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Original Article

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lncRNA ENST00000422059 promotes cell proliferation and inhibits cell apoptosis in breast cancer by regulating the miR-145-5p/KLF5 axis

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Abstract

Krüppel-like zinc-finger transcription factor 5 (KLF5) is a vital regulator of breast cancer (BC) onset and progression. The mechanism by which KLF5 regulates BC is still not clearly known. In this study, bioinformatics analysis shows that BC-affected individuals with elevated KLF5 expression levels have poor clinical outcomes. We further verify that miR-145-5p regulated KLF5 expression to promote cell apoptosis and inhibit cell proliferation in BC via dual-luciferase reporter assay, western blot analysis, qRT-PCR, CCK-8 assay and cell apoptosis assay. In addition, based on bioinformatics analysis, the binding of ENST00000422059 with miR-145-5p is confirmed by dual-luciferase reporter assay. Subsequently, FISH, western blot analysis, qRT-PCR, CCK-8 and cell apoptosis assays verified that ENST00000422059 increases KLF5 protein expression by sponging miRNA to promote cell proliferation and inhibit cell apoptosis. Finally, ENST00000422059 is found to accelerate tumor progression by regulating the miR-145-5p/KLF5 axis *in vivo*. In conclusion, this study suggests that ENST00000422059 upregulates KLF5 by sponging miR-145-5p to promote BC progression.

Key words ENST00000422059, miR-145-5p, KLF5, breast cancer

Introduction

Breast cancer (BC) is a malignant gynecologic tumor prevalent worldwide [1]. The majority of cancer-associated deaths in females are caused by BC, which is typically diagnosed at an advanced stage [2,3]. Surgical resection, adjuvant chemoradiotherapy, endocrine therapy, targeted therapy, and immunotherapy have significantly improved clinical outcomes in BC patients. Unfortunately, the survival rate of individuals suffering from metastatic breast cancer (MBC) remains dismal [4]. The underlying reason for this is that the complexity of the molecular regulation of BC development and progression remains elusive [5,6]. Therefore, it is necessary to further explore new biomarkers to provide potential therapeutic targets for BC patients.

The onset and progression of BC are significantly influenced by Krüppel-like zinc-finger transcription factor 5 (KLF5) [7]. Previous research has confirmed that the elevated KLF5 expression level is an effective biomarker for poor prognosis in individuals suffering from cancer [8,9]. In BC, however, the molecular mechanism of KLF5 is intricate and currently unknown [10]. Long noncoding RNAs (lncRNAs), with a length greater than 200 nt, are vital regulators of cancer progression (including BC) [11]. For example, lncRNA-CDC6 promotes BC development by sponging miR-215 [12]. lncRNA HOTAIR affects BC progression by sponging miR-20a-5p [13]. KLF5 can also increase lncRNA IGFL2-AS1 expression and promote basal-like BC progression [14]. However, whether lncRNA regulates KLF5 gene expression in BC and its molecular mechanism have yet to be explored.

Herein, miR-145-5p was found to target KLF5 in BC based on

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bioinformatics analyses. miR-145-5p was identified to regulate KLF5 expression and be involved in cell proliferation and apoptosis. ENST00000422059 was further confirmed to enhance BC growth via regulation of the miR-145-5p/KLF5 axis. This study confirms that ENST00000422059 promotes BC progression *in vivo* by regulating the miR-145-5p/KLF5 axis.

Materials and Methods

Bioinformatics analysis

The ENST00000422059-specific sequence matching with miR-145-5p was identified by searching the RNAhybrid database (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) [15]. The miR-145-5p targets were identified by utilizing TargetScanHuman 8.0 (https://www.targetscan.org/vert_80/).

Cell lines

The BC cell lines MDA-MB-231 and MCF-7 employed in this study were supplied by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cell lines were maintained in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, USA) containing 10% fetal bovine serum (A3160802; Gibco). Cell culture was conducted at 37°C in a 5% CO_2 atmosphere.

Cell transfection

BC cell lines MDA-MB-231 and MCF-7 were seeded in 6-well plates and cultured for approximately 24 h prior to transfection. At 70%–80% confluence, cells were transfected with si-KLF5, si-ENST00000422059, miR-145-5p mimic or inhibitor transfection using Lipofectamine 2000 (Invitrogen, Beijing, China) according to the manufacturer's instructions. The KLF5 plasmid vector (KLF plasmid), control plasmid for KLF-5 (control plasmid), siRNAs specifically targeting KLF5 or ENST00000422059, miR-145-5p mimic, and inhibitor were provided by GenePharma (Suzhou, China). The scramble sequence for KLF5 (control plasmid) and scramble sequence for ENST00000422059 (si-ENST00000422059) are used as controls. The sequence information of siRNA, miR-145-5p mimic and controls are shown in Table 1.

qRT-PCR

Total RNA extraction was carried out using the TRIzol® Plus RNA Purification Kit (12183-555; Invitrogen) following the instructions provided by the manufacturer. SuperScript™ III First-Strand Synthesis SuperMix (11752-050; Invitrogen) was then employed for 1st-strand cDNA synthesis for qRT-PCR. RT-qPCR was performed on a CFX384 multiplex real-time PCR instrument (Bio-Rad, Hercules, USA) using Power SYBR® Green PCR Master Mix (4367659; Applied Biosystems, Carlsbad, USA). The reaction conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 58°C for 30 s and 72°C for 30 s. The relative expression level of the ENST00000422059 gene was quantified using the $2^{-\Delta\Delta Ct}$ method. *GAPDH* and *U6* were utilized as internal references. The primer sequences are shown in Table 2.

CCK assay

MDA-MB-231 and MCF-7 cells (1×10^3 cells/well, 100 μL) at logarithmic growth phase were added to 96-well plates (5 duplicate wells) and incubated at 37°C and 5% CO_2. At indicated time points, 7 μL of CCK-8 solution (BL001B 500T; Biomiky, Beijing, China) was added to each well and incubated 2 h. The absorbance was measured at 450 nm using a microplate reader (Infinite F50; Tecan, Morgan Hill, USA).

EdU assay

MDA-MB-231 and MCF-7 cells (1×10^4 cells/well, 200 μL) at

Table 1. The sequence of siRNAs and miR-145-5p mimics

Name	Sequence (5′→3′)
si-ENST00000422059-1	Sense: GGCGAUCUUGACUUAACCUGUTT
	Antisense: ACAGGUUAAGUCAAGAUCGCCTT
si-ENST00000422059-2	Sense: GAAACACCAUCGCAGACAUGUTT
	Antisense: ACAUGUCUGCGAUGGUGUUUCTT
si-ENST00000422059-3	Sense: GCAGCUAUGAGCCGAGAAAUATT
	Antisense: UAUUUCUCGGCUCAUAGCUGCTT
si-ENST00000422059-negative control	Sense: UUCUCCGAACGUGUCACGUTT Antisense: ACGUGACACGUUCGGAGAATT
si-KLF5-1	Sense: GCUCCAGAGGUGAACAAUATT
	Antisense: UAUUGUUCACCUCUGGAGCTG
si-KLF5-2	Sense: GCAGAGAUGCUCCAGAAUUTT
	Antisense: AAUUCUGGAGCAUCUCUGCTT
si-KLF5-3	Sense: GCAUCCACUACUGCGAUUATT
	Antisense: UAAUCGCAGUAGUGGAUGCGT
si-KLF5-negative control	Sense: UUCUCCGAACGUGUCACGUTT Antisense: ACGUGACACGUUCGGAGAATT
miR-145-5p mimic	GUCCAGUUUUCCCAGGAAUCCCU
miR-145-5p mimic-negative control	Sense: UUCUCCGAACGUGUCACGUTT Antisense: ACGUGACACGUUCGGAGAATT
miR-145-5p inhibitor	AGGGAUUCCUGGGAAAACUGGAC
miR-145-5p inhibitor-negative control	CAGUACUUUUGUGUAGUACAA

Table 2. Sequences of primers used in this study

Gene	Primer sequence (5′→3′)
qPCR	
GAPDH	Forward: CCATGACAACTTTGGTATCGTGGAA
	Reverse: GGCCATCACGCCACAGTTTC
ENST00000422059	Forward: GGTGGGTTCTTGGTCTCACT
	Reverse: CTGGAAGAGTAGCTGGTGGAT
miR-145-5p	CGGTCCAGTTTTCCCAGGAA
U6	CGCTTCGGCAGCACATATACTAA
micro-R	AGTGCAGGGTCCGAGGTATT
Reverse transcription	
miR-145-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGAT
U6	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTCATCC

logarithmic growth phase were added to 48-well plates. After transfection and culture for 24 h, EdU reagent (RiboBio,Guangzhou, China) was used to measure viability following the manufacturer's instructions. Finally, a BX51 fluorescence microscope (Olympus, Tokyo, Japan) was used to image the stained cells.

Cell apoptosis assