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ORIGINAL RESEARCH

miR-145-5p Inhibits HER2-Positive Breast Cancer Cells via Targeting ARF6

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Background: The objective of this study was to examine how miR-145-5p contributes to inhibiting the growth and movement of breast cancer cells by targeting and modulating the ADP-ribosylation factor 6 gene, as well as to clarify the mechanisms involved.

Methods: Bioinformatics analysis was used to study miR-145-5p expression in breast cancer samples from the TCGA database. RT-qPCR was performed on 25 pairs of HER2-positive breast cancer tissues and adjacent normal tissues, as well as in SK-BR3 and MCF10A cell lines. The effects of miR-145-5p overexpression on cell viability, migration, and invasion were assessed using CCK-8, scratch, and Transwell assays in SK-BR3 cells. A dual luciferase reporter assay was used to confirm miR-145-5p binding to the 3'-UTR of ARF6 mRNA. Additionally, the combined effects of miR-145-5p and ARF6 overexpression on SK-BR3 cell proliferation, migration, and invasion were evaluated.

Results: The examination of the TCGA database indicated that the expression levels of miR-145-5p were reduced in both paired and unpaired breast cancer tissues in comparison to normal control breast tissues. Notably, miR-145-5p showed a remarkably lower expression in HER2-positive breast cancer tissues versus paraneoplastic tissues. When the cells were transfected with a miR-145-5p mimic, there was a significant reduction in SK-BR3 cell proliferation, migration, and invasion in vitro. Conversely, the transfection of the cells with a miR-145-5p inhibitor led to a notable increase in SK-BR3 cell proliferation, migration, and invasion. Furthermore, miR-145-5p was found to suppress the expression of ARF6 mRNA by directly interacting with its 3'-untranslated region.

Conclusion: Overall, this study reveals that miR-145-5p suppresses the proliferation, migration, and invasion of breast cancer cells by interacting with ARF6 mRNA. Consequently, this miRNA might serve as a new target for accurate diagnosis and treatment of breast cancer.

Keywords: miR-145, ARF6, breast cancer, HER2-positive

Introduction

Breast cancer ranks as the most prevalent malignant tumor among females, comprising roughly 22% of all newly diagnosed cancer cases globally, with more than 1.05 million new diagnoses each year.[1] In the year 2021, there were approximately 281,550 new instances of female breast cancer reported worldwide, which represented 30% of all cancer cases in women globally.[2] This illness is characterized by its heterogeneity, featuring a diverse range of clinical, pathological, and prognostic subtypes. Traditionally, the progression of breast cancer has been conceptualized as a continuum beginning with normal epithelial cells, advancing through stages of hyperplasia, then in situ carcinoma, and ultimately leading to invasive carcinoma, culminating in metastasis.[3] Consequently, a deeper investigation into the molecular mechanisms underlying tumorigenesis and the progression of breast cancer is essential, alongside the identification of new therapeutic targets to establish innovative treatment strategies.[4] Thus, it is crucial to discover new therapeutic targets and clarify their roles and clinical significance.

MicroRNAs (miRNAs or miRs) represent a category of small non-coding single-stranded RNAs, typically ranging from 18 to 22 nucleotides in length. They are involved in the degradation of target mRNAs or the inhibition of protein translation by forming either complete or partial complementary pairs with the RNA of their target genes.[5] The post-

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transcriptional regulation exerted by miRNAs on their target mRNAs can stimulate cell proliferation, suppress apoptosis, and increase cell invasion, thereby influencing tumor progression.[6]

The involvement of various miRNAs in the tumor development and progression of breast cancer is evident, including miR-622,[7] miR-4759[8] and miR-181c.[9] In addition, miR-145-5p has been shown to play a profibrotic role in several diseases. For example, in recessive dystrophic epidermolysis bullosa (RDEB), overexpression of miR-145-5p in skin fibroblasts promotes a profibrotic phenotype, including increased cell contraction, proliferation, and migration, which is associated with the upregulation of contractile proteins such as α-smooth muscle actin and transgelin.[10] Additionally, in melanoma, miR-145-5p, as part of the miR-143/145 cluster, mediates a profibrotic reaction by targeting Fascin actin-bundling protein 1 (FSCN1), promoting a drug-tolerant mesenchymal-like phenotype.[11] miR-145-5p is located at chromosome 5q33.1 and is typically co-transcribed with miR-143. Epigenetic silencing or deletion of this region is a common event associated with cancer phenotypes. Research has indicated that multiple miRNAs are expressed abnormally across a variety of human cancers, displaying inconsistent expression patterns in distinct cancer types. Notably, a decrease in miR-145 levels has been observed in cancers of the colorectal,[12] lung[13] and gastric cancer.[14] Nonetheless, the specific biological role of miR-145-5p in breast cancer has yet to be clarified.

The researchers involved in this study performed data mining utilizing The Cancer Genome Atlas (TCGA) database. The study focused on the HER2-positive breast cancer cell line, SK-BR3, and manipulated the levels of miR-145-5p through transfection with either a mimic or an inhibitor. The aim was to explore how miR-145-5p influences key functions of HER2-positive breast cancer cells, including proliferation, invasion, and migration. Additionally, a bioinformatics method was applied to forecast the target genes of miR-145-5p and conduct an initial examination of the mechanisms by which miR-145-5p may affect the development of HER2-positive breast cancer.

Materials and Methods

Cell Line

The SK-BR3 cell line, known for its HER2-positive breast cancer characteristics, along with the MCF10A normal breast epithelial cell line, were sourced from Procell Life Science & Technology Co., Ltd (https://www.procell.com.cn/).

Clinical Tissues

A total of 25 pairs of breast cancer tissues that were positive for HER2 and matched adjacent non-tumor tissues were collected from patients (ages ranging from 28 to 65 years; 25 females) who underwent surgical treatment at the Breast Surgery department of Xingtai People's Hospital in Xingtai, China, from January to August in 2021. All of these patients received a pathological diagnosis of breast cancer. Furthermore, none had undergone any treatment before surgery, and written informed consent was secured from all individuals. The clinicopathological details of these patients are displayed in Table 1. Tissue specimens were cryopreserved in liquid nitrogen and kept at −80°C in a refrigerator. The Ethics Committee of Xingtai People's Hospital approved this study. Furthermore, the study adhered to the ethical principles outlined in The Declaration of Helsinki. Throughout the data collection process and after its completion, all authors had access to information that could identify individual participants. Sensitive data were masked or replaced with unique tokens or references that have no intrinsic value. The original data were stored securely in a separate location.

Main Reagents and Consumables

Fetal bovine serum (FBS) from Gibco, a subsidiary of Thermo Fisher Scientific, along with Lipofectamine 3000 transfection reagent (Thermo Fisher Scientific), 1640 medium also provided by Gibco, and serum-free transfection medium OPTI-MEM (Gibco; Thermo Fisher Scientific) were employed. Additionally, we utilized the Cell Counting Kit-8 (CCK-8) from Dojindo Laboratories, Transwell invasion chambers (with a pore size of 8.0 μm and PC membrane from Corning), a 25 cm^2 asymptomatic phase flask, and a 96-well cell culture plate sourced from Corning. The following miRNA reagents were obtained: miRNA mimic, inhibitor, and negative control[15] from Shanghai GenePharma Co., Ltd., based in Shanghai, China (https://www.genepharma.com/en/). The specific sequences for the reagents are as follows: miR-145-5p mimic, 5'-GTCCAGTTTTTTCCCAGGAATCCCT-3'; miR-145-5p inhibitor, 5'-AGGGATTCCTCCCAAAACTGGAC-3'; NC, 5'-

Table 1 Characterization of the Patients With Breast Cancer in the Present Study

Factors	No. of Patients	High	Low	χ^2	P-value
Age (years)					
≤50	13	6	7	0.371	0.695
>50	12	7	5		
Tumor size (cm)					
≤2	18	12	6	5.540	0.030
>2	7	1	6		
Grade					
II	19	13	6	2.339	0.175
III	6	2	4		
TNM stage					
I + II	16	10	6	3.744	0.097
III	9	2	7		
ER status					
Positive	20	13	7	1.042	0.615
Negative	5	2	3		
PR status					
Positive	19	10	9	0.365	0.661
Negative	6	4	2		
Ki-67					
≤20%	8	5	3	0.031	0.999
>20%	17	10	7		
Lymph node status					
Positive	8	1	7	5.940	0.030
Negative	17	11	6		

GUAGGAGUAGUGAAAGGCC-3'. It is important to note that the NC sequence was non-homologous to the human genome, thus it did not interfere with any miRNA functions. Furthermore, for the purpose of ARF6 overexpression, the C-3FLAG-ARF6 plasmid from Shanghai GenePharma was utilized.

Instruments and Equipment

The current research employed several essential pieces of equipment to facilitate the experiments conducted. Among these, a low-speed centrifuge was utilized, which plays a critical role in the separation of components within various biological samples. The study also made use of a CO2 incubator from Thermo Electron Corporation, which is specifically designed to create optimal growth conditions for cell cultures by maintaining appropriate levels of carbon dioxide and temperature. Additionally, an enzyme marker provided by Thermo Fisher Scientific was implemented, serving as a vital tool for detecting specific biochemical substances within the

samples. Finally, observations were conducted using a phase contrast inverted microscope from Olympus Corporation, which allows for the visualization of live cells and other transparent specimens without the need for staining.

Cell Culture

SK-BR3 cells were maintained in 1640 medium supplemented with 10% FBS in an incubator with saturated humidity, set to a stable temperature of 37°C and 5% CO_2. One day before the inoculation and transfection of the cells, the SK-BR3 cultures were distributed into a six-well plate to achieve approximately 60% confluency, and were then placed in the same incubator conditions of 37°C and 5% CO_2 with saturated humidity.

Transfection

The dry powder forms of miR-145-5p mimic, inhibitor, and negative control[15] were dissolved to create a working solution with a final concentration of 20 μmol/l. Transfection occurred within 24 hours after plating, following the guidelines included with the Lipofectamine 3000 transfection reagent. At 6 hours post-transfection, the culture medium was switched to normal complete medium, and the culture was maintained for the following experiments.

CCK-8 Assay for the Detection of Cell Proliferation

The cells were transfected with either NC, miR-145-5p inhibitor, or a mimic group of miR-145-5p. After 48 hours of transfection, trypsin was used to digest the cells, which were then counted and distributed into 96-well plates, ensuring that each group had six wells. Once the cells were plated, 10 μL of CCK-8 solution was introduced to each well. The optical density[10] of each sample was assessed at 490 nm using microplate readers (Multiskan Sky; Thermo Fisher Scientific, Inc.) following an incubation period of 1 hour in a 5% CO_2 environment. A cell growth curve was created by plotting the time as the horizontal axis and the OD values of the samples as the vertical axis, allowing for an analysis to identify any significant differences in cell activity across the three groups.

Scratch Assay

After the transfection process was completed and the cell density attained 90%, the cells were subjected to a serum starvation procedure using serum-free 1640 medium for a duration of 12 hours. Subsequently, a scratch was introduced in each well by employing a 20 μL pipette tip. To document the initial state of the scratch, several distinct fields of view were captured using a phase contrast microscope (Olympus, Tokyo, Japan) at the 0-hour mark. The cultures were then maintained at a temperature of 37°C within a 5% CO2 incubator, and subsequent photographic documentation of the cells was conducted every 24 hours. The analysis focused on measuring the width of the scratch across various fields of view as depicted in the images collected.

Transwell Invasion and Migration Assay

A total of 20,000 cells were introduced into the upper sections of 24-well culture plates, and assays for cell migration and invasion were conducted using uncoated chambers for migration and chambers coated with Matrix gel for invasion. After a 24-hour incubation period, the cells adhering to the chamber surfaces were gently removed with cotton swabs. The cells located beneath the chambers were then fixed with cold methanol for 15 minutes and subsequently stained with a 0.1% crystalline violet solution for half an hour. An inverted microscope (Olympus, Tokyo, Japan) was utilized to observe the morphology and count of the cells.

RNA Extraction and Reverse Transcription-Quantitative PCR (RT-qPCR)

TRIzol reagent (Invitrogen) was utilized to extract total RNA from both tissues and cells. Subsequently, 1 μg of RNA underwent reverse transcription into cDNA with the aid of a reverse transcription kit (cat. no. RR047; Takara Bio, Inc.) under specific reaction conditions: 37°C for 15 minutes, followed by 85°C for 5 seconds, and a final hold at 4°C. To evaluate gene expression, SYBR-Green qPCR premix (Roche Diagnostics) was employed. The conditions for the PCR reaction included an initial step at 95°C for 2 minutes, followed by 40 cycles with denaturation at 95°C for 10 seconds,

Table 2 Primer Sequences Used for Reverse Transcription-Quantitative PCR

Gene	Sequence (5'-3')
miR-145-5p	F: CAGTCTTGTCCAGTTTTCCCAG
	R: TATGCTTGTTCTCGTCTCTGTGTC
U6	F: CTGGTAGGGTGCTCGCTTCGGCAG
	R: CAACTGGTGTCGTGG AGTCGG
ARF6	ATTACTACACCGGGACCCAGGGTCT
	AGGTCCTGCTTGTTGGCGAAGATG
GAPDH	F: GCACCGTCAAGGCTGAGAAC
	R: GGATCTCGCTCCTGGAAGATG

annealing temperature set at 60°C for 30 seconds, and extension at 70°C for 5 seconds. U6 and GAPDH served as internal reference genes for miRNAs and mRNAs, respectively. The primers utilized are detailed in Table 2. The relative expression levels of noncoding RNA and mRNA were determined using the $2^{-\Delta\Delta}$CT method.

Luciferase Reporter Assay

The complementary DNA fragment that includes the 3'-untranslated region (3'-UTR) of ADP-ribosylation factor 6 (ARF6) was successfully sub-cloned downstream of the luciferase gene within the pGL3-Basic luciferase reporter vector. This specific arrangement allows for the investigation of the regulatory effects of the 3'-UTR on luciferase activity. To evaluate the potential interaction between miR-145-5p and the 3'-UTR of ARF6, mammalian cells were co-transfected with the constructed reporter plasmid and either miR-145-5p or its corresponding negative control. This co-transfection was facilitated using Lipofectamine 3000, a widely-used transfection reagent that enhances cellular uptake of nucleic acids. After a period of 48 hours post-transfection, the levels of luciferase activity were quantified employing a dual luciferase assay kit provided by Promega Corporation, allowing for an accurate assessment of the effects of miR-145-5p on ARF6 expression through its 3'-UTR.

Target Gene Prediction and Gene Function Classification

TargetScan was used for searching, predicting their target genes and performing preliminary validation.

