24 %	19 76 %	
TNM stage	**I+II (58)**		0.51 (0.45-0.56)	**.01***	٢٦ 83.9%	٥ 16.1%	**0.000***	١٨ 58.1%	١٣ 41.9%	**.000****
	III+IV(42)		0.45 (0.42-0.49)		٩ 39.1%	١٤ 60.9%		5 21.7%	١٨ 78.3 %	
Molecular subtypes	**luminal a (46)**		0.52 (0.49-0.57)	**.001****	37 80.4%	9 19.6%	**0.000***	34 73.9%	12 26.1%	**<.001****
	luminal b (23)	Her2 -ve (15)	0.49 (0.45-0.55)		15 65.2%	8 34.8%		11 47.8 %	12 52.2 %	
		Her2 +ve(8)	0.48 (0.48-0.58)							
	her2(14)		0.45 (0.40-0.52)**		4 28.6%	10 71.4%		2 14.3 %	12 85.7 %	
	TNBC (17)		0.43 (0.41-0.47)		4 23.5%	13 76.5%		1 5.9 %	16 94.1 %	
KI67	**<14% (43)**		0.53 (0.49-0.58)	**.001****	33 76.7%	10 23.3%	**.003***	28 65.1%	15 34.9 %	**.003***
	>14% (57)		0.46 (0.42-0.49)		27 47.4%	30 52.6%		20 35.1 %	37 64.9 %	
Total	100				60%	40%		48%	52%	

EVI1: Ecotropic viral integration site 1, CRT:Calreticulin , TNBC: Tripple negative breast cancer , * significant, **highly significant.

Table 4: Correlation between miR1, EVI1 and Calreticulin in the studied breast cancer cases

Studied tissues Marker		miR1 Low (N.=93)	miR1 High (N.=7)	P value
EVI1	**Low (60)**	53 88.3%	7 11.7%	**.02***
	High (40)	40 100.0%	0 0.0%	
Calreticulin	**Low (48)**	41 85.4%	7 14.6%	**.000****
	High (52)	52 100.0%	0 0.0%	

EVI1: Ecotropic viral integration site 1 ,CRT:Calreticulin , * significant ,**highly significant.

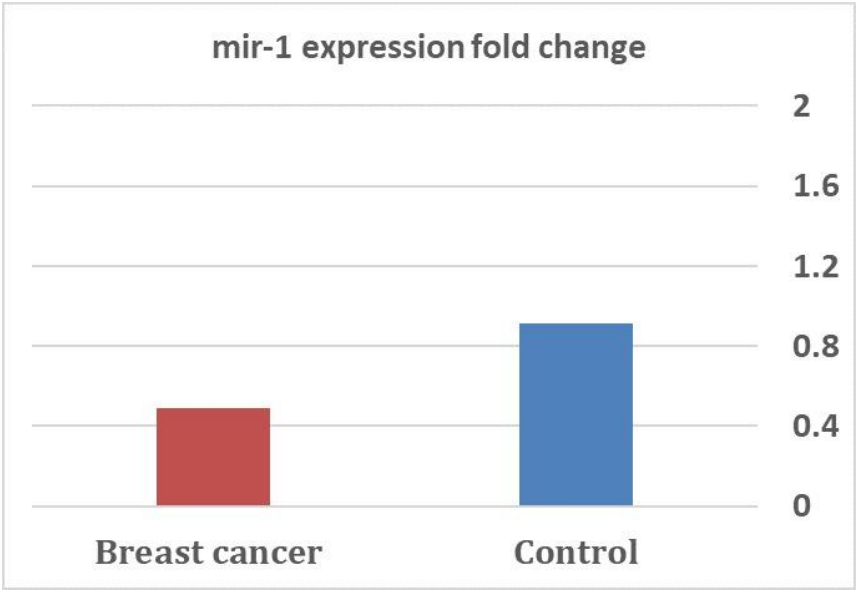

Figure 1: MiR-1 expression fold change.

