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MicroRNA-1291–Mediated Regulation of KLF6: Integrating Protein Expression and Post-Transcriptional Control in Breast Cancer Progression

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Abstract

MicroRNAs (or miR) are a group of RNA transcripts that post-transcriptionally mediate protein-coding genes. When the miR expression was aberrant, different types of cancer were developed by altering their target gene expression. This study aimed to determine the role of *miR-1291* expression and its targeted *KLF6* gene in breast cancer (BC). Here, *KLF6* was determined applying putative target sits as a direct target of *miR-1291*. Real-time quantitative PCR outcomes showed that the expression of *miR-1291* was significantly up (P value=0.003) and *KLF6* was significantly down (P value=0.001) in 50 BC participants and 50 normal adjacent to tumor (NAT). Furthermore, 3' UTR luciferase reporter validated that *miR-1291* expression level in BC cell lines and tissues was elevated. While *miR-1291* mimics are up-regulated, the BC cell growth and metastasis are enhanced by directly suppressing *KLF6* in BC cell lines and tissues. In conclusion, the consequences suggest that *miR-1291* acts as an oncogene, promoting the growth and metastasis of BC cells. *KLF6* is a direct target gene for *miR-1291* in BC cells, and upregulation of *KLF6* is likely to reverse the abnormal growth phenotype induced by *miR-1291*.

Keyword: Breast cancer, Cell growth, Invasion, Metastasis, *miR-1291*, *KLF6*

Introduction

Breast cancer (BC) is the most prevalent malignancy among women globally [1] and a complicated illness that is impacted by a number of environmental factors, as well as heterogeneity of genetic changes. Dysregulation of oncogenes often occurs in the later stages of tumor progression and is associated with increased tumor aggressiveness [2, 3]. As a result, tumor cells may migrate from the original tumor to other locations [4, 5]. Numerous proteins are involved in growth factor signaling, angiogenesis, adhesion molecules, proteases, and the regulation of proliferation and metastasis [5]. Therefore, early detection and treatment intervention may benefit from an understanding of the alterations in gene and protein expression that occur throughout the development of breast cancer.

MicroRNAs (miRNAs) are characterized as small, non-coding RNAs (19–25 bp) that function as master regulators of gene expression by causing target mRNAs to be broken down or blocked translationally[6]. A completely new level of gene regulation has been uncovered by the identification of miRNAs and their mode of function. To control the expression of their mRNA targets, miRNAs must come together to form a complex referred to as the RNA induced silencing complex (RISC). After they are assembled together, they attach to the 3' untranslated region (3'-UTR) and cause transcriptional suppression or degradation [7]. Hundreds of different mRNAs can be regulated by a single miRNA, and over 5,000 human miRNAs have been found that may affect about one-third of the coding genes in the human genome[8].

There is evidence that a correlation has been made between human cancers and miRNAs dysregulated expression. Since almost one-third of the coding genes in the human genome are either oncogenes or tumor suppressor genes, miRNAs with oncogenic or tumor suppressor characteristics have been detected. The involvement of miRNAs in the proliferation, apoptosis, invasion/metastasis, and angiogenesis of normal and cancer cells are being thoroughly studied [9, 10]. The aberrant expression patterns of miRNAs have been researched in a variety of malignancies, including breast cancer[11].

According to many researches, the expression profiles of miRNAs in human malignancies differ from those in normal tissues. Numerous studies have shown that miRNAs are dysregulated in breast cancer, with clusters of miRNAs often being either over-expressed or down-regulated.

Furthermore, the regulatory function of miRNAs affects several biological processes, and dysregulation of miRNAs has been linked to a number of human diseases, including cancer, and plays a crucial role in different stages of the metastatic process.

SNORA34 (small nucleolar RNA H/ACA box 34) is the source of *miR-1291* in the pancreatic cancer cell line *PANC-1* [12]. *MiR-1291* was shown to have many roles in earlier research. In pancreatic cancer clinical samples, *miR-1291* expression was markedly decreased [13]. By focusing on multidrug resistance-associated protein 1 (*MRP1*/*ABCC1*), *miR-1291* has been demonstrated to influence cellular drug chemosensitivity and disposition [13]. Additionally, *miR-1291* directly influences a number of metabolic pathways, including glucose transporter 1 (*GLUT1*) and forkhead box protein A2 (*FOXA2*), to influence tumor cell invasion and proliferation [14]. Nevertheless, it is still unclear what molecular processes underlie these significant roles.

