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MiR-1249-5p suppresses Breast Cancer Cell Migration and Invasion by Targeting *MSI1* gene

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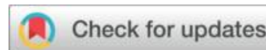

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Abstract

MicroRNAs are identified to post-transcriptionally regulate the expression level of protein-coding genes. Aberrant microRNAs expression led to the suppression and promotion of different types of cancer by changing target gene expression. In this experiment, *miR-1249-5p* expression level in relation with breast cancer (BC) was studied to identify if it influences this type of cancer. Fresh samples from in 50 BC tissues and 50 normal adjacent tissue (NAT). Bioinformatics analysis showed that the putative targets of *miR-1249-5p* was *MSI1*. RT-qPCR findings displayed that the expression level of *miR-1249-5p* was significantly down (P value=0.0001) and *MSI1* was significantly up-regulated (P value=0.001), as compared to NAT. In addition, 3'UTR luciferase reporter confirmed that the *miR-1249-5p* expression level in BC cell lines and tissues was low. When *miR-1249* -5pmimics are increased, the cell growth and migration in BC are stimulated by directly promoting *MSI1* in breast cancer cell lines and tissues. To sum up, the consequences recommend that *miR-1249*-5p roles as a tumor suppressor, promoting the growth and migration of breast cancer cells. *MSI1* is target genes for *miR-1249-5p* in breast cancer cells, and *MSI1* overexpression may reverse the invasive phenotype regulated by *miR-1249-5p*.

Keywords: Breast cancer, *miR-1249-5p*, *MSI1*, cell growth, cell invasion, cell migration.

Introduction

In 2025, breast cancer (BC) accounted for 15.4% of all cancer deaths and 23.8% of all new cases, making it the most common disease diagnosed in women globally [1]. It is still unknown what exactly causes breast cancer. It has long been known that genetic and epigenetic changes play a role in the development and spread of cancer [2-4]. Epigenetics has been prolonged to include microRNAs (miRNAs) in addition to DNA methylation and histone modification.

MicroRNAs are a class of small (usually 18–24 nt) regulatory RNAs that control post-transcriptional mRNA expression by attaching to the 3′ untranslated region (3′-UTR) of the target mRNA sequence, causing mRNA cleavage or mRNA degradation or translational repression [5]. They function as negative regulators of gene expression and are essential in controlling the proliferation, invasion, migration, and metastasis of breast cancer[6]. Numerous miRNAs have been identified as important regulators in breast cancer, including miR-1275[4], miR-4510[3], miR-23 [7, 8], miR-191[9], miR-1185-5p[5], miR-200 [10], miR-132[7], and miR-378 [11]. Nevertheless, it has seldom been shown that miR-1249-5p is implicated in breast cancer. The mechanism by which miR-1249-5p controls breast cancer cells remains unknown.

Musashi1 gene (*MSI1*), an RNA-binding protein, is essential for proper cell division and proliferation as well as the development of several organs [12, 13]. Numerous malignancies have been shown to have abnormal expression of the *MSI1* protein, which is abundantly expressed in stem cells from various organs[14]. Increased expression of *MSI1* has been linked to a number of malignancies, including lung, pancreatic, glioma, breast, and colon cancers. *MSI1* is regarded as an activator in carcinogenesis [15, 16]. Nevertheless, nothing is known about the role and mode of action of *MSI1*. A study demonstrated that by competing with *eIF4G* for binding to *PABPC1*, *MSI1* prevents translation initiation of *MSI1* target mRNAs [17]. *MSI1* stimulates cell proliferation and maintains the stemness state of cancer cells by activating the NOTCH and WNT pathways. Numerous studies have demonstrated that *MSI1* knockdown decreases malignant characteristics such as invasion, radioresistance, and cell proliferation. In glioblastoma and medulloblastoma cells, *MSI1* knockdown decreased the cancer cells' ability to self-renew and survive. On the other hand, by focusing on the Notch pathway, lowering the expression of *MSI1* protein in triple-negative breast cancer cell lines increased the rate of apoptosis and decreased cell proliferation [18, 19].

The current study demonstrated that *miR-1249-5p* was commonly downregulated in breast cancer tissues compared to normal tissues. In vitro and in vivo, overexpression of *miR-1249-5p* dramatically reduced the growth, invasion, migration, and metastasis of breast cancer cells. Additional findings showed that in breast cancer cells, *miR-1249-5p* might inhibit *MSI1* translation through its 3′ UTR. Furthermore, the *miR-1249-5p*-suppressed tumor malignancy in breast cancer cells was eliminated by overexpressing *MSI1*. According to an examination of clinical data, individuals with breast cancer who had elevated *MSI1* expression had a worse overall survival rate. These findings therefore indicated that *miR-1249-5p* targeted *MSI1* to control the aggressiveness of breast cancer cells.