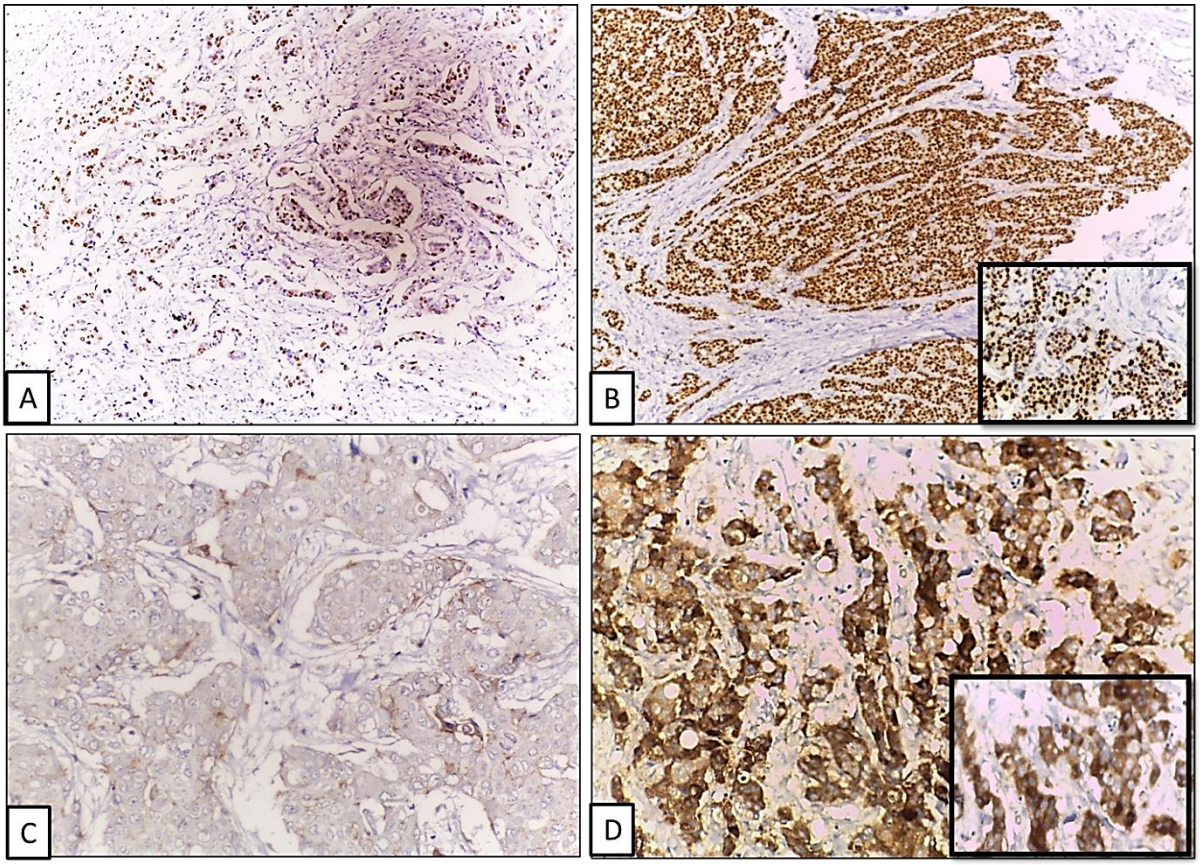

Figure 2: Immunohistochemical expression of EVI1 & Calreticulin in invasive breast carcinoma. (a) Lower positivity of EVI1 in invasive breast carcinoma, grade 2, while it showed diffuse expression in higher grade carcinomas (b). (c)Low expression of calreticulin in carcinoma of breast, grade 2. While strong diffuse expression was seen in higher grade carcinoma (d), Inset: X400 to show localization of Ab (IHC, x200).

Discussion

Breast cancer represents the most often identified malignancy among adult females globally, encompassing the population of Egyptian women [2]. Morbidity and mortality rates among breast cancer patients exhibit considerable variation across the globe, largely attributable to differences in tumor biology and genomics, as well as disparities in the quality and standard of cancer care [28].

miRNA expression levels have been connected to human malignancies severity and clinical outcomes, indicating that miRNA expression profiles in cancer tissues- as opposed to normal tissues- could be extremely important in the diagnosis and prognosis of breast cancer. Therefore, there is a necessity for new and reliable biomarkers, and these changes in miRNA expression profiles may address this requirement [٢٩].

In this study, we noted a marked downregulation of miR-1 in breast cancer tissues as opposed to normal breast tissue (p = 0.000). This outcome corroborates the research conducted by previous study [٣٠]. Furthermore, miR-1 demonstrated an inverse correlation with tumor differentiation, increased tumor sizes, positive lymph node metastasis, and the advanced stages of breast cancer. Analogous observations were documented by previous studies [٣١ ,٣٢], Previous study [٣٣] substantiated these outcomes, with pancreatic ductal adenocarcinoma (PDAC). Aforementioned study [٣٤]

corroborated these observations, demonstrating a significant downregulation of miR-1 in colorectal carcinoma (CRC).