In the present study, the RT-qPCR outcomes showed that *miR-1291* was significantly increased in breast cancer tissues, as compared with fresh tissue. In breast cancer cell lines and tissues, the expression value of *miR-1291* was found to be increased. Ectopic expression of *miR-1291* mimics contributed to promoting cell growth, invasion, and migration. Using target predicted sites, it was discovered that the *KLF6* 3'UTR had a potential *miR-1291* binding site. The 3'UTR luciferase reporter experiment was used to establish that *miR-1291* may directly target *KLF6* mRNA. The endogenous expression of *KLF6* is suppressed by the forced expression of *miR-1291*. Additionally, the results showed that *KLF6* was down-regulated in tissues and cell lines associated with breast cancer. The rescue experiment showed that overexpression of *IGF1R* attenuates *miR-1291*-promoted cell growth, whereas overexpression of *KLF6* attenuates *miR-1291*-promoted cell invasion and migration. When considered collectively, this research shows that *miR-1291* functions as an oncogene to encourage the growth, invasion, and migration of breast cancer, potentially making it a crucial therapeutic target for the treatment of breast cancer.

Material and Method

Sample collection

This study was conducted with the consent of all cases for the analysis of their specimens. This procedure is obtained approval by the local Human Research Ethics Committee (HREC) at Science College in Salahuddin University-Erbil (Reference no. SU2026HREC/59). Moreover, all programs while performing the study were conducted in accordance with the 1964 Helsinki Declaration. The fresh tissue specimens were harvested from 50 cases with breast cancer (BC) at the private CMC hospital. For each case, two fresh tissue samples (one BC tissue and one normal adjacent to tumor (NAT)) were harvested and stored at -80°C. The NAT was gotten about 4 cm distal to the malignant margine. The NAT were diagnosed and separated from the BC tissues by histopathologist at the histopathological laboratory. The including criteria required that these patients were not taken any radiotherapy or chemotherapy. By a questionnaire, the information about clinical characterizations of the 50 cases were obtained. Table 1 demonstrates the clinical characterizations.

Total RNA Extraction for mRNAs and miRNAs Detection

Total RNA transcripts were extracted applying RNA/DNA Purification Plus kit (Cat. No. 54300, NORGEN BIOTEK CORP, Canada) from human breast cancer and normal adjacent tissues for determination the expression level of *miR-1291* or *KLF6* . Two μg of each total RNA sample was aliquoted to generated complementary (c)DNA using the miRNA All-In-One cDNA Synthesis Kit (Cat. No. G898, abmgood company, US) according to the manufacturer's instructions.

The qRT-PCR was used to evaluate *KLF6* and *GAPDH* in order to quantify the mRNA expression value. In accordance with the manufacturer's recommendations, all qRT-PCR products were amplified using a SYBR green PCR Master Mix kit (Qiagen) on the Bio-Rad CFX96 Real Time PCR Machine, 96 wells. GAPDH was utilized as the internal control to measure PURB mRNA in transfected cells and fresh tissues. The mRNA expression level was measured based on the ratio of *KLF6* mRNA/GAPDH mRNA applying the equation of 2-ΔΔCt method where ΔΔCt = ΔCtBC – ΔCtCon = (CtBC-target - CtBC-*GAPDH*) - (CtCon-target - CtCon-*GAPDH*), in which "BC" represents the BC tissue, "Con" the negative control group, and "target" the desired gene.

Two microliters of cDNA template, one microliter of each reverse and forward primer (Cat. No. MAH01227), ten microliters of BrightGreen miRNA qPCR MasterMix-ROX (Cat. No. MasterMix-mR), and six microliters of nuclease-free water made up the twenty microliter total volume for each solution well used to quantify the miR expression value. The amount of miR

expression was measured using the U6-2 primers (Cat. No. MPH0001) as a control. As previously explained, the 2-ΔΔCt method formula was used to assess and normalize the *miR-1291* level in human tissue samples. The following three-step cycle protocol was used to carry out the qRT-PCR reaction. The enzyme was activated at 95°C for ten minutes, followed by 35 cycles of denaturing at 95°C for ten seconds, annealing at 60°C for fifteen seconds, and extension at 72°C for twenty-five seconds.

Cell Culture and Transfection

IraqiLab in Baghdad provided the non-malignant breast epithelial cell (MCF-10A) and breast cancer cell lines (MCF-7 and MDA-MB-468) utilized in this study. To culture them, Dulbecco's Modified Eagle Medium (DMEM) was supplemented with 10% fetal bovine serum (FBS). Each cell line was grown at 37°C with 5% CO2. The cells were transfected with *miR-1291* mimics (MNH01227), *miR-1291* inhibitors (MIH01227), and pcDNA3-*KLF6* (Invitrogen, China) in accordance with the manufacturer's instructions. Lipofectamine 2000 (Invitrogen) was used for transfection, as explained in the manufacturer's protocol.

Observation of cell phenotype

Using the MTT solution and colony formation assays, the impact of *miR-1291-5p* on breast cancer cell proliferation was assessed. Here, 96-well culture plates containing 3×103 MCF-7 and MDA-MB-468 cells were used. The cells were incubated for 24 h. Transfection of *miR-1291* mimics, *miR-1291* inhibitors, and their controls were then performed into the cells for 12, 24, 36, and 48 hours. Next, twenty µl of MTT (0.5 mg/ml; Sigma-Aldrich, USA) were applied to each well. The MTT solution was removed, 200 milliliters of DMSO (Sigma, USA) were added, and the plates were gently shaken after an additional 4 hours of incubation. An ELISA reader was used to measure the absorbance at a wavelength of 570 nm. The Cells were counted and seeded at a rate of 100 cells per 12-well plate (in triplicate) to carry out the colony formation experiment.

