RESEARCH ARTICLE

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The Role of miRNA-182 and FOXO3 Expression in Breast Cancer

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Abstract

Objective: evaluating the role of FOXO3 mRNA and mi RNA 182-5P expression levels in BC patients. **Method:** 25 Samples of breast cancer and paired samples of non-cancerous tissues from the same resected breast were obtained from 25 female patients suffering from breast cancer and examined and analyzed by real time PCR to detect the expression levels of FOXO3 mRNA and mi RNA 182-5P. Patients' data were collected from patients medical records. **Results:** Foxo3 m RNA expression was down regulated in BC tissues (1.37± 1.96) as compared to control group (23.62 ± 54.39) and decreased FOXO3 expression was associated with larger tumor size (p= 0.046), late histopathological grading (p= 0.002), late TNM staging (<0.001) and increased miR-182 expression (p= 0.025). We found that expression level of miR-182 was significantly higher among breast cancer group (1.10±1.15) as compared to the control group (0.58±0.96) with p value = 0.017. We noted a significant increased expression associated with larger tumor size (p= 0.002), late histopathological grading (p= 0.008), late TNM staging (p= 0.002) and decreased FOXO3 expression (p= 0.025). A significant negative correlation between miR-182 and FOXO3 mRNA fold expression with r = - 0.447, and a p value of 0.025, this could be attributed to miRNA targeting FOXO gene. **Coclusion:** Down regulation of FOXO3 and up regulation of miR-182 expression was associated with advanced breast cancer. The negative correlation between miR-182 and FOXO3 mRNA could be attributed to miRNA targeting FOXO gene.

Keywords: miRNA-182- FOXO3m RNA- breast cancer

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Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among females worldwide (Siegel et al., 2018). Although early diagnosis and more effective treatment strategies have improved patient outcomes over the past few decades, a substantial portion of patients are refractory to current chemotherapeutic strategies (Iwamoto and Pusztai, 2010).

Reports are suggesting that the major determinant of tumor response to treatment is not the anatomical prognostic factors but rather the intrinsic molecular characteristics (Perou et al., 2000; Sotiriou and Pusztai, 2009). There has been a growing interest in breast cancer characterization based on the gene expression profiling for a better prognostication of the disease (Jansson and Lund, 2012).

MicroRNAs (miRNAs) are small, non-coding RNA formed nearly of 22 nucleotides. miRNAs function through post-transcriptional modulation of gene expression. This occurs by miRNA specifically binding to its target RNA, suppressing its translation. Aberrant miRNA expression levels are observed in numerous cancers (Bedewy et al., 2017).

Recently, there has been a paradigm shift from studying about the genetics of breast cancer to exploring the epigenetic factors and this has led to the linking of the miRNA expression profiles with the different stages of tumor growth in terms of local spread, invasion, progression and metastasis, thus making miRNAs an important tumor biomarker (Schwartz et al., 2016). In breast cancer, some miRNAs have been shown to upregulate the functions of oncogenes while others stimulate the tumor suppressors (Serpico et al., 2014). Many miRNAs, particularly miR-182 that has been proven to be encoded in cancer-related gene regions, thereby revealing that alteration of miRNA expression may have a causal relationship with tumorigenesis, aggressive disease and chemo resistance

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(Du and Pertsemlidis, 2012).

Breast cancer stem cells (BCSCs), are small subset of tumor cells that are capable of self-renewal. They have been isolated from human breast cancers (Al-Hajj et al., 2003). Due to their intrinsic stem cell-like properties, BCSCs play great role in tumor progression, therapeutic resistance, and the ineffectiveness of conventional chemotherapy to eradicate BCSCs thus resulting in therapy failure (Cojoc et al., 2015). So, understanding the regulation mechanisms of BCSCs might help in the development of new targeted strategies for eliminating BCSCs, resulting in improving the clinical outcomes of patients with breast cancer.

Epigenetic programs contribute to gene expression regulation and have been proposed as key regulators of CSC self-renewal and differentiation. (Easwaran et al., 2014) Aberrant DNA methylation is one of the most common defects in epigenetic regulation noticed in tumorigenesis (Klutstein et al., 2016). Aberrant DNA hypermethylation at CpG islands, that leads to the loss of expression of genes specific to the differentiated state and regaining of stem cell-specific characteristics, has been reported to be crucial for CSC properties in BCSCs (El Helou et al., 2014).

CpG methylation is catalyzed by DNA methyltransferases (DNMTs), including DNMT1, DNMT3a, and DNMT3b.(Subramaniam et al., 2014) A new study results has just shown an essential role for DNMTs in mammary stem/progenitor cell and BCSC maintenance (Pathania et al., 2015). DNMT deletion or inhibition of DNMT activity by a low dose of DNA demethylating agents (decitabine or azacitidine) has been shown to durably eradicate BCSCs (Pathania et al., 2016; Tsai et al., 2012). But, the molecular mechanisms by which DNMTs regulate BCSCs remain unknown.

Forkhead box O3a (FOXO3a), a transcription factor of the FOXO protein family, that has been highlighted as an important transcriptional regulator of crucial proteins associated with cell cycle progression, apoptosis, metastasis, angiogenesis, and metabolism (Liu et al., 2020; Liu et al., 2015; Yadav et al., 2018). Downregulation of FOXO3a leads to tumorigenesis, progression, and poor prognosis in many human cancers (Jiang et al., 2013; Ma et al., 2018; Yang et al., 2008). Thus, many studies have suggested that FOXO3a plays an important role in regulating cancer stem cell (CSC) properties (Dubrovska et al., 2009; Sunayama et al., 2011). For example, over expression, pharmacological activation of FOXO3a inhibits stem-like properties and tumor initiation and suppresses drug resistance in lung cancer cells and colorectal cancer (Chiu et al., 2016; Prabhu et al., 2015). More recently, an integrated genomic approach revealed that FOXO3a is involved in breast cancer initiation (Smit et al., 2016). However, the biological function and detailed molecular mechanism of FOXO3a in breast cancer stem cells (BCSCs) are still unclear. The aim of this study was to evaluate the role of miRNA-182 and FOXO3 expression in breast cancer.

Materials and Methods

Twenty-five breast cancer patients were enrolled in the present study from March 2020 to May 2021, after approval of the Ethical Committee of the Medical Research Institute, Alexandria university. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. Informed consent was obtained from all eligible subjects before starting the study. Confidentiality of patients' data was ensured for all the patients.

All the studied cases were subjected to detailed history taking as well as comprehensive physical examination.

From each patient two biopsies were taken, one from the cancerous tissue, representing the patient group, and the other from adjacent non-cancerous tissue corresponding to control group.

Surgically resected BC tissue and adjacent non-cancerous tissue were directly submerged in sterile Eppendorf tubes containing RNA later (Ambion; Applied Biosystems USA) to preserve the RNA till time of analysis. At least 10 volumes of the reagent (or approximately 10 ml reagent per 1mg of tissue) was required. They were incubated in the refrigerator at 4^{o}C for 24 hours to allow full permeation of the preservative into tissue, then transferred to -80^{o}C freezer till the time of molecular analysis.

Histopathological examination

The resected tissues were formalin fixed and embedded in paraffin and were stained with hematoxylin and eosin and were pathologically examined to determine the type of the tumor, grading and clinical TNM staging in addition to assessment of the hormonal status of the tumor including estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2.

RNA isolation and qPCR

Total RNA extraction was performed by utilizing the TRIzol reagents "QIAGEN miRNeasy" Mini Kit, Catalogue number 217004 (Qiagen). And the procedure was applied according to the manufacturer's instructions.

The concentration and purity of RNA were assessed using NanoDrop Spectrophotometer (Thermo Scientific, USA), where the absorpance at 260nm (A_{260}), at 280 (A_{280}) and at 230 (A_{230}) were measured. The ratio of (A_{260}/A_{280}) was used to indicate the purity of RNA extract, where a ratio between 1.9-2.1 indicates pure extract. If the ratio is lower in either case, it may indicate the presence of protein, phenol, or other contaminants that absorb strongly at or near 280nm. The ratio (A_{260}/A_{230}) is used as a second measure of nucleic acid purity. Expected values are commonly in the range of 2.0-2.2. If the ratio is lower than expected, it may indicate the presence of contaminants which absorb at 230nm. RNA integrity and DNA contamination were detected by gel electrophoresis.

The RNA extracted was reverse transcribed into cDNA using the miScript starter kit, for reverse transcription, Catalogue number 218193 (Qiagen).

Thermocycling was carried out using PCR thermocycler

(Applied Biosystems) as outlined in the Table 1.