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Material and Method

Sample collection

All methods while performing the research were done in accordance with the local Human Research Ethics Committee (HREC) at Science College in Salahuddin University-Erbil (Reference no. SU2026HREC/58) and the 1964 Helsinki Declaration. In this experiment, all individuals included provided the written informed-consent and permission. Fresh samples were gathered from 50 individuals with breast cancer (BC) at the theater of Erbil International Hospital. The inclusion criteria required that these patients had not received any radiotherapy or chemotherapy. From the same participant, two fresh samples (one BC tissue and one normal adjacent to tumor (NAT)) were gathered and kept at -80°C. NAT was gotten about 3cm away from the malignant margin. The NAT were histopathologically diagnosed and separated from the BC tissue prior to total RNA extraction performed at the laboratory. Through a questionnaire, clinical features of the 50 patients were gained, as shown in Table 1.

Table 1: The clinical characterization of participant.

Variable	HG (*n*=30) Percentage (%)	LG (*n*=20) Percentage (%)	*p*- value
Age-Median, *IQR	50 (21–67)	30 (19–49)	0.08
***BMI-Median, IQR**	30 (24–43)	29 (25–40)	**0.003**
Menopausal status			**0.04**
Pre	16 (53.3%)	13 (65%)	
Post	14 (46.7%)	7 (35%)	
Hypertension status			0.07
Yes	10 (33.3%)	3 (15%)	
No	20 (66.7%)	17 (75%)	
Diabetes			0.08
Yes	19 (63.3%)	-	
No	11 (36.7%)	20 (100%)	
Smoking status			0.06
Never smoker	23 (73.7%)	20 (100%)	
Earlier smoker	6(20%)	-	
Current smoker	1(3.3%)	-	
Ethnicity			0.07
Kurdish	30 (100%)	11 (55%)	
Non-Kurdish	-	9 (45%)	
Stage			0.07
I (LG)	-	1 (5%)	
II (LG)	-	19 (95%)	
III (HG)	15 (50%)	-	
IV (HG)	15 (50%)	-	
Histological subtype			**0.001**
*ILC	20(66.7%)	1 (5%)	
*IDC	10(33.3%)	19 (95%)	
Metastasis state			**0.005**
Yes	30 (100%)	-	
No	-	20(100%)	

*BMI= body mass index, *IQR= interquartile range, *IDC=Invasive lobular carcinoma, *IDC=Invasive ductal carcinoma.

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Extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA molecules were extracted from human breast cancer and normal adjacent tissues for determining the expression level of *miR-1249-5p* or *MSI1* according to the manufacturer's instructions in RNA/DNA Purification Plus kit (Cat. No. 54300, NORGEN BIOTEK CORP, Canada). Two µg of each total RNA sample was reversely transcribed into complementary (c)DNA applying the miRNA All-In-One cDNA Synthesis Kit (Cat. No. G898, abmgood company, US) according to the manufacturer's instructions.

To measure the mRNA expression value, *MSI1* and *GAPDH* were assessed using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). All qRT-PCR products were amplified on the Bio-Rad CFX96 Real Time PCR Machine in 96 wells using a SYBR green PCR Master Mix kit (Qiagen) in accordance with the manufacturer's instructions. The internal control for measuring *MSI1* mRNA in transfected cells and fresh tissues was *GAPDH*. The mRNA expression level was calculated based on the ratio of MSI1 mRNA/GAPDH mRNA applying the equation of 2-ΔΔCt method where ΔCtBC=CtBC-target - CtBC-GAPDH and ΔCtNC = CtNC-target - CtNC-GAPDH ΔΔCt = ΔCtBC – ΔCtNC, in which "BC" represents the breast cancer group, "NC" the negative control group, and "target" the wanted gene.

The 20 µl total volume for each solution well used to measure the miR expression value consisted of 2 µl of cDNA template, 0.5 µl of each reverse and forward primer (Cat. No. MPH02111), 10 µl of BrightGreen miRNA qPCR MasterMix-ROX (Cat. No. MasterMix-mR), and 7 µl of nuclease-free water. The U6-2 primers (Cat. No. MPH0001) were used as a control to determine the level of miR expression. As previously mentioned, the *miR-1249-5p* level in human tissue samples was measured and normalized using the 2-ΔΔCt method formula. The qRT-PCR reaction was performed using the subsequent three-step cycle procedure. After 10 min of activation at 95°C, the enzyme underwent 35 cycles of denaturing at 10 s for 95°C, annealing at 15 s for 60°C, and elongation at 25 s for 72°C.

Cell Culture

The non-malignant breast epithelial cell (MCF-10A) and breast cancer cell lines (MCF-7 and MDA-MB-468) used in this experiment were obtained from IraqiLab in Baghdad, Iraq. Ten percent fetal bovine serum (FBS) was added to Dulbecco's Modified Eagle Medium (DMEM) to cultivate them. Every cell line was cultured in 5% CO_2 at 37°C.

Transfection

Following the manufacturer's instructions, the cells were transfected with *miR-1249-5p* mimics (MCH01133), *miR-1249-5p* inhibitors (MIH01131), and pcDNA3-MSI (Invitrogen, China). Lipofectamine 2000 (Invitrogen) was used for transfection, as mentioned in the manufacturer's instruction.

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Observation of cell phenotype

For detection of the phenotype cells, the impact of *miR-1291-5p* on breast cancer cell proliferation was assessed using the MTT solution (0.5 mg/ml; Sigma-Aldrich, USA) and colony formation assays. This experiment used 96-well culture plates containing 3×103 MCF-7 and MDA-MB-468 cells. Incubation time of the cells was 24 h. After the transfection of miR-1249-5p inhibitors, and miR-1249-5p mimics into the cells, they were controlled for 12, 24, 36, and 48 hours. After the cells incubation for 4 hours, 20 µl of the MTT was added to each well and then removed. After that, 200 milliliters of DMSO (Sigma, USA) were added, and the plates were gently shaken. An ELISA reader was used to measure the absorbance at a wavelength of 570 nm. The cells were counted and stored at a rate of 100 cells per 12-well plate (in triplicate) to carry out the colony formation test.

Western blotting

Whole-cell proteins were extracted utilizing RIPA lysis buffer. Then, Western blotting tool was used to determine the *MESI1* expression level. SDS-PAGE (SDS-polyacrylamide gel electrophoresis) followed by Western blotting were executed to separate the molecule of protein lysates. Next, they were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). The transferred proteins on membranes were blocked with 5% non-fat milk using a blocking peptide (sc-516214). Then, incubation with antibodies against *MESI1* (1:1000, Millipore, sc-135721, Santa Cruz, CA) was performed. *GAPDH* (1:1000, Santa Cruz, CA) was utilized as an internal control. According to the manufacturer's protocols, horseradish peroxidase (HRP) conjugated secondary antibody (sc-516102) was used to visualize the blot.

Cell proliferation assay

The cells of MCF-7 and MDA-MB-468 were placed into 96-well plates at a density 1×10^4 cells/well. After 24 h, they were transfected and then cultivated for about 48 h. According to the manufacturer's instruction, Cell proliferation was measured 72 h after transfection applying the CCK8 (Cell Counting Kit-8, Beyotime, China). Ten microliters of CCK8 solution for each well was used and then kept at 37°C for four hours. The absorbance of 450 nm was used to read with the microplate reader.

Migration and invasion assay

Migrating and invasive cells were observed using a 24-well transwell plate with 8 mm pore polycarbonate membrane inserts, according to the manufacturer"s protocol (BD Biosciences, USA). At 48 h after transfection, 5×10^4 cells of MCF-7 and MDA-MB-468 were placed into the top chamber in serum-free DMEM medium at the concentration of 1.5x105/ml. Complete growth media was used for twelve hours to replace. For the invasion assay, the matrigel (14.8 µg/ml) was used to the upper surface of the membranes. Methanol was used to fix the cells that moved or invaded through surface of the membrane were fixed. Then, hematoxylin stain was used to stain

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them. Five random microscope fields per filter were determined for counting of migrating or invasive cells.

Bioinformatics prediction and statistics analysis

In this experiment, Six predicted target sites, Mirbase, Mirnamap, MirTarBase, DIANA, MirWalk, Mirmap, were used to determine the targets of *miR-1249 5p*. Statistical analysis was executed for *miR-1249-5p* expression value applying the software GraphPad Prism (V. 8.0.1) to compare the differential expression levels across sample tissues and cells. A Bonferroni adjustment was applied to the p values for the pair-wise comparisons. Results were delineated as means ± S.D., differences were tested for significance using 2-sided Student's t-test.

Results

The differential expression value of *miR-1249-5p*

To determine the differential expression value of *miR-1249-5p* in the progression of breast cancer, Real-Time quantitative PCR (RT-qPCR) was used to quantify the expression levels of *miR-1249-5p* in breast cancer cell lines and sample tissues. In the present study, 50 sample tissues with different stages were studied. The RT-qPCR results showed that the expression of *miR-1249-5p* was significantly lower (2.3-fold, $p \leq 0.001$) in cancer tissues (BCs), when compared with normal to adjacent tissues (NATs) (Figure 1I). Moreover, the *miR-1249-5p* expression level in non-metastatic tissues was significantly higher (0.7-fold, $p \leq 0.05$) than in metastatic tissues (Figure 1II). Tumor cells expressed lower amounts of *miR-1249-5p* with malignancy stage (Figure 1III). Due to funding constraints, two cell lines, MCF-7 and MDA-MB-468, were utilized. The result revealed that the *miR-1249-5p* expression value was much lower in highly metastatic MDA-MB-468 cells (0.4-fold, $p \leq 0.05$) than in low metastatic MCF-7 cells (2.1-fold) (figure 1IV).

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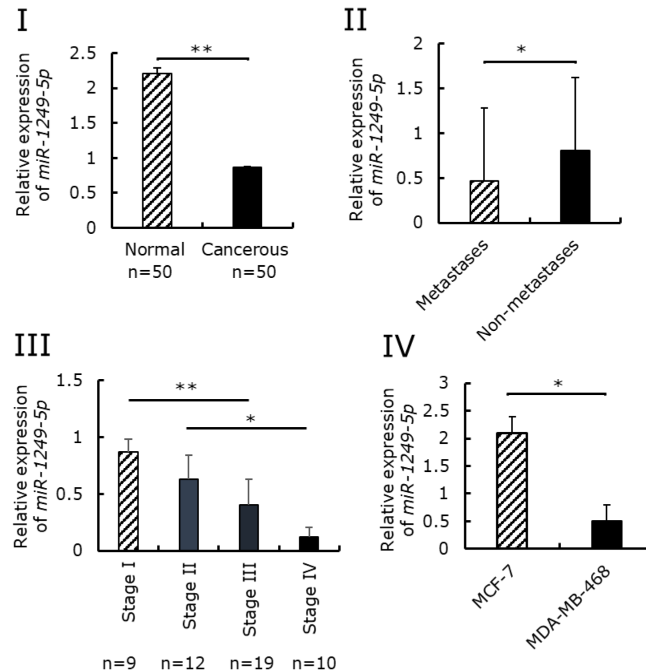

Figure 1. Differentially expressed *miR-1249-5p* in breast cancer cells and tissues. **I**: Relative expression of *miR-1249-5p* in cancerous and normal to adjacent tissues. **II**: Relative expression of *miR-1249-5p* with breast cancer staging. **III**: Differential expression of *miR-1291* in non-metastases and metastases tissues. **IV**: *miR-1249-5p* with decreased expression in MCF-7 and MDA-MB-468 cells. Statistical significance is denoted as follows: * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

miR-1291 boosts breast cancer cell development

To determine the function of *miR-1249-5P* in cell growth regulation, MCF-7 or MDA-MB-468 cells were transfected with either a *miR-1249* (mimic) or an anti-*miR-1249* (inhibitor). Compared to the control group, the transfection of *miR-1249* significantly decreased *miR-1249* expression level in MCF-7 cells (Figure 2I), while it significantly boosted in the MDA-MB-468 cells (Figure 2II). Subsequently, the effects of *miR-1249* or anti-*miR-1249* on cell growth were assessed. The outcomes from MTT and colony formation assays discovered that introducing miR-1249 prevented the growth of MCF-7 cells (Figures 2III and 2IV). Conversely, when the anti-miR-1249 increased the expression of *miR-1249-5p*, it stimulated the proliferation of MDA-MB-468 cells (Figures 2V and 2VI). Furthermore, the results of the Annexin V experiment showed that the level of cell death (apoptosis) was significantly higher in miR-1249-5p cells than in the control group (Figure 2VII). In contrast, there was a clear reduction in apoptosis of MDA-MB-468 cells when anti-miR-1249 was present (Figure 2VIII). In addition, confirmation was achieved via the GRP64 gene (G-protein coupled receptor 64), which encodes an oncoprotein essential for regulating cell development and apoptosis. The results of the western blot revealed that the miR-1249 raised GRP64 expression value (Figure 2IX).