Statistical Analysis

Data analysis was conducted using SPSS version 23.0 (IBM Corp.) along with GraphPad Prism version 8 (GraphPad Software, Inc). Results are displayed as mean ± standard deviation (SD). Unpaired Student's t-tests were utilized for comparisons between two distinct cell groups. For comparisons between paired tumor tissues and their adjacent non-tumor counterparts, paired Student's t-tests were applied. To analyze data from the dual-luciferase reporter assay, two-way ANOVA followed by the Bonferroni post hoc test was implemented. Fisher's exact tests were conducted to compare the frequency of events across different groups. The correlation in gene expression between miR-145-5p and ARF6 was evaluated using the Pearson correlation coefficient. Each experiment was carried out a minimum of three times. A P-value of less than 0.05 was deemed to signify a statistically significant difference.

Results

miR-145-5p is Downregulated in Breast Cancer Tissues and Cells

The current investigation focused on the expression levels of miR-145-5p in invasive breast cancer utilizing data from the TCGA database. The findings indicated that miR-145-5p expression was markedly reduced in breast cancer tissues compared to normal breast tissues (see Figure 1A and B). Additionally, the expression levels of miR-145-5p were analyzed in 25 pairs of HER2-positive breast cancer tissues alongside their para-neoplastic normal counterparts. It was revealed that HER2-positive breast cancer tissues exhibited a notably lower expression of miR-145-5p (refer to

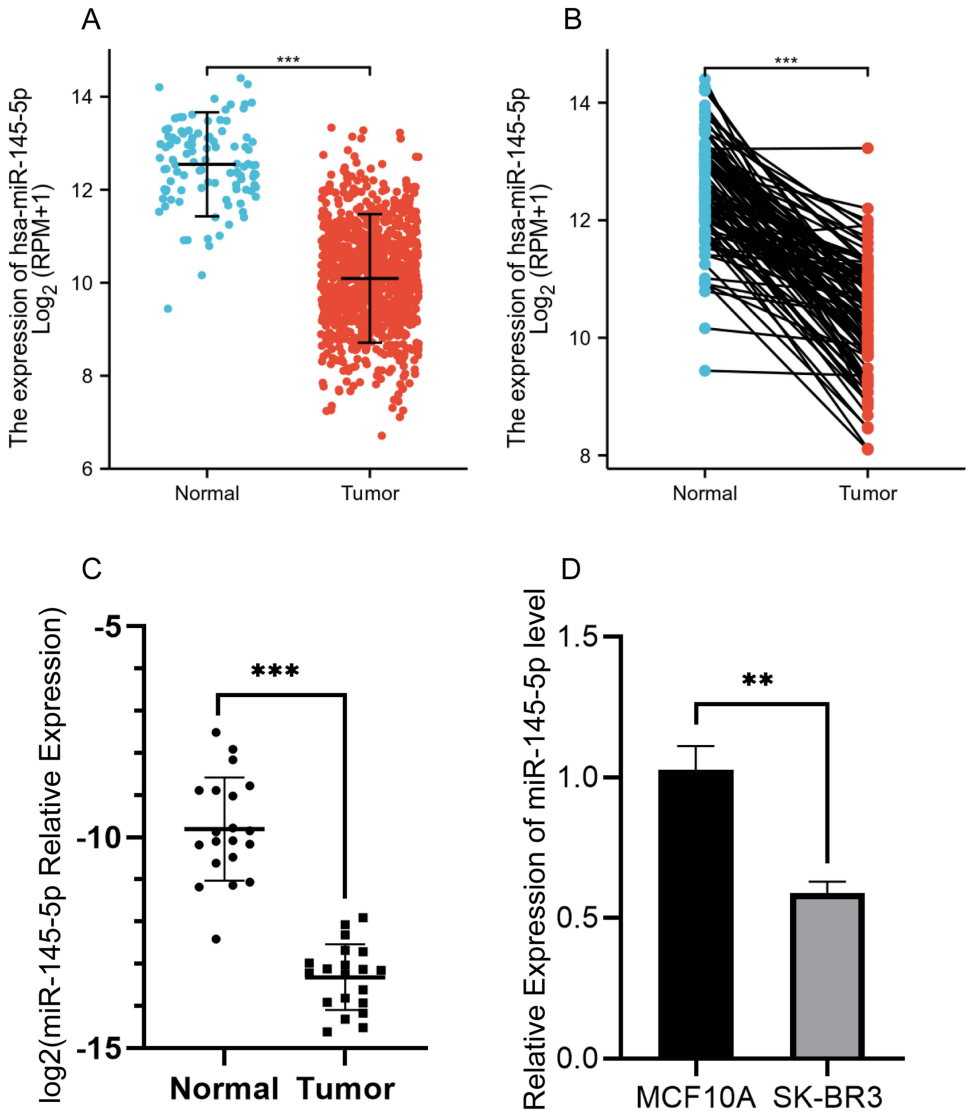

Figure 1 miR-145-5p is downregulated in breast cancer lines and tissues. (**A**) miR-145-5p expression in breast cancer and normal tissue (TCGA datasets). (**B**) miR-145-5p expression in breast cancer and adjacent normal tissue (TCGA datasets). Reverse transcription-quantitative PCR analysis of miR-145-5p expression levels in (**C**) HER2-positive breast cancer cell lines and (**D**) HER2-positive breast cancer tissues. **P<0.01, ***P<0.001.
Abbreviation: TCGA, The Cancer Genome Atlas.