The cDNA was divided into two aliquots, one for assessing mRNA FOXO3 and the other one for miRNA 182-5p

Real-time PCR was performed using Rotor-Gene Q system to assess expression levels of mRNA FOXO3, miRNA 182-5p, housekeeping Human RNU6B (U6) and GAPDH 15a.

The reaction mixture was prepared as follows: adding 12.5 µL 2x Quanti Tect SYBR Green PCR master mix, 2.5 µL 10x miScript Universal Primer (Catalogue number 218193, Qiagen), 2.5 µL miRNA-182-5p miScript primer assay (Catalogue number 218300, Qiagen), or 2.5 µL FOXO3a mRNA Quanti Tect primer assay (Catalogue number 249900, Qiagen) for miRNA-182-5p and mRNA FOXO3 assays, respectively, then 2.5 µL template cDNA were mixed, and finally nuclease-free water was added to reach a final volume of 25 µL.

The housekeeping RNU6B (U6) miScript primer assay was used as an internal reference control for both miRNA-182-5p and GAPDH 15a as an internal control for mRNA FOXO3 assays. The cycling conditions were as described in the Table 2.

Relative quantification (RQ) of miRNA expression = $2^{-\Delta\Delta Ct}$:

For each sample, the difference in Ct values for each target and its internal control gene is calculated (ΔCt). Next, subtraction of the mean Δ Ct of control group from the mean ΔCt of the target group yields the ΔΔCt, then the negative value of this subtraction, the −ΔΔCt, is used as the exponent of 2 in the equation. The final result of this method is presented as the fold change of target gene expression in a target sample relative to a reference sample, normalized to a reference gene.

- ΔCT = CT (a target gene) – CT (a reference gene)
- ΔΔCT = mean ΔCT (a target sample) – mean ΔCT (a reference sample)
- Then calculate $2^{-\Delta\Delta CT}$

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. Quantitative data were described using mean and standard deviation. Significance of the obtained results was judged at the 5% level. The student t-test was used to compare between two studied groups. Wilcoxon signed-rank test was used to compare between two related samples. Correlations between quantitative continuous variables parameters were evaluated by linear regression with analysis of Pearson's coefficients to measure of the strength of the association between the two variables.

Results

The study was conducted on 25 paired cancerous and adjacent non-cancerous tissues as patient and control groups, respectively.

Demographic and clinicopathological data

Sixteen patients (64%) were above 50 years old, whereas 9 patients (36%) were below 50 years. The premenopausal patients were 13 (52%) of the cases while 12 patients (48%) were postmenopausal. All studied cases were diagnosed as invasive ductal carcinoma (IDC) (Figure 2). 16 patients (64%) were graded histopathologically as GI and GII while 9 (36%) of the cases were of GIII. Regarding tumor stagging 19 (76%) cases were in the early stages (0, I, IIA, II) while only 6 (24%) were in late stage (III) as shown in Table 3.

Expression levels of FOXO3 mRNA and miR-182

The fold expression level of miR-182 was significantly higher among tumor tissues as compared to the non-cancerous adjacent tissues with p value = 0.017, where 84% of the non-cancerous adjacent tissues showed decreased fold expression levels with a mean value of 0.58±0.96, as compared to tumor tissues having a mean fold expression value of 1.10 ±1.15, as shown in Table 4.

However, the fold expression of its target mRNA FOXO3 was significantly lower in the tumor tissues as compared to the non-cancerous adjacent tissues (p = 0.021), with a mean fold expression value of 1.37± 1.96, and 23.62 ± 54.39, respectively as shown in Table 4.

Relationship between FOXO3 mRNA and miR-182 and tumor characteristics

There were a significant positive association between tumor size and the fold expression of miR-182, so that as the tumor size increases the fold expression of miR-182 increases. On the other hand, it was inversely correlated with FOXO3 mRNA fold expression with a p value of 0.002 and 0.046 respectively as shown in Table 5.

Regarding histopathological grading, tumor tissues of grade I and II showed significantly lower fold expression level of miR-182 and significantly higher fold expression of FOXO3 mRNA, as compared to (GIII) with a p value of 0.008 and 0.002, respectively. Table 5.

Moreover, grouped tumor stages (stage 0, I, IIA, IIB) were significantly negatively correlated with miR-182-fold expression as compared to the late stage (stage III) with a p value of 0.001. Meanwhile, FOXO3 mRNA fold expression was significantly increased among cases of tumor stage (stage 0, I, IIA, IIB), when compared with late stage (stage III), having a p value of less than 0.001.