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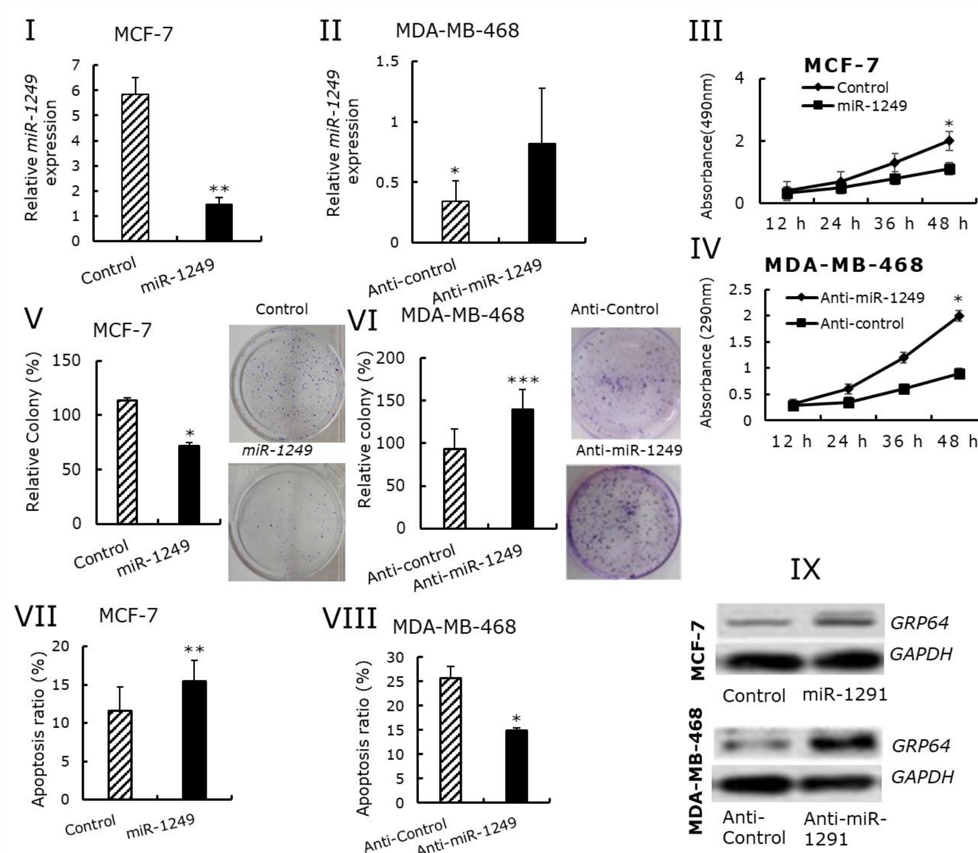

Figure 2. miR-1249-5p prevent the cell growth of breast cancer cells and boosted cellular death (apoptosis). **I** and **II**: RT-qPCR was used to determine the effectiveness of miR-1249 and anti-miR-1249 in MCF-7 and MDA-MB-468 cells, respectively. **III** and **IV**: The viability of MCF-7 cells transfected with miR-1249 or MDA-MB-468 transfected with anti-miR-1249 for 12, 24, 36, and 48 hours was assessed using the MTT method. **V** and **VI**: To evaluate the cells' capacity for long-term proliferation, MCF-7 cells transfected with miR-1249 or MDA-MB-468 cells transfected with anti-miR-1249 were employed in the colony formation experiment. **VII** and **VII**: The Annexin V assay was performed to detect cell apoptosis in MCF-7 cells transfected with miR-1249 or MDA-MB-468 cells transfected with anti-miR-1249. **IX**: The impact of miR-1249 and anti-miR-1249 on *GRP64* expression in MCF-7 and MDA-MB-468 cells, respectively, was evalauted by Western blot. The star symbols indicating statistical significance are: * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

Breast cell invasion and migration decreased by *miR-1249-5p*

In the present study, the role of *miR-1249-5p* on cell metastasis was determined. Both Transwell invasion and Wound Healing assays were utilized. The findings showed that miR-1249 (mimic) could decreased MCF-7 cell invasion, as compared to the control group (Figure 3I). In contrast, ant-miR-1249-5p (inhibitor) could boost the cell division in MDA-MB-468 cell line (Figure 3II). Besides, the outcomes of Wound Healing assay showed that the *miR-1249-5p* played a key role in cell migration. Figures 3III and 3IV showed that whereas miR-1249 (mimics) reduce the mobility

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of MCF-7 cells, but anti-miR-1249 (inhibitor) increased the cell mobility capacity in MDA-MB-468 cells. These consequences confirmed that the down-regulation of *miR-1249-5p* could decrease the invasion and migration of the breast cancer cell. Wound healing assay was applied to observe the role of miR-1249 on cell migration. The miR-1249 (mimics) boosted the migration potential of MCF-7 cells, whereas anti-miR-1249 prevented the migration potential of MDA-MB-231 cells (Figure 3V and 3VI). These consequences proved that miR-1249-5p has the ability to promote the cells invasion and migration of breast cancer.

***miR-1249-5p directly targets MSI1* gene**

Here, six computational predicted sites, including MirMap, MirBase, PicTar, MirTar2, MirPath, and DIANA, were applied to propose *MSI1* gene as the direct target of *miR-1249-5p.* The subsequent step involved investigating the potential mechanism through which *miR-1291* influences cell migration and invasion.

The 3'UTR luciferase reporter assay was applied to determine if *miR-1249-5p* directly targets *MSI1*. The result showed that the luciferase activity of the wild-type *MSI1* 3'UTR was significantly down by miR-1249 (mimics) in MCF-7 cells, whereas the significant inhibition was up when the seed sequences of the target were mutated in the *MSI1* 3'UTR, as shown in Figure 3VII, . The cells in MCF-7 and MDA-MB-468 were transfected with miR-1249 (mimics) or anti-miR-1249 (inhibitors) to measure the influence of *miR-1249-5p* on the *MSI1* expression in order to confirm if *miR-1249* directly targets *MSI1* gene. The Western blot outcomes showed that transfection with miR-1249 mimics considerably decreased *MSI1* expression in MCF-7 cells, while ant-miR-1249-5p inhibitor increased *MSI1* protein expression (Figure 3VIII and 3IX). These outcomes indicated that *miR-1249-5p* inhibits the *MSI1* protein synthesis by directly targeting the 3'UTR of *MSI1* mRNA.

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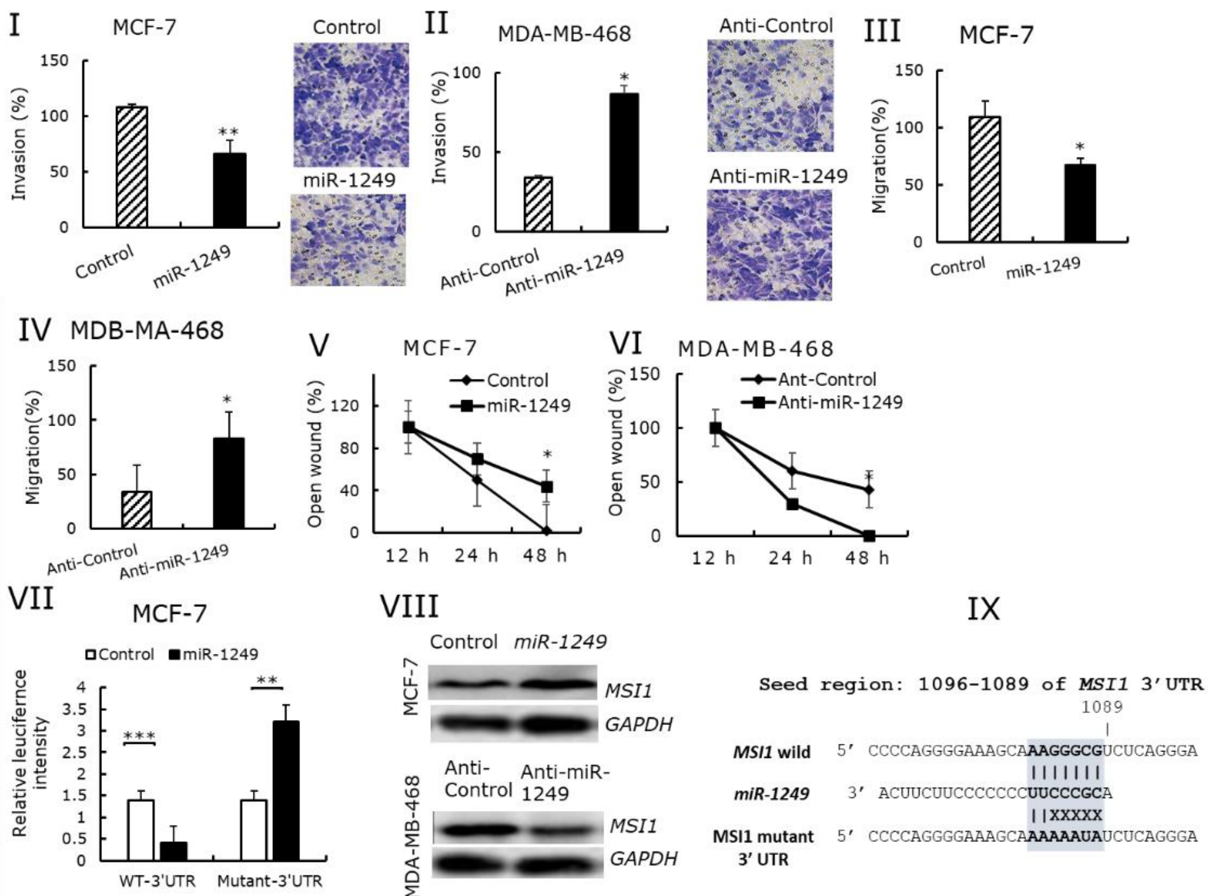

Figure 3. Inhibition of *MSI1* through *miR-1249-5p*, resulting in improved cell invasion. **I** and **II**: Transwell invasion experiment was conducted applying MCF-7 cells transfected with miR-1249 or MDA-MB-468 cells transfected with anti-miR-1249. **III** and **IV**: A Wound healing assay was carried out applying MCF-7 cells transfected with miR-1249 or MDA-MB-468 cells transfected with anti-miR-1249. **V** and **VI**: The viability of MCF-7 cells transfected with miR-1249 or MDA-MB-468 transfected with anti-miR-1249 for 12, 24, 36, and 48 hours was evaluated applying the wound healing assay. **VII**: 3'UTR luciferase reporter experiment was performed using MCF-7 cells co-transfected with either miR-1291 plus WT-3'UTR or *miR-1249-5p* plus mutant-3'UTR. **VIII**: Western blot experiment was performed using MCF-7 cells transfected with miR-1249 or MDA-MB-468 cells transfected with anti-miR-1249, and *MSI1* protein expression was quantified with *GAPDH* as an expression control. **IX**: The *miR-1249*-5p seed sequence binding site in *MSI1* 3'UTR (site: 1069-1089bps) was emphasized (bold color and grey background). Statistical significance is represented as follows: * for p ≤ 0.05, ** for p ≤ 0.01, and *** for p ≤ 0.001.

Down regulation of *GRP64* and *MSI1* considerably reduces the stimulation of cell growth and metastasis by *miR-1249-5p*.

The *MSI1* expression as a direct target of *miR-1249-5p* in breast cancer cell lines and tissues was confirmed. The RT-qPCR was utilized to measure *MSI1* mRNA expression values in breast cancer cell lines, which showed that *miR-1249-5p* had higher expression from MCF-7 and lower

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expression from MDA-MB-468 cells (Figure 4I). Furthermore, *MSI1* mRNA and protein expression was significantly upregulated in breast cancer (BC) tissue compared to normal to adjacent tissues (NATs) (Figure 4II and 4III). To confirm that the effects of *miR-1249-5p* on cellular invasion, proliferation, and migration are mediated by the inhibition of *GRP64* and *MSI1*, a rescue experiment was performed. Transfection of pcDNA3-*GRP64* promoted the growth effects of *miR-1249-5p* on MCF-7 cells (Figure 41V). Furthermore, transfection of pcDNA3-*MSI1* in MCF-7 cells was confirmed to promoted the invasion and migration of cells induced by miR-1249 mimics (Figure 4V and 4VI). The findings illustrated in Figure 4VII demonstrated that *GRP64* and *MSI1* boosted the proliferation, invasion, and migration of breast cancer cells triggerd by miR-1249-P5.

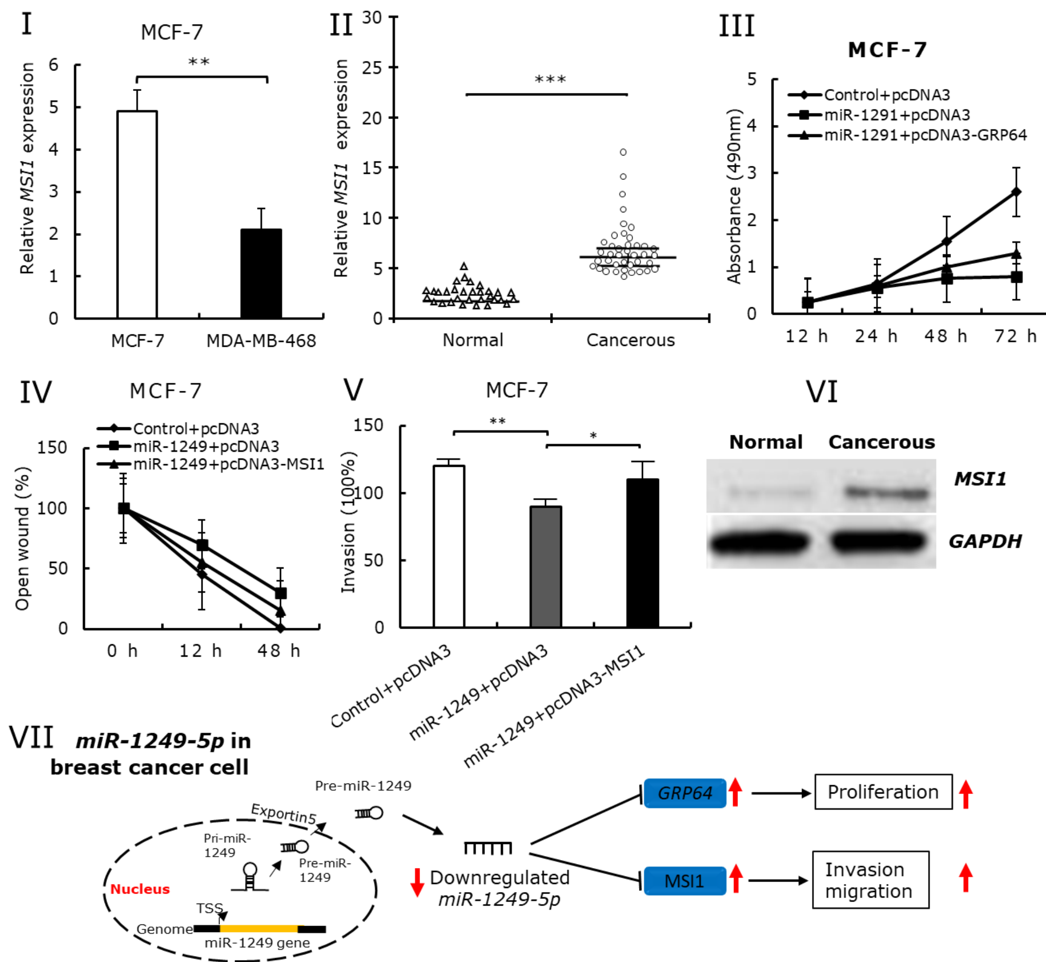

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Figure 4. *GRP64* and *MSI1* could reverse the *miR-1249-5p* caused cell invasion, migration, and proliferation. (I) Relative *MSI1* Expression in MCF-7 and MDA-MB-468 cells. (II) Relative mRNA expression of *MSI1* in breast cancer (BC) and normal to adjacent tissues (NATs). (III) The effect of GRP64 downexpression on miR-1249-5p caused cell viability detected with MTT. IV and V: The effect of *MSI1* downexpression on miR-1249-5p caused cell invasion and migration detected with the transwell and wound

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healing assay. (VI) *MSI1* protein expression in NATs and BC tissues. (VII) An illustration of miR-1249 mechanism on breast cancer cells proliferation, invasion and migration. Statistical significance is represented as follows: * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

Discussion

There is evidence that almost all human malignancies are associated with aberrant miRNA expression, which plays a crucial role in controlling target genes by either stimulating or inhibiting their translation. Therefore, the expression levels of miRNA in normal and cancerous cells or tissues may differ. For example, compared to normal breast and matching non-tumor breast tissues, the level of miR-205 is considerably lower in breast cancer cells and tissues[20]; in Hp-infected gastric cancer, miR-210 is down-regulated[21].

According to this study, breast cancer tissues and cells have a significant downregulation of miR-1249-5p. These results prompted researchers to investigate the possibility that miR-1249-5p may control cellular phenotypes. Recently, a number of miRNAs, such as *miR-7*[22], *miR-15a*[23], *miR-34a*[24],miR-140[25], *miR-146a*[26], *miR-191*[27], *miR-200c*[28], *miR-204*[29], *miR-205*[30], *miR-210*[31], *miR-214*[32], *miR-335*[33], *miR-638*[34], *miR-1301*[35], *miR-1275*[4], etc., have been experimentally confirmed to be linked to cellular proliferation, invasion, and migration. We discovered that upregulation of miR-130a caused the suppression of proliferation, invasion, and migration in MCF-7 and MDA-MB-468 cells, which allowed us to experimentally confirm that *miR-1249-5p* is a potential tumor suppressor of breast cancer cells. Our discovery broadened the list of miRNA members implicated in the pathophysiology of breast cancer.

Target gene expression can be upregulated or downregulated by miRNA in a variety of ways. Using bioinformatics and functional knowledge related to miR-1249-5p, the *MSI1* gene was selected as a potential gene for more research in order to determine the miR-1249-5p target genes responsible for its effects on breast cancer cells. In the 3'UTR luciferase reporter test, *miR-1249-5p* suppressed the expression of the luciferase reporter plasmid carrying the *MSI1* 3'UTR; this effect was eliminated by the mutant *MSI1* 3'UTR. Additionally, *miR-1249-5p* reduced *MSI1* mRNA and protein expression levels in breast cancer cells when compared to the control, according to qRT-PCR and Western blot analysis. These findings collectively imply that *miR-1249-5p* binds to the 3'UTR of *MSI1* to downregulate its expression. Breast cancer [19], colon cancer[36], gastric cancer [37], hepatocellular carcinoma [38], glioblastoma [39], ovarian cancer, and cervical carcinomas [40] have all been shown to have substantial overexpression of *MSI1*. Additionally, our study's immunohistochemical analysis revealed that *MSI1* was elevated in breast cancer tissues. This result further showed that the elevation of MSI1 in breast cancer tissues may be caused by the downregulation of *miR-1249-5p*. The *miR-1249-5p/MSI1* axis in the control of Wnt and the PI3K/AKT pathway in the pathophysiology of breast cancer requires further exploration, as the findings of the present study indicate an additional layer of post-transcriptional *MSI1* regulation via *miR-1249-5p*.

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Conclusion

The findings of this study demonstrate that *miR-1249-5p* inhibits the growth, invasion, and migration of breast cancer cells. *MiR-1249-5p* was found to directly target the gene *MSI1*. Understanding the *miR-1249-5P/MSI1* regulation network presents a significant opportunity for the clever multitargeted development of novel treatments for breast cancer.

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