The effect of *miR-1291-5p* on proliferation of breast cancer cells was evaluated by MTT and colony formation assays. MCF-7 and MDA-MB-468 cells were plated in 96-well culture plates (3×103 per well). After 24 h incubation, the cells were transfected with *miR-1291* mimics, *miR-*

1291 inhibitor and their controls for 12, 24, 36 and 48 hours. Then the MTT (0.5 mg/ml; Sigma-Aldrich, USA) was added to each well (20 µl/well). After 4 hours of additional incubation, MTT solution was discarded and 200 ml of DMSO (Sigma, USA) was added and the plates shaken gently. The absorbance was measured on an ELISA reader at a wavelength of 570 nm. For colony formation assay, cells were counted and seeded in 12-well plates (in triplicate) at 100 cells per well. Fresh culture medium was replaced every 3 days. The number of viable cell colonies was determined after 14 days and colonies were fixed with methanol, stained with crystal violet, photographed and counted. Each experiment was performed in triplicate.

Invasion assay

The Transwell chamber with 8 µm pores (Corning, USA) was used in this assay. On the inner surface, 50 µl diluted matrigel (2 mg/ml) was put on. For 48 h, cells were transfected and separated to obtain a final concentration at 2×105/ml. Then, they were on on the top chamber. With a cotton-tipped swab, non-invasive cells were isolated from the top of the Matrigel after twenty-four hours. At the bottom of the Matrigel, invasive cells were fixed in methanol. Crystal violet was used to stain the invasive cells. For invasiveness determination, a microscope at ×200 magnification of five random fields was used to count the invasive cells in each well. Each test was conducted in triplicate.

Migration assay

The process of cells transfection was performed for twenty-four hours. The cells were then harvested and placed in 12 well plates (3×105/well) for twenty-four hours. When the cells got ninety-percent confluence, sterile pipette tips were used to scratch the wound regularly. Assessment of cell movement was performed by measuring the cells motility into a scraped wound. By assessing the distance of the wound from 0 h, the speed of wound closure after seventy-two hours was scrutinized. Each test was performed in twice.

Western blotting

In the present study, the total proteins were extracted according to the manufacturers protocol RIPA (sc-24948) in 48 hours after transfection. Western blotting was performed to measure *KLF6* expression level. Phosphate Buffered Saline (PBS, sc-24946) was used to wash the cells twice. Then, the protein concentration in the supernatants was measured by Bradford protein dye reagent

(Bio-Rad, Hercules, CA). Then, the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was prepared to separate the molecule of protein lysates. After that, Towbin, with SDS, 10X (sc-24954) was used to the transfer protein lysates to a polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). Then, 5% non-fat milk using blocking peptide (sc-516214) used to block the membranes. The membranes were incubated with antibodies against *KLF6* (1:1000, sc-462 AF488). After transfer, the blot was probed with *KLF6* (1:1000) and GAPDH (1:1000) antibody and visualized by Horseradish peroxidase (HRP) conjugated secondary antibody (sc-516102).

Data analysis

To select the target of *miR-1291*. The target predicted websits, such as Mirmap, Mirbase, RNA22, PicTar, MirWalk and MirTarBase, were applied. To analyze the distribution of expression level of *miR-1291* and its target across sample tissues and cells, the software GraphPad Prism (V. 8.0.1) was used. The p values for the pair-wise comparisons were adjusted applying a Bonferroni adjustment. The differences were two-sided and the significance level was determined at $P<0.05$. The findings were shown as the mean ± S.D (Standard division).

Results

The expression level of *miR-1291*

Here, quantitative real-time PCR (RT-qPCR) was employed to quantify the expression levels of *miR-1291* in breast cancer cell lines and tissues in order to examine the function of *miR-1291* in the pathogenesis of breast cancer. The cell lines of MCF-7 and MDA-MB-468 were used. Figure 1I revealed that the expression of *miR-1291* was much higher in highly metastatic MDA-MB-468 cells (3.6-fold, $p \leq 0.01$) than in low metastatic MCF-7 cells (0.9-fold).

The qRT-PCR was used to measure the expression value of *miR-1291* in breast cancer and normal tissues in order to further validate the significance of *miR-1291* during breast cancer progression. In comparison to normal breast tissues, the expression of *miR-1291* was considerably higher (2.2-fold, $p \leq 0.001$) in breast cancer tissue (Figure 1II). In addition, tumor cells expressed increasing amounts of *miR-1291* with malignancy stage (Figure 1III). The expression of *miR-1291* in metastatic tissues was significantly higher (3.1-fold, $p \leq 0.05$) than in non-metastatic tissues (Figure 1IV).