Figure 1C), a result that aligned with the data from the TCGA database. Moreover, analysis showed that SK-BR3 cells had a significantly decreased level of miR-145-5p in comparison to MCF10A cells (illustrated in Figure 1D).

miR-145-5p Inhibits the Proliferation, Migration and Invasion of BC Cells

Experiments were conducted to assess the efficiency of transfecting mimics and inhibitors. SK-BR3 cells were subjected to transfection with either the miR-145-5p mimic or inhibitor, and these were compared to the cells transfected with the negative control[15] group. The findings demonstrated that transfection led to a significant upregulation or downregulation of miR-145-5p levels (Figure 2A). Following this, additional related experiments took place. To explore the impact of miR-145-5p on breast cancer cell proliferation, SK-BR3 cells were either overexpressed or subjected to knockdown of miR-145-5p, with a CCK-8 assay employed to evaluate cell proliferation. The results indicated a marked reduction in relative absorbance after transfection with the miR-145-5p mimic in comparison to the NC group. Conversely, relative absorbance significantly increased following transfection with the miR-145-5p inhibitor, implying that the proliferation of breast cancer (BC) cells was notably diminished due to the overexpression of miR-145-5p (Figure 2B). The current study focused on investigating the

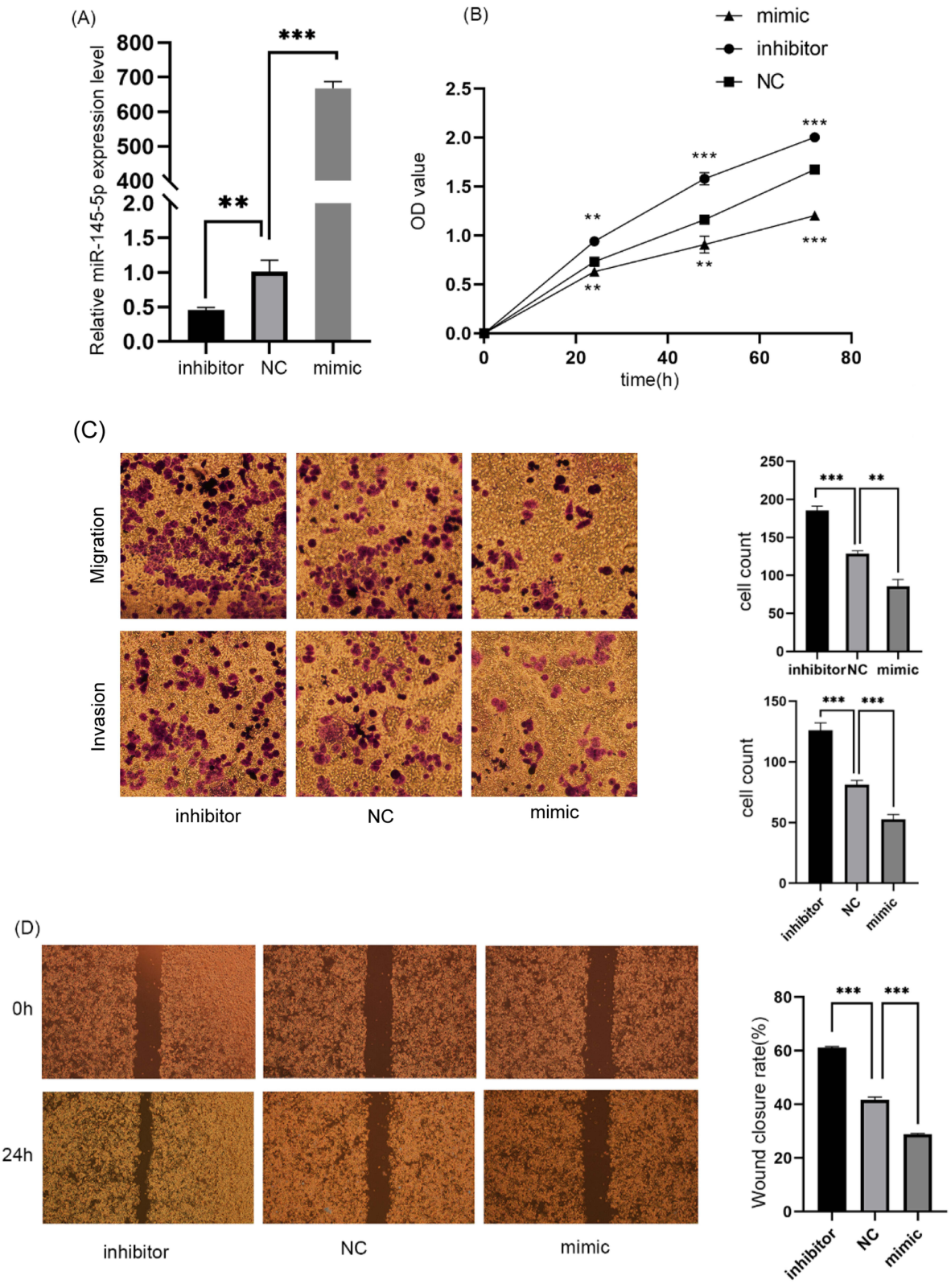

Figure 2 Overexpression of miR-145-5p inhibits the proliferation, migration and invasion of SK-BR3 cells. (**A**) Reverse transcription-quantitative PCR analysis of miR-145-5p expression levels in SK-BR3 cells transfected with miR-145-5p or miR-NC mimic. (**B**) Cell proliferation in SK-BR3 cells transfected with miR-145-5p mimic or miR-NC mimic. (**C**) Transwell assay of SK-BR3 cells transfected with miR-145-5p mimic or inhibitor, respectively, or the corresponding NCs. (**D**) Wound healing assays for SK-BR3 cells transfected with miR-145-5p mimic or inhibitor, respectively, or the corresponding NCs. **P<0.01, ***P<0.001.

Abbreviation: NC, negative control.

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role of miR-145-5p in regulating the migration and invasion of breast cancer cells. To assess these effects, scratch assays and Transwell assays were employed. The findings revealed that overexpression of miR-145-5p led to a marked reduction in both the migration and invasion capabilities of SK-BR3 cells when compared to the negative control[15] group. In contrast, the knockdown of miR-145-5p resulted in a significant increase in the migration and invasion of SK-BR3 cells, as illustrated in Figure 2C. Overall, these results underscore the ability of miR-145-5p to effectively suppress the proliferation, migration, and invasion of SK-BR3 cells in an in vitro setting, as shown in Figure 2D.

ARF6 is a Direct Target of miR-145-5p

Given that miR-145-5p suppresses the growth, movement, and invasion of breast cancer cells, an investigation was conducted into the mechanisms underlying its inhibitory effects on these malignantly biological behaviors. An analysis through the TargetScan database identified ARF6 as a target of miR-145-5p (Figure 3A). To confirm this finding, the expression levels of ARF6 were assessed in human breast cancer tissues, adjacent non-cancerous tissues, and breast cancer cell lines. Results indicated that ARF6 expression was notably increased in breast cancer tissues compared to para-neoplastic normal tissues, and also in SK-BR3 cells relative to MCF10A normal mammary epithelial cells (Figure 3B and C). In contrast, the expression levels of ARF6 appeared to decrease or increase in SK-BR3 cells after the overexpression or knockdown of miR-145-5p, respectively, as revealed by RT-qPCR results (Figure 3D). To verify that ARF6 served as a target for miR-145-5p, a mimic of miR-145-5p was co-transfected into SK-BR3 cells alongside either the wild-type (WT) or mutant (Mut) 3'-UTR of the ARF6 gene, followed by a luciferase reporter assay. The outcomes of the co-transfection assays indicated that the luciferase activity in SK-BR3 cells co-transfected with the miR-145-5p mimic and ARF6 WT plasmid was notably reduced, whereas the luciferase activity in cells with the ARF6 Mut plasmid remained largely unchanged (Figure 3E). Collectively, these findings illustrate that the ARF6 gene is a direct target of miR-145-5p, contributing to the inhibition of proliferation, migration, and invasion in SK-BR3 cells.

miR-145-5p Inhibits the Proliferation of BC Cells in vitro by Targeting ARF6

To validate the impact of ARF6 on the proliferation of breast cancer (BC) cells, SK-BR3 cells were co-transfected with miR-145-5p alongside either ARF6 or its corresponding control. This approach aimed to evaluate the expression levels of ARF6 in the cells and to analyze BC cell proliferation. Findings from RT-qPCR indicated that co-transfection with ARF6 led to a partial restoration of ARF6 expression levels and an enhancement of the proliferative abilities of BC cells (Figure 4A and B). These findings imply that miR-145-5p inhibits the proliferation of SK-BR3 cells through its targeting of ARF6.

Discussion

Numerous prior investigations have established the role of miRNAs in the progression of various malignant tumors, including pancreatic,[16] thyroid,[17] breast,[18] esophageal,[19] gastric[20] and colon cancer.[21] Typically, miR-145 is recognized for its function as an oncogene, situated on chromosome 5q32-33.[22] The expression levels of miR-145-5p, an essential member of the miR-145 family,[23] are often diminished in several cancer types, such as thyroid cancer,[17] glioma[24] and lung cancer.[22] It is regarded as a biological marker for tumors, significantly associated with tumorigenesis, progression, metastasis, drug resistance, and overall prognosis. Research focusing on miR-145-5p in breast cancer has primarily targeted triple-negative breast cancer,[25] while limited studies have explored its implications in HER2-positive breast cancer. Thus, this study aimed to analyze the expression and significance of miR-145-5p in HER2-positive breast cancer. The findings indicated a significant downregulation of miR-145-5p expression in both HER2-positive breast cancer tissues and cell lines. Additionally, miR-145-5p was shown to substantially hinder the proliferation, migration, and invasion of the SK-BR3 cell line, which is associated with HER2-positive breast cancer. Consequently, this study suggests that miR-145-5p could serve as a potential novel biomarker for HER2-positive breast cancer.

MicroRNAs (miRNAs) primarily fulfill their biological roles by influencing and regulating their target genes.[5] Consequently, this study conducted an analysis and validation to identify the target genes of miR-145-5p. A bioinformatics approach was employed to screen for miR-145-5p target genes using the TargetScan database, where the ARF6 gene was recognized. The ARF protein family (ARF1-6) consists of small GTPases involved in the regulation

A

Position 973-979 of ARF6 3'UTR	WT	5'...GUGACUUUUGGGCAAAACUGGAA...3'
miR-145-5p		3'...CCCUAAGGACCCUUUUUGACCUG...5'
Position 2171-2177 of ARF6 3'UTR	WT	5'...AGGUAUAAAUGCCUAAACUGGAG...3'
miR-145-5p		3'...UCCCUAAGGACCCUUUUGACCUG...5'

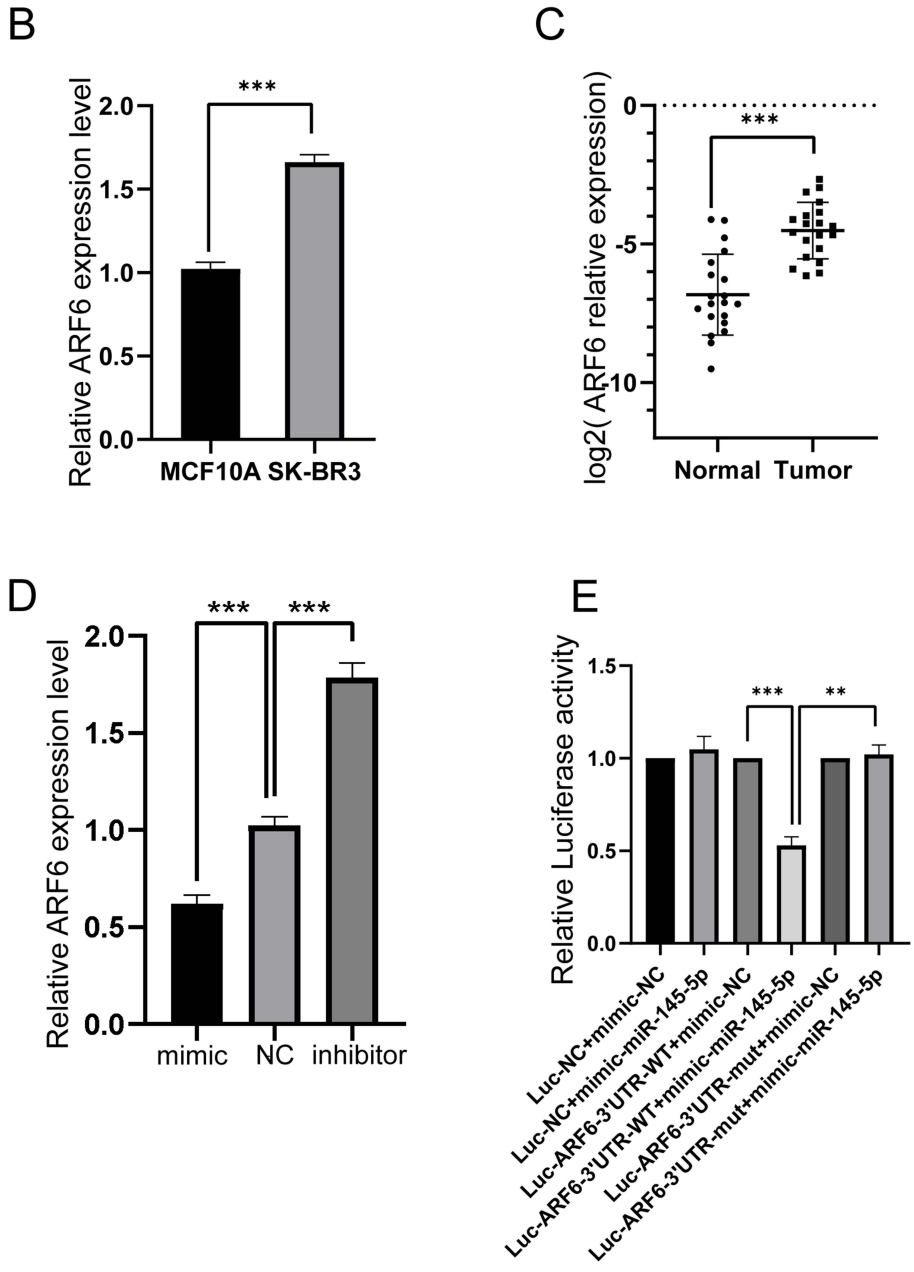

Figure 3 ARF6 is a direct target of miR-145-5p. (**A**) Schematic representation of ARF6 3'-UTR demonstrating the putative miR-145-5p target site. RT-qPCR analysis of ARF6 mRNA expression levels in breast cancer (**B**) cell lines and (**C**) tissues. (**D**) Reverse transcription-quantitative PCR analysis of ARF6 expression levels in SK-BR3 cells transfected with miR-145-5p mimic or inhibitor, respectively, or the corresponding negative control. (**E**) Analysis of the relative luciferase activities of ARF6-WT and ARF6-mut. **P<0.01, ***P<0.001.

Abbreviations: ARF6, ADP-ribosylation factor 6; WT, wild-type; mut, mutant type.

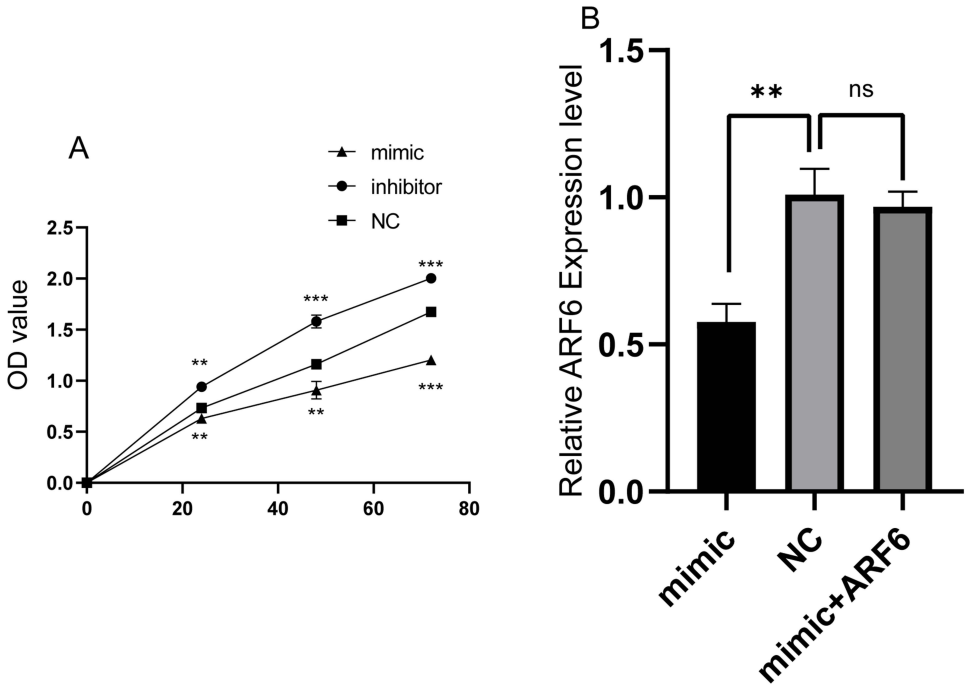

Figure 4 Rescue effects of miR-145-5p and ARF6 on SK-BR3 cells. (**A**) CCK-8 assay was used to evaluate the rescue effects of miR-145-5p mimics on SK-BR3 cells proliferation following ARF6 overexpression. (**B**) Reverse transcription-quantitative PCR assay of the expression level of ARF6 to evaluate the rescue effect of miR-145-5p mimics on SK-BR3 cells proliferation following ARF6 overexpression. **P<0.01, ***P<0.001.
Abbreviation: ARF6, ADP-ribosylation factor 6.

of protein transport across the cell membrane and endocytosis.[26] Notably, ARF6 has been implicated in the invasion of tumor cells in both breast cancer and brain tumors.[27] Prior research has demonstrated that miR-145 inhibits cell migration and invasion in upper tract urothelial carcinoma through the targeting of ARF6.[28] miR-145-5p has also been documented to influence the progression of hepatocellular carcinoma and the proliferation of epithelial ovarian cancer by targeting SPATS2 and SMAD4.[26,27] To investigate the connection between miR-145 and ARF6 in breast cancer (BC), additional experiments were carried out. The current study further validated that ARF6 serves as a direct target gene of miR-145-5p through a dual luciferase reporter assay. Moreover, overexpression of ARF6 was shown to significantly counteract the repressive effects of miR-145-5p on the proliferation, migration, and invasion of breast cancer cells compared to control groups. These findings indicated that miR-145-5p modulates the proliferation, migration, and invasion of HER2-positive breast cancer cells by targeting and suppressing ARF6 expression levels. Nevertheless, there are certain limitations to our research. First, the sample size in our clinical study was limited, necessitating the collection of additional samples along with an extended follow-up period to gather more thorough data. Additionally, this investigation was exclusively focused on HER2-positive breast cancer patients, and related studies involving other types, such as triple-negative and Luminal type breast cancer patients, are currently underway, with results to be shared in future publications.

Conclusion

In summary, miR-145-5p has the potential to play a role in the oncogenic processes associated with HER2-positive breast cancer by inhibiting the expression of the ARF6 gene. This may offer fresh perspectives for the clinical treatment of HER2-positive breast cancer.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

The present study was approved by the Ethics Committee of Xingtai People's Hospital [approval no. 2021(035)]. The patients provided written informed consent for the use of their samples in research. The present study was conducted in accordance with the principles described in the Declaration of Helsinki. Throughout the study, participants were identified by unique numbers instead of their names to protect their privacy. None of the completed study materials which were kept by the main researcher contained the names of the participants. Study materials were not accessed by other persons outside the researcher's team to ensure confidentiality.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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