The implementation of miR-1 has demonstrated a pronounced capability to inhibit neoplastic cell proliferation, through its potential participation in cell cycle regulation or the induction of cellular senescence within malignant cells. Prior investigations have elucidated that miR-1 can precipitate cell cycle arrest at the G0/G1 phase in neoplastic cells via the downregulation of CDK4 [٣٥].

The overexpression of miR-1 correlated with a diminution in proliferation markers such as phosphorylated AKT (p-AKT), phosphorylated ERK1/2 (p-ERK1/2), binding immunoglobulin protein (BIP), and myeloid cell leukemia 1 (Mcl-1). Moreover, there was upregulation in the expression of apoptotic markers such as Bax, Bad, cleaved poly ADP-ribose polymerase (PARP), and cleaved caspase-3. miR-1 may exert its tumor suppressor role in breast cancer by facilitating apoptotic processes and repressing epithelial-mesenchymal transition [٣٠].

Numerous studies have elucidated various pathways through which miR-1 influences the oncogenesis of breast cancer cells. For example, miR-1 has been demonstrated to decelerate cancer stem cells proliferation by modulating EVI-1 [٣٦]. Furthermore, miR-1 has the capability to impede the advancement of triple-negative breast cancer via suppressing Slug's expression [٣٧].

In this research, miR-1 was proved to be down-regulated in triple-negative breast cancer and HER2-positive molecular subtypes, whereas its levels were elevated in luminal A and luminal B cancer cases, exhibiting a significant correlation (*p*-value **<0.001**). This outcome aligns with the outcomes of previous study [٣٥], who observed that miR-1 expression in 45 breast cancer tissue samples and their corresponding serum samples was significantly diminished in HER2-positive tumors relative to luminal A and B tumors, yet markedly elevated compared to basal-like tumors.

Contrary to our results, a previous study [٣٨] identified miR-1 localization within carcinoma cells in 20% of breast carcinoma cases, while its presence was minimal in non-neoplastic mammary glands or stroma. The miR-1 ISH status demonstrated a significant association with Ki-67, histological grade, lymph node metastases, distant metastases, clinical stage, and pathological T factor in breast cancer. This discrepancy may be due to differences in methodologies, interpretative approaches, and the number of cases analyzed.

The EVI1 oncogene, positioned on chromosome 3q26, exhibits specific binding affinity to promoter DNA sequences and is integral to transcriptional regulation, as elucidated in a previous research [٣٩]. CRT, a chaperone protein localized in endoplasmic reticulum's lumen, has been connected to several pathological circumstances, like autoimmune disorders and specific cancers. Nevertheless, its involvement in pathogenesis of breast cancer stays ambiguous, as suggested by the research conducted by previous study [18].

In this investigation, EVI1 and Calreticulin were significantly upregulated in breast carcinoma cases rather than non-cancerous tissue (p=0.01 & p=0.03). This aligns with previous investigation [27] who stated that 59.1% of breast cancer cases exhibited high expression levels of EVI-1, while 72.7% showed elevated CRT expression. In contrast, both markers were expressed at low levels in all nearby healthy tissues, with these variations being statistically significant (P<0.05).

In this present investigation, EVI1 was associated significantly with larger T stage, positive lymph node metastasis, higher stage & high ki67(p=0.01, p=0.000, p=0.000, p=0.003, respectively). It was overexpressed in 19% &;34% of hormone receptor positive cases, while it was

overexpressed in 70% of Tripple negative cases.

EVI1 is crucial in enhancing cell proliferation, migration, and invasion while concurrently inhibiting apoptosis in breast cancer cells. Conversely, silencing EVI1 expression leads to reduced cell proliferation, migration, and invasion, and an increase in apoptosis in these cells. Moreover, abnormal EVI1 expression has been demonstrated to modulate genes linked to breast cancer cells' apoptotic process [٤٠].

Previous study [27] substantiate our results, revealing a significant association of EVI1 with an advanced T stage, lymph node metastasis, elevated staging, and high Ki67 index. Furthermore, they noted EVI1 overexpression predominantly in triple-negative breast cancer cases.

CRT—a protein integral to calcium homeostasis and protein chaperoning—has been connected to the development and spread of cancer, though the specific mechanisms remain largely undefined [٤١]. Surface-displayed CRT functions as an "eat-me" signal across a spectrum of human malignancies, thereby facilitating a process known as immunogenic cell death in cancerous cells [٤٢]. However, this prophagocytic function of CRT can be offset by the anti-phagocytic signal CD47 [٤٣], which is upregulated on surfaces of cancer cells to evade immune-mediated phagocytosis [٤٤].

Calreticulin has also been associated with anoikis, a type of cell death that is brought on by separation from the extracellular matrix [٤٠]. Thrombospondin 1, a matricellular protein, can bind to calreticulin and promote resistance to anoikis in fibroblasts [٤٥]. Cancer cells, which exhibit enhanced survival mechanisms, regulate calcium homeostasis in the ER to mitigate hypoxia and ER stress [٤٦]. CRT, essential in alleviating ER stress, may thus have a vital part in cancer cell survival [٤٧]. Furthermore, CRT has been connected with regulating cell adhesion and motility. For instance, in a study on kidney cancer, CRT overexpression induced EMT in renal epithelial cells [٤٨].

The dual roles of CRT in both promoting tumor progression and eliciting an antitumor immune response present a paradox [٤٩]. While some studies have illustrated that CRT can provoke an antitumor immune response and impede tumor growth, others have correlated CRT expression with development of tumors and cellular changes.

A notable inverse connection between miR-1 and EVI1 was identified in the studied cancer cases (p=0.02), corroborating the findings of previous study [٣٦], Their research demonstrated that EVI-1 is a direct target of miR-1, showing that increased miR-1 levels led to decreased EVI-1 expression in breast cancer stem cells (BCSCs) at both transcriptional and post-translational levels. Additionally, miR-1 overexpression inhibited BCSC proliferation and induced apoptosis, effects that were reversed by EVI-1 overexpression. Aberrant miR-1 expression has additionally been demonstrated to influence EMT-associated genes in breast cancer stem cells (BCSCs). Immunohistochemical analysis demonstrated a reduction in EVI-1 expression in BCSC tumors after intra-tumoral administration of miR-1 agomir as opposed to control groups. These findings imply that miR-1 may hold therapeutic promise for breast cancer treatment.

Calreticulin expression is modulated by multiple transcription factors in the embryonic heart, as demonstrated by previous work [٥٠], they identified GATA6 and EVI-1 as novel regulators of calreticulin gene during cardiac embryogenesis. Nonetheless, the function of EVI-1 in transcriptional regulation remains insufficiently elucidated, with a limited repertoire of its target genes identified thus far. The purpose of EVI-1 as a repressor or activator is contextually dependent, varying with the specific target gene, tissue type, developmental stage, and

the prevailing physiological or pathological conditions.

According to previous study [27], there is increased expression of both EVI1 and Calreticulin in triple-negative breast cancer, suggesting a link between the two proteins. Additional investigation is required to better understand their part in breast cancer and to develop targeted therapies.

In our current research, we discovered that miR-1 is negatively connected to CRT, exhibiting a statistically significant correlation (p=0.000). Several proteins, identified as miR-1 targets and possessing muscle-related functions that may be relevant in vivo, include ACTR3, TPM3, and CRT [٥١]. miR-1 may impede the growth, invasion, and metastasis of breast cancer by inhibiting EVI1-mediated CALR expression, a hypothesis that requires further investigation.

This finding aligns with the findings of previous study [٥٠], who indicated that miR-206 downregulates CRT expression, resulting in the suppression of proliferation and metastasis of breast cancer stem cells, along with induction of apoptosis. Additionally, it was noted that the overexpression of CRT effectively mitigated the cytotoxic effects exerted by miR-206. Furthermore, additional studies indicated that EVI1 could serve as a key regulator of miR-206-mediated CRT expression, with increased EVI1 levels reversing the effects of miR-206 on CRT induction.

Conclusion

To sum up, our research elucidates that miR-1 is downregulated in breast carcinomas and is connected to tumor progression, invasion, and migration, thereby suggesting its function as a tumor suppressor. Thus, enhancing miR-1 expression might possibly prevent tumor cell proliferation and metastasis, underscoring the considerable promise of miR-1 replacement therapy in cancer treatment. Both EVI1 and CRT are crucial contributors to the development and progression of invasive breast carcinomas. Additionally, CRT-mediated immune mechanisms present a viable avenue for the development of novel anticancer treatments. EVI1 also emerges as a potentially useful target for treatment for breast cancer treatment.

The results suggest a potential connection between miR-1, EVI1, Calreticulin, and E-cadherin inducing epithelial-mesenchymal transition. Therefore, additional research is needed to investigate this hypothesis. Further research is also needed to elucidate the functions of EVI1 and Calreticulin proteins in breast cancer and to explore their potential as therapeutic targets. This may open the door to the creation of more focused and efficient treatments for patients with breast cancer.

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Competing interests

The authors have disclosed no relevant conflicts of interest, either financial or non-financial.

Data availability

The corresponding author can provide the data supporting the study's conclusions upon request.

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