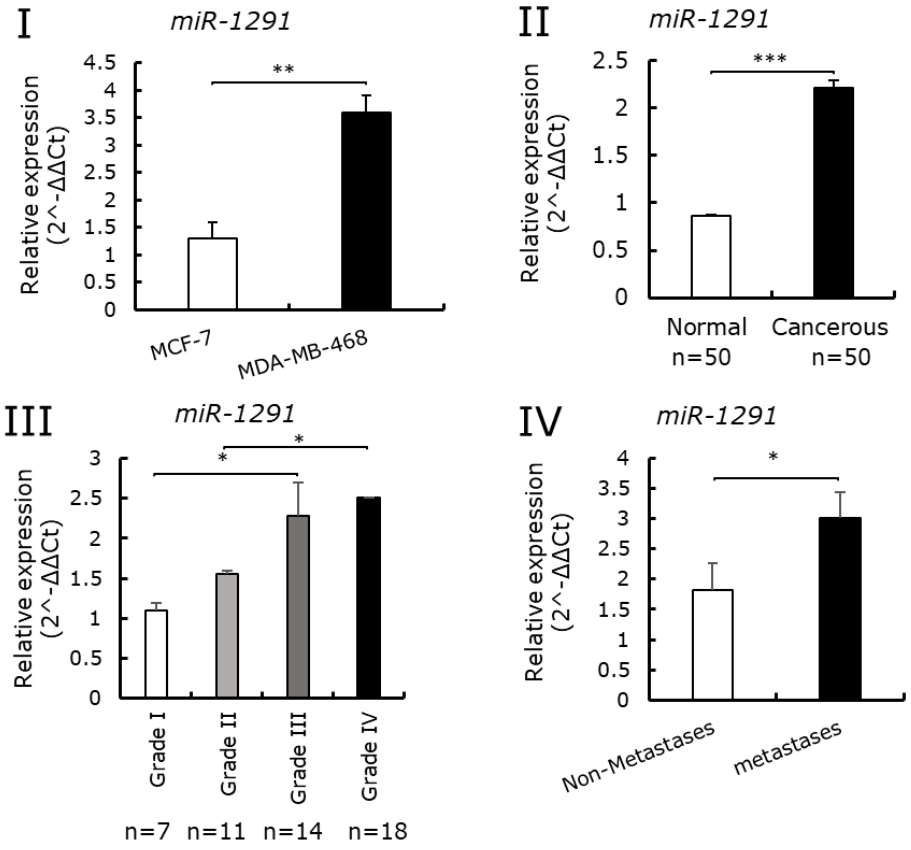

Figure 1. *miR-1291* differential expression in breast cancer cells and tissues. **I**: *miR-1291* with increased expression in MCF-7, and MDA-MB-468 cells. **II**: Relative expression of *miR-1291* in cancerous and fresh tissues. **III**: Differential expression of *miR-1291* with breast cancer staging. **IV**: Differential expression of *miR-1291* in non-metastases and metastases tissues. Statistical significance is represented 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 examine the role of *miR-1291* in regulating cell growth, MCF-7 or MDA-MB-468 cells were transfected with either a *miR-1291* activator (mimic) or an anti-*miR-1291* (inhibitor). In comparison to the control group, the transfection of *miR-1291* mimic (1291 mimic) significantly boosted the level of *miR-1291* in MCF-7 cells (Figure 2I). However, the expression value of *miR-1291* in the MDA-MB-468 cells was significantly decreased (Figure 2II). Next, the effects of *miR-1291* mimic or *miR-1291* inhibitor (1291 inhibitor) on cell growth were investigated. The outcomes of the MTT and colony formation tests revealed that the introduction of 1291 mimic boosted the growth of MCF-7 cells (Figures 2III and 2IV). However, when the 1291 inhibitor inhibited the expression of *miR-1291*, the proliferation of MDA-MB-468 cells was decreased (Figures 2V and 2VI). Moreover, the Annexin V experiment revealed that the 1291 mimics significantly reduced cell death (apoptosis), as compared to the control group (Figure 2VII), but the 1291 inhibitor clearly elevated MDA-MB-468 cell apoptosis (Figure 2VIII). Moreover, confirmation was gained by means of the *IGF1R* gene (Insulin-like Growth Factor 1 Receptor), which codes a tumor suppressor protein crucial for controlling cell development and apoptosis. The western blot outcome demonstrated that the 1291 mimics inhibited *IGF1R* expression (Figure 2IX).