Concerning lymph node (LN) metastasis, hormonal status of the tumor and the molecular subtypes, there were no association between them and the fold expression of neither miR-182 nor FOXO3 mRNA, as seen in Table 5 and Table 6.

Table 1. Reverse Transcription PCR Cycling Protocol

Step	Temperature	Time
Reverse transcription step	16°C	30 minutes
	42°C	30 minutes
Stop reaction	85°C	5 minutes
Hold	4°C	Hold

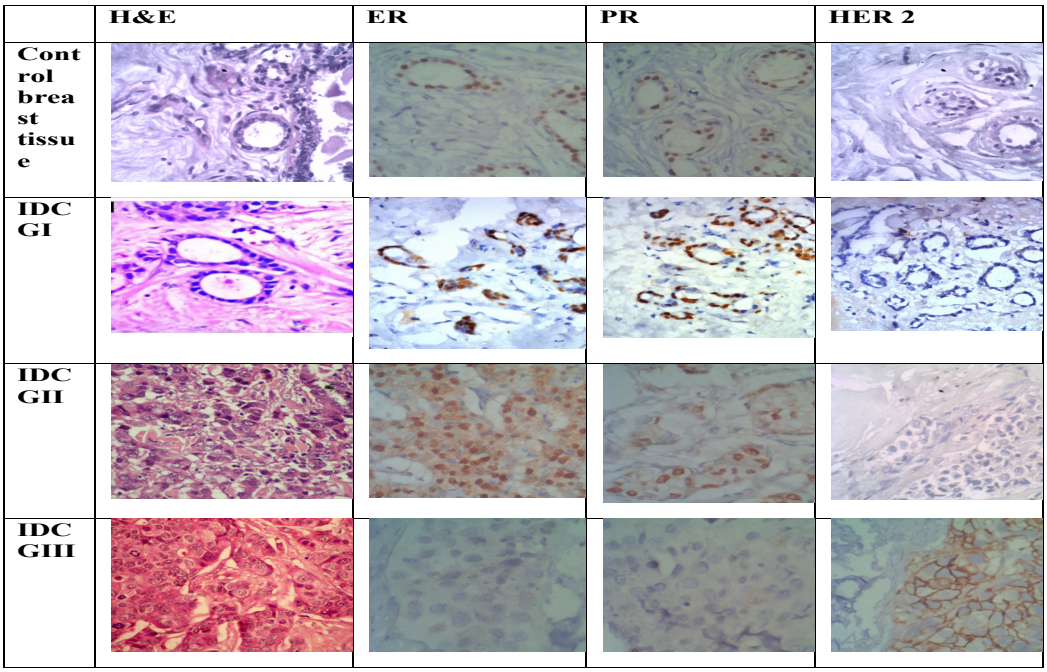

Figure 1. Paraffin Sections Photomicrograph of Breast Tissue Showing, • Control Breast Tissue, (a) HandE Stain, Benign Breast Ducts; (b) +ve Nuclear ER Stain; (c) +ve Nuclear PR Stain; (d) –ve Membranous HER2/neu Stain. (x400)• IDC (GI), (a) HandE stain: GI ductal carcinoma; (b) +ve nuclear ER stain; (c) +ve nuclear PR stain; (d) –ve membranous HER2/neu stain. (x400)• IDC (GII), (a) HandE stain: GII ductal carcinoma; (b) +ve nuclear ER stain; (c) +ve nuclear PR stain; (d) –ve membranous HER2/neu stain. (x400)• IDC (GIII), (a) HandE stain, GIII ductal carcinoma; (b) -ve nuclear ER stain; (c) -ve nuclear PR stain; (d) +ve membranous HER2/neu stain. (x400)

Correlation between miR-182 and FOXO3 mRNA

There is a significant negative correlation between miR-182 and FOXO3 mRNA fold expression with r = - 0.447, and a p value of 0.025. Figure 2.

Discussion

Breast cancer (BC) is considered the most common cancer in females. It is caused by complex, interconnected risk factors. One of the most important factors is the genetic and epigenetic factors (El-Attar et al., 2020). The progression of BC depends on intrinsic factors related to the tumor cells, such as transcription factors. The FOXO family are a group of transcription factors that have been incriminated in tumor development (Yang et al., 2008). FOXO3 is a member of the FOXO family with tumor suppressor function (Accili and Arden, 2004). It is involved in many processes, such as cell cycle, cellular proliferation, apoptosis, malignant transformation, cellular differentiation, epithelial-to-mesenchymal transition, DNA damage response and longevity (Jiang et al., 2013; Weidinger et al., 2008). This led to the possibility of its beneficial role as an anticancer treatment in resistant cases (Lam et al., 2013).

Micro RNAs (miRNAs) are series regulators of gene expression, and their disruption is related to carcinogenesis and metastasis (Zhang et al., 2017). MIR182 is an oncogene, acting by down regulation of multiple tumor suppressor genes like BRCA1, FOXO1 and FOXO3 (Segura et al., 2009). It has been implicated in some malignancies as prostate cancer, melanoma and gliomas (Schwartz et al., 2016). The role of FOXO3 mRNA and miR-182 have not been fully elucidated in the previous research, which encouraged us to further investigate their involvement in BC.

In the present study we evaluated the expression levels of both FOXO3 mRNA and miR-182 in 25 paired cancerous (tumor tissues) and adjacent non-cancerous breast tissues, aiming to evaluate their role in BC prognosis and other related clinicopathological findings. The expression level of FOXO3 mRNA was significantly downregulated in the tumor tissues when compared to the adjacent non-cancerous breast tissues with a p value p 0.021, Table 4.

This finding comes in agreement with Liu etal (Liu et al., 2020). They compared the expression levels of FOXO3a, FOXM1, SOX2, and DNMT1 in a tissue microarray in one hundred primary breast tumor samples

Table 2. Cycling Protocol of Quantitative Real-time Polymerase Chain Reaction (QRT/PCR)

Cycle	Cycle Point
Hold	Hold @ 95°C, 15min 0s (for initial activation)
Cycling (40 repeats)	Step 1: Hold @ 94°C, 15s (for denaturation)
	Step 2: Hold @ 55°C, 30s (for annealing)
	Step 3: Hold @ 70°C, 30s (for extension)

Table 3. Distribution of Different Clinicopathological Variables among the Staudied Cases

		No.	%
Age	>50	16	64
	<50	9	36
Menstrual history	Post-menopause	12	48
	Pre-menopause	13	52
Focality	Unifocal	21	84
	Multifocal	4	16
Tumor size	< 2cm	12	48
	> 2 cm	13	52
Histopathological type	IDC	25	100
Histological grade	GI	4	16
	GII	12	48
	GIII	9	36
Grouping of Histological grade	Group1: GI, GII	16	64
	Group 2: GIII	9	36
Lympho-vascular invasion	Negative	5	20
	Positive	20	80
LN metastasis	No LN metastasis	14	56
	LN metastasis	11	44
Stage	No stage	2	8
	IA	3	12
	IIA+IIB	14	56
	III	6	24
Stage	Group I: (0, IA,IIA, IIB)	19	76
	Group II: (III)	6	24
ER	Positive	17	68
	Negative	8	32
PR	Positive	18	72
	Negative	7	28
HER2/neu	Positive	18	72
	Negative	7	28
Molecular subtypes	luminal A	5	20
	Luminal B	12	48
	Her2/neu enriched	8	32

and 32 adjacent normal breast tissues. They found that, adjacent normal tissues showed increased FOXO3a expression levels and decreased expression levels of FOXM1, SOX2, and DNMT1 and vice versa in the BC tissues.

In accordance with our results, Dilmac et al, assessed FOXO proteins and mRNA expression levels in benign and malignant breast cancer cell lines, they reported that their levels were significantly higher among the benign cell lines, when compared to the malignant ones. Moreover, their levels were significantly lower in the metastatic tumor cell lines. ($p<0,0005$), denoting that the absence of FOXO proteins might promote metastatic progression. Additionally, they reported decreased FOXO proteins from resected primary BC as compared to metastatic BC in mice by western blot and confirmed their results by immunohistochemistry. They also analyzed the expression level FOXO mRNA in The Cancer Genome Analysis (TCGA), revealing a decreased expression level in tumor triple negative breast cancer (TNBC) compared to normal tissues (Sayra et al., 2022). Similarly, Jiang et al, evaluated FOXO3 expression in 70 BC tissues using immunohistochemical techniques and compared it with some clinicopathological data and concluded that FOXO3 positive expression could be considered as an independent indicator of good prognosis in BC.

Moreover, Pellegrino et al, demonstrated a significantly reduction in both FoxO3a mRNA and protein expression, in tamoxifen resistant BC cell line with respect to MCF-7 cells (Pellegrino et al., 2019).

These findings were consistent with other researchers who investigated FOXO3 expression in other malignancies. Yang et al, reported a decreased expression of FOXO3 mRNA in gastric cancer tissues with respect to adjacent non-tumorous tissues ($p = 0.03$) and stated that patients having increased expression of FOXO3 had superior

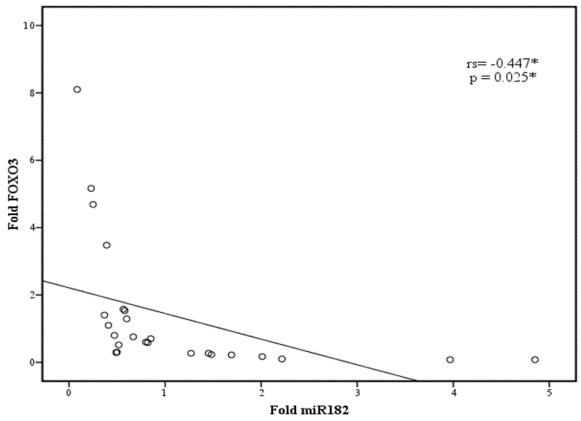

Figure 2. Correlation between miR-182 and FOXO3 mRNA Fold Expression in BC.

overall survival than those with lower expression levels (Yang et al., 2013). Zhou et al., (2019) also revealed that decreased level of Foxo3 gene was associated with poor prognosis in AML patients. Zhang et al., (2018) also observed a decreased expression of FOXO3 in non-small cell lung carcinoma (NSCLC).

Similarly, Fei et al., (2009); Shen et al., (2020); Xing et al., (2020) and Wang et al., (2019) reported a decreased FOXO3 expression in ovarian, prostate, esophageal squamous cell cancer and bladder cancer, respectively.

One possible explanation of such findings is that FOXO3 is a downstream target in the Akt/PI3K pathway. Thus, regulating the expression of apoptosis genes, genes regulating cell cycle by stimulating the expression of genes that block cell cycle progression like cyclin dependent kinase (CDK) inhibitors, leading to cell cycle arrest (Habashy et al., 2011).

On the other hand, Guttilla and White, (2009) stated that expression of FOXO3 did not vary significantly between normal and tumor breast tissue, attributing this finding to the fact that FOXO3 expression might vary in response to certain circumstances. Interestingly Hornsveld et al., (2018) clarified that FOXOs can lead to both promotion and repression of metastatic breast cancer, thus they should not be considered as typical tumor suppressors. They concluded that FOXO activity is necessary for tumor proliferation and spreading, implying the potential role of FOXO inhibitors in cancer therapeutics (Hornsveld et al., 2018). Also, Storz et al., (2009) pinpointed the importance of FOXO3a in stimulating tumor growth and metastasis by controlling matrix metalloproteinase (MMP) expression and cell invasiveness in BC cell lines.

Many researchers investigated the role of FOXO3 in other malignancies. For example, Sung et al., (2020) revealed a significantly increased FOXO3 expression in hepatocellular carcinoma (HCC) when compared with that in non-cancerous liver tissue (P<0.00) suggesting that this may denote that the expression of FOXO3 in HCC is tissue specific. Others observed that FOXO3 level was upregulated in glioblastoma, (Zhang et al., 2019) gastric cancer (Xiang et al., 2020) and prostate cancer (Kong et al., 2020).

Contradicting to our results, increased expression of FOXO3 has been accused of other nonmalignant conditions, for instance Wong et al., (2013) and Su et al., (2009) discovered an increased FOXO3 expression in Alzheimer's disease and Parkinson's disease, respectively.

The present study assessed the correlation between FOXO3 expression and various clinicopathological data, we observed a significant decreased FOXO3 expression associated with larger tumor size (p= 0.046), high histopathological grading (p= 0.002), late TNM staging (<0.001) and increased miR-182 expression (p= 0.025). However, we did not find a significant association between FOXO3 expression and LN metastasis, hormonal status, or molecular subtypes.

Our results were in accordance with Jiang et al., (2021) regarding histopathological grading, and tumor staging. Conversely, they reported a significant association between FOXO3 expression and lymph node status and ER status, while they did not find a significant association with tumor size (Perou et al., 2000). They concluded that Foxo3a expression is an attractive predictive factor in breast cancer especially in ER-positive patients, having better overall survival and could be used in stratification of patients.

In agreement with our results, Jin et al., (2021) stated that decreased FOXO3 expression was associated with late tumor stage but contradicting to our findings he observed that FOXO3 expression was correlated with lymph node invasion.

MicroRNAs usually have an essential role in regulating gene expression. This is achieved epigenetically, through post-transcriptional modification leading to down regulation of their target genes (O'Brien et al.,

Table 4. Comparison between the two Studied Tissues According to miR-182 and FOXO3 mRNA Fold Expression

	Adjacent noncancerous tissues (n=25)	Tumor tissues (n=25)	p
Fold miR182			
< 1	21 (84.0%)	17 (68.0%)	p=
≥ 1	4 (16.0%)	8 (32.0%)	0.289
Mean ± SD.	0.58±0.96	1.10±1.15	0.017*
Fold FOXO3			
< 1	11 (44.0%)	16 (64.0%)	p=
≥ 1	14 (56.0%)	9 (36.0%)	0.18
Mean ± SD.	23.62 ± 54.39	1.37± 1.96	0.021*

Table 5. Association between Different Clinicopathological Variables and miR-182 and FOXO3 mRNA Fold Expression in Breast Cancer Tissues (BC)

	Fold miR182 (n=25)	p	Fold FOXO3 (n=25)	p
Tumor size				
< 2cm		0.002*		0.046*
Mean ± SD	0.5 ± 0.3		2.2 ± 2.6	
> 2 cm				
Mean ± SD	1.6 ± 1.4		0.6 ± 0.6	
Histological grade				
GI		0.032*		0.009*
Mean ± SD	0.5 ± 0.3		2.1 ± 2.1	
GII				
Mean ± SD	0.6 ± 0.4		1.9 ± 2.4	
GIII				
Mean ± SD	2.0 ± 1.5		0.4 ± 0.4	
Histological grade				
Group I (GI, GII)		0.008*		0.002*
Mean ± SD	0.6 ± 0.4		1.9 ± 2.3	
Group II				
Mean ± SD	2.0 ± 1.5		0.4 ± 0.4	
Stage				
0		0.009*		0.004*
Mean ± SD	1.2 ± 0.5		0.4 ± 0.3	
IA				
Mean ± SD	0.5 ± 0.0		0.7 ± 0.4	
IIA+IIB				
Mean ± SD	0.6 ± 0.4		2.2 ± 2.3	
III				
Mean ± SD	2.5 ± 1.6		0.2 ± 0.2	
Median (Min – Max)	2.1 (0.5 – 4.9)		0.1 (0.1 – 0.5)	
Stage				
Group I		0.001*		<0.001*
Mean ± SD	0.6 ± 0.4		1.7 ± 2.1	
GroupII				

2018). Several miRNAs have been reported to have a fundamental role in carcinogenesis (Loh et al., 2019). MiR-182 has revealed carcinogenic features via regulating the expression of various tumor suppressor genes (Schwartz et al., 2016).

The stimulatory role of miR-182 on tumor cells was formerly tested in breast cancer (Lei et al., 2014), ovarian cancer (Moazzeni et al., 2017), pancreatic cancer (Wang et al., 2016), and colorectal cancer (Jia et al., 2017) in several studies (Yu et al., 2016).

In the present study, we found that expression level of miR-182 was significantly higher among tumor tissues as compared to the adjacent non-cancerous breast tissues with p value = 0.017. We evaluated the association between miR-182 expression and different clinicopathological findings, we noted a significant increased expression associated with larger tumor size (p= 0.002), high histopathological grading (p= 0.008), late TNM staging (p= 0.002) and decreased FOXO3 expression (p= 0.025). However, we did not find a significant association between miR-182 expression and LN metastasis, hormonal status, or molecular subtypes.

Similar results were observed by Zhang et al., (2020) who reported increased expression of miR-182 in TNBC cells and tissues. Also, they demonstrated that miR-182 expression promotes tumor progression and metastasis in TNBC, thus acting as a possible metastatic miRNA (Schwartz et al., 2016). In accordance with our results Bajaj et al., (2020) showed that miR-182 overexpression was associated larger tumor size but opposing to our findings they also demonstrated a positive association with LN affection. They deduced that miR-182 could be used to predict the outcomes and prognosis in locally advanced TNBC patients. Also, Chiang et al., (2013) confirmed that miR-182 is overexpressed by β-catenin signaling pathway in breast cancer thus increasing oncogenicity and invasive

Table 6. Relationship between Hormonal Status and Molecular Subtypes of BC and miR-182 and FOXO3 mRNA fold Expression

	Fold miR182 (n=25)	p	Fold FOXO3 (n=25)	p
ER				
Positive		0.315		0.238
Mean ± SD	0.7 ± 0.4		1.3 ± 1.9	
Negative				
Mean ± SD	1.9 ± 1.7		1.5 ± 2.2	
PR				
Positive		0.297		0.389
Mean ± SD	0.9 ± 0.9		1.3 ± 1.9	
Negative				
Mean ± SD	1.7 ± 1.6		1.6 ± 2.3	
HER2/neu				
Positive		0.423		0.158
Mean ± SD	1.0 ± 1.1		1.7 ± 2.2	
Negative				
Mean ± SD	1.4 ± 1.3		0.5 ± 0.5	
Molecular subtypes				
luminal A		0.643		0.534
Mean ± SD	1.2 ± 1.5		0.6 ± 0.6	
Luminal B				
Mean ± SD	0.7 ± 0.4		1.4 ± 2.2	
Her2/neu enriched				
Mean ± SD	1.6 ± 1.5		1.8 ± 2.3	

capacity by inhibiting RECK. Later they described that miR-182 promoted cell cycle progression, proliferation, and clonal expansion of breast cancer cells (Chiang et al., 2016).

Zhang et al., (2017) too clarified that miR-182 expression levels in TNBC tissues were significantly higher than those in the adjacent normal breast tissues suggesting its oncogenic role in BC by promoting cellular proliferation and other important signaling pathways.

On the other hand, other researchers demonstrated a decreased expression of miR-182 in several tumors. For instance, Kong et al., (2012) denoted a decreased expression of miR-182 in gastric adenocarcinoma signifying its tumor suppressive nature which led to increased expression of downstream oncogenes such as cAMP-responsive element-binding protein 1 (CREB1) leading to cellular proliferation. They summarized that miR-182 inhibits cancer cell viability and clonal expansion by targeting CREB1. Additionally, Poell et al., (2012) and Hu et al., (2015) proposed that miR-182 might have suppressive effect on human melanoma cells and osteosarcoma respectively, owing to their down regulation promoting tumor proliferation, progression, and invasion, offering a promising treatment for such patients.

It's worth mentioning that the observed negative correlation between FOXO3 and miRNA 182-5p expression in our study could be attributed to miRNA targeting FOXO gene. Segura et al., (2009) utilized a luciferase reporter gene assay to confirm that FOXO3a was a direct target gene of miR-182. They reported that miR-182 over-expression in melanoma cells promotes invasion, migration and aggravates metastatic capacity. They added that these effects necessitate inhibition of the FOXO3. Whereas enhanced expression of FOXO3 blocks miR-182's proinvasive effects.

This implies that miRNA silencing may be a valuable therapeutic strategy.

These findings need to be further studied to elucidate the probable therapeutic potential of silencing miRNA in treating various cancers.

Author Contribution Statement

Noha Said Kandil: Selection of the research idea and research design, participation in the writing and reference gathering of the paper, participation in the performance of molecular laboratory tests, data interpretation, plagiarism checking and responsible for the international publishing process.

Lamia Said Kandil: Participation in the writing and reference gathering of the paper, participation in the performance of molecular laboratory tests, results and data interpretation, statistical analysis, plagiarism checking and paper revision.

Radwa Mohamed: Participation in the writing and literature review and reference gathering of the paper, participation in the performance of histopathological examinations.

Mohamed Selima: Participation in patient selection and participation in the writing and reference gathering

of the paper.

Mohamed El Nemr: Participation in patient selection and collection of clinical data and participation in the writing and reference gathering of the paper.

Yasmine Nagy Alwany: Participation in patient selection and collection of clinical data and participation in the writing and literature review and reference gathering of the paper.

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Ethics approval

All patients were recruited from medical research institute teaching hospital. The study was approved by the local ethics committee of the Medical Research Institute, in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) (reference number: IORG 0008812), for research involving humans, and written informed consent was obtained from all included patients before the acquisition of the tissues, explaining the investigational nature of this study.

The study is approved by the Chemical Pathology Department Council, Medical Research Institute, Alexandria University.

The study is not a part of an approved thesis or scientific body.

Availability of data

All data are supplied in the provided tables.

Conflict of interests

All authors declare that they have no conflict of interests.

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