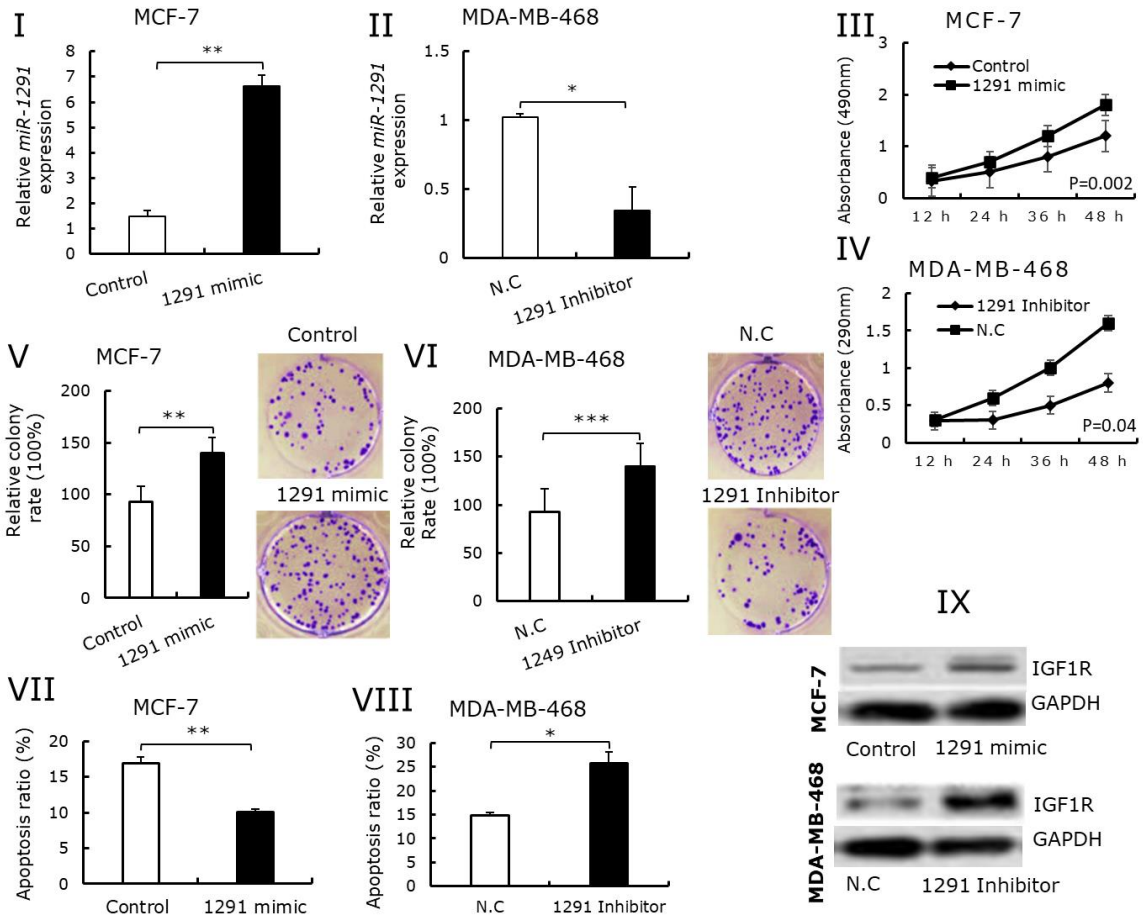

Figure 2. *miR-1291* blocked cellular apoptosis and boosted the development of breast cancer cells. **I** and **II**: The efficacy of 1291 mimic and 1291 inhibitor in MCF-7 and MDA-MB-468 cells, respectively, was assessed by RT-qPCR. **III** and **IV**: The MTT assay was used to measure the viability of MCF-7 cells transfected with *miR-1291-5p* or MDA-MB-468 transfected with 1291 inhibitor for 12, 24, 36, and 48 hours. **V** and **VI:** MCF-7 cells transfected with 1291 mimic or MDA-MB-468 cells transfected with 1291 inhibitor were used in the colony formation experiment to measure the cells' long-term proliferation potential. **VII** and **VII**: Using MCF-7 cells transfected with 1291 mimic or MDA-MB-468 cells transfected with 1291 inhibitor, the Annexin V test was used to identify cell apoptosis. **IX**: The impact of 1291 mimic and 1291 inhibitor on *IGF1R* expression in MCF-7 and MDA-MB-468 cells, respectively, was assessed by Western blot.Statistical significance is represented as follows: * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

MiR-1291 boostes the invasion and migration of breast cancer cell lines.

To further investigate whether *miR-1291* affects cell metastasis, the Transwell invasion and wound healing experiments were employed. In contrast to the control group, 1291 mimics increased MCF-7 cell invasion (Figure 3I), whereas 1291 inhibitor decreased MDA-MB-468 cell invasion (Figure 3II). Additionally, the function of *miR-1291* in cell migration was ascertained using the wound healing experiment. Figures 3III and 3IV showed that whereas 1291 mimics increased the migratory capacity of MCF-7 cells, 1291 inhibitor reduced the migration potential of MDA-MB-468 cells. These results showed that *miR-1291* might boost breast cancer cell invasion and migration.

KLF6 gene determination as a direct target of *miR-1291*

The subsequent step involved investigating the potential mechanism through which *miR-1291* influences cell migration and invasion. Based on bioinformatic analyses employing four computational algorithms; Including Mirbase, MirTarBase, PicTar, MirPath, and MirTar2. The *KLF6* (Krüppel-like factor 6), a previously identified protein known to regulate invasive cell migration, was proposed as a potential target of *miR-1291*.

Evidence suggests that miRNAs induce degradation of mRNA or translational inhibition by mismatched base pairing to the 3'UTR of target genes (Figure 3VII). The 3'UTR luciferase reporter assay was applied to determine if *miR-1291* directly targets *KLF6*. As shown in Figure 3V, the luciferase activity of the wild-type *KLF6* 3'UTR was significantly decreased by 1291 mimics in MCF-7 cells, but the significant inhibition was abrogated when the seed sequences of the *miR-1291* target sequences were mutated in the *KLF6* 3'UTR.

The cells in MCF-7 and MDA-MB-468 were transfected with 1291 mimics or 1291 inhibitors to evaluate the effect of *miR-1291* on the expression of *KLF6* protein in order to confirm if *miR-1291* directly targets *KLF6*. When compared to the control group, the Western blot findings demonstrated that transfection with 1291 mimics dramatically prevented *KLF6* expression in MCF-7 cells, while 1291 inhibitor considerably boosted *KLF6* protein expression (Figure 3VI). These results showed that *miR-1291* suppresses the synthesis of *KLF6* protein by specifically targeting the 3'UTR of *KLF6* mRNA.

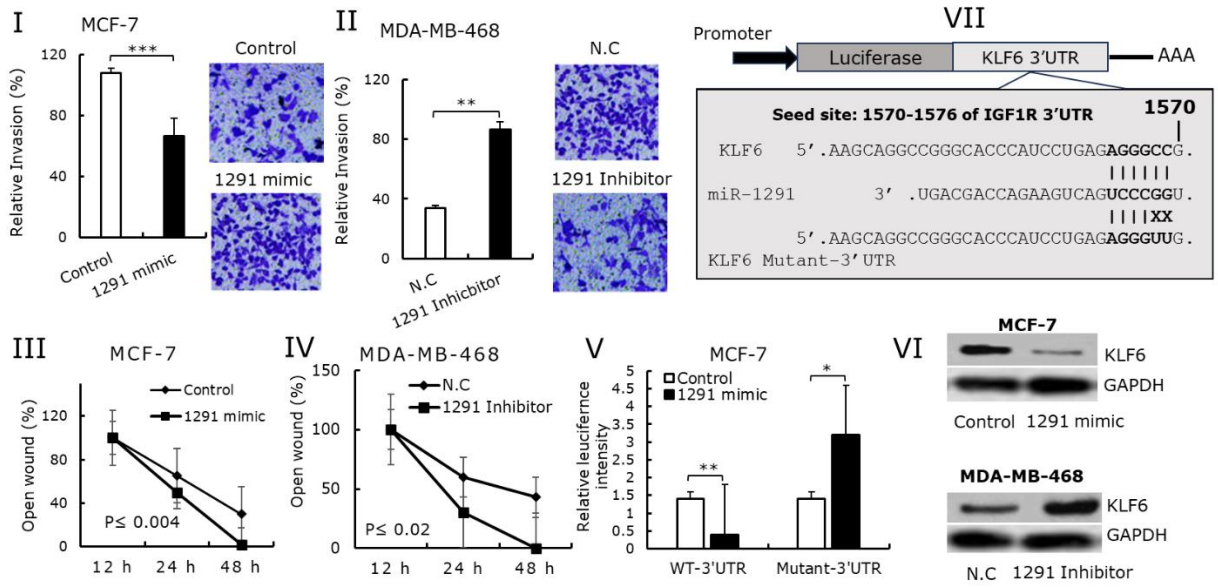

Figure 3. *miR-1291* inhibits *KLF6*, resulting in increased cell spread. **I** and **II**: Transwell invasion experiment was performed using MCF-7 cells transfected with 1291 mimic or MDA-MB-468 cells transfected with 1291 inhibitor. **III** and **IV**: A Wound healing experiment was performed using MCF-7 cells transfected with 1291 mimic or MDA-MB-468 cells transfected with 1291 inhibitor. **V**: The *miR-1291* seed sequence binding site in *KLF6* 3'UTR (position: 1570-1576 bps) was highlighted (bold site). **VI**: 3'UTR luciferase reporter experiment was performed using MCF-7 cells co-transfected with either *miR-1291* plus WT-3'UTR or *miR-1291* plus mutant-3'UTR. **VII**: Western blot experiment was performed using MCF-7 cells transfected with 1291 mimic or MDA-MB-468 cells transfected with 1291 inhibitor, and *KLF6* protein expression was quantified with *GAPDH* as an expression control. Statistical significance is represented as follows: * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

The promotion of cell growth and metastasis by *miR-1291* is significantly reduced when *IGF1R* and *KLF6* are upregulated.

The *KLF6* expression as a direct target of *miR-1291* in breast cancer cell lines and tissues was confirmed. The RT-qPCR was utilized to measure *KALF6* mRNA expression values in breast cancer cell lines, which showed that *miR-1291* had lower expression from MCF-7 and MDA-MB-468 cells (Figure 4I). Furthermore, *KLF6* mRNA and protein expression was significantly downregulated in breast cancer (BC) tissue compared to normal to adjacent tissues (NATs) (Figure 4II and 4III). To confirm that the effects of *miR-1291* on cellular invasion, proliferation, and migration are mediated by the inhibition of *IGF1R* and *KLF6*, a rescue experiment was performed.

Transfection of pcDNA3-*IGF1R* blocked the growth-promoting effects of *miR-1291* on MCF-7 cells (Figure 41V). Furthermore, transfection of pcDNA3-*KLF6* in MCF-7 cells was confirmed to inhibit the invasion and migration of cells induced by 1291 mimics (Figure 4V and 4VI). These results showed that, as shown in Figure 4VII, *IGF1R* and *KLF6* promoted the growth, invasion, and migration of breast cancer cells induced by *miR-1291*.

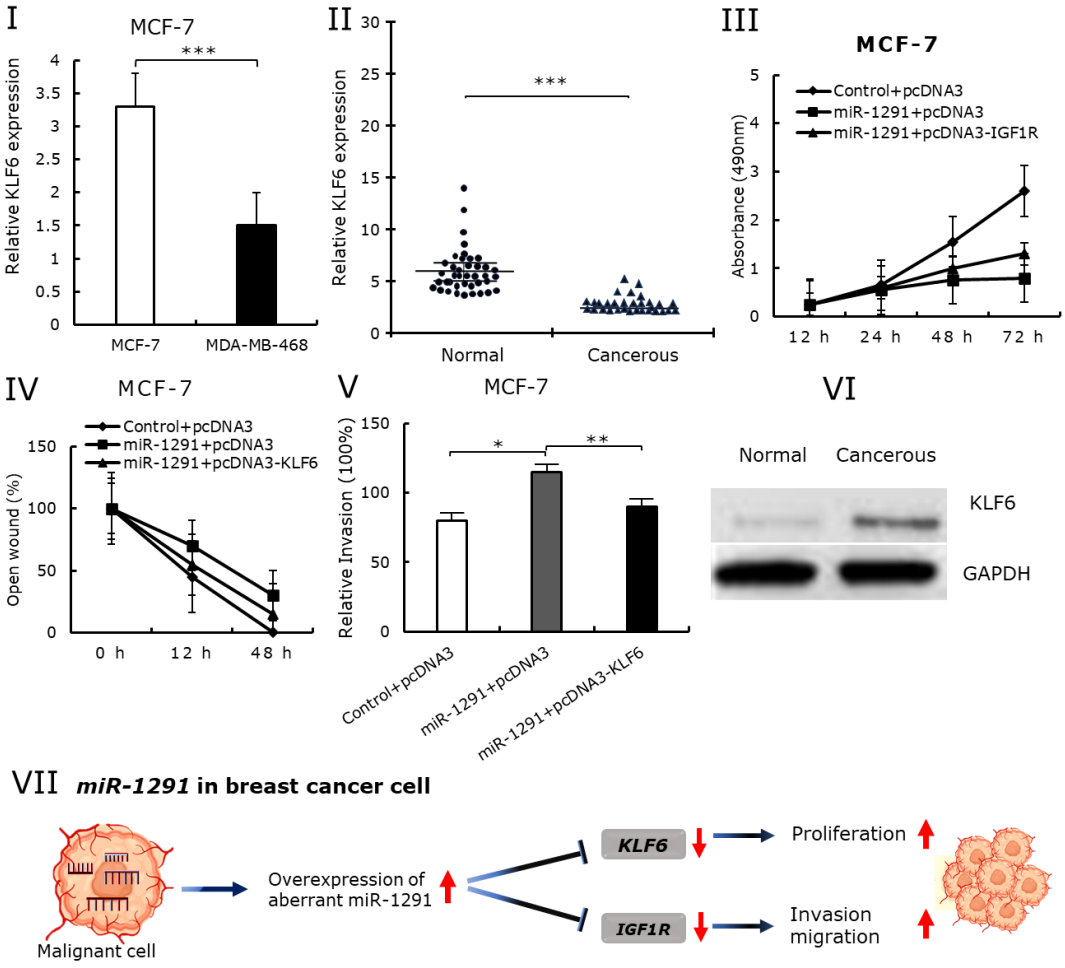

Figure 4. *IGF1R* and *KLF6* reversed the *miR-1291*-mediated cell invasion, migration, and proliferation. (I) Expression of *KLF6* in MCF-7 and MDA-MB-468 cells. (II) Relative mRNA expression of *KLF6* in breast cancer (BC) and normal to adjacent tissues (NATs). (III) Protein expression of *KLF6* in surrounding NATs and BC tissues. (IV) Overexpression of *IGF1R* reversed *miR-1291*-mediated inhibition of MTT-detected cell viability. (V and VI) Overexpression of *KLF6* reversed *miR-1291*-induced cell invasion and migration, as determined by Transwell and wound healing assays. (VII) *miR-1291* promoted the invasion, migration, and proliferation of breast

cancer cells. Statistical significance is represented as follows: * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

Discussion

MicroRNAs, which are small noncoding regulatory RNAs and about 18-22 nucleotides, have been studied in various types of cancer. Many miRNAs have been demonstrated to regulate pro-metastatic or anti-metastatic processes, along with the epithelial to mesenchymal transition (EMT) [10, 15, 16]. It has been demonstrated that numerous miRNAs are expressed at different levels in breast cancer cells and tissues, which suggests that they are involved in the progression and growth of breast cancer. While *miR-1291* has been demonstrated to regulate genes in various other cancer types, its roles in breast cancer have not yet been identified [17, 18]. This present research provided the initial indication that *miR-1291* plays a role in the proliferation, invasion, and metastasis of breast cancer cells. The potential effectiveness of *miR-1291* in relation to breast cancer was investigated. In the highly metastatic MDA-MB-468 cells, there was a significant increase in *miR-1291* expression level, whereas in non-cancerous breast epithelial cells (MCF-7), its expression was significantly up. In breast cancer tissues at the highest malignancy stage, *miR-1291* exhibited the greatest levels of expression, and its expression was found to be higher in metastases compared to non-metastases. Therefore, *miR-1291* might contribute to the development of breast cancer by enhancing proliferation and metastasis. There is no evidence that *miR-1291* suppresses cellular apoptotic signaling by targeting the genes of *IGF1R* (Insulin-Like Growth Factor 1 Receptor) and *KLF6* (Kruppel-like factor 6). The results indicate that *miR-1291* might inhibit apoptosis in MCF-7 and MDA-MB-468. It was also confirmed that in breast cancer cells, *miR-1291* suppresses the expression of *IGF1R*. Various computational methods were used to explore additional potential targets of *miR-1291*, leading to the identification of the *KLF6* gene as a possible target. The 3'UTR luciferase reporter experiment demonstrated that *KLF6* is the direct target of *miR-1291*. As the expression of *miR-1291* increases, the expression of *KLF6* is reduced. The type 1 insulin-like growth factor receptor (*IGF1R*) signaling pathway is crucial for cell growth and survival and is often exploited by various cancers, including breast cancer.

Several miRNAs function as tumor suppressors by reducing the levels of *IGF1R*. These miRNAs, when present at normal levels, assist in regulating cell proliferation. The expression of these proteins is frequently diminished in breast cancer, resulting in elevated levels of *IGF1R* and contributing to tumor growth and therapy resistance. A study demonstrated that miR-122 directly interacts with the 3'-untranslated region (3'-UTR) of *IGF1R* mRNA, thereby restricting cell proliferation and tumor progression. In breast cancer cells, its expression is often reduced[19]. The upregulation of miR-375 contributes to enhancing the sensitivity of cancer cells to anti-HER2 treatments (such as Herceptin). Cells that are resistant to Herceptin frequently exhibit a downregulation of miR-375, leading to an increase in *IGF1R* expression [20]. As it targets *IGF1R*, decreased levels of miR-145 in cancer tissues correlate with heightened cell growth and mobility. *miR-15b* [21] and *miR-630* [22]also aim at *IGF1R*, playing a role in the control of cancer cell proliferation and drug sensitivity. The *KLF6* transcription factor, along with factors such as ERα, c-Jun, and E2F1, can function as an enhancer to boost the transcription of the *IGF1R* gene. The *KLF6* gene, recognized as a tumor suppressor, can be involved in cancer advancement and metastasis through an oncogenic splice variant known as *KLF6*-SV1 [23]. miR-191-5p is often found to be upregulated (overexpressed) in breast cancer cells and the blood of patients with the disease. *MiR-191-5p* has a negative impact on the expression of *KLF6*, which normally acts as a tumor suppressor, by directly targeting it [24]. This interaction fosters the epithelial-mesenchymal transition (EMT) in breast cancer cells, resulting in a more aggressive malignant progression and heightened metastatic potential. In conclusion, the relationship between microRNAs, *IGF1R*, and *KLF6* is intricate: certain microRNAs reduce the levels of the tumor-promoting *IGF1R*, while others increase the levels of the oncogenic variant of *KLF6*. This intricate regulatory network presents potential pathways for creating targeted diagnostic and treatment strategies for individuals with breast cancer.

Conclusion

This study showed that *KLF6* was the direct target of *miR-1291*. Increasing the expression of *miR-1291* can reduce the expression of *KLF6*. Moreover, *miR-1291* promoted the invasion, migration, and proliferation of breast cancer through *IGF1R* and *KLF6*. These results may therefore provide new insights into the pathophysiology and treatment of breast cancer.

Acknowledgements

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Conflict of interest

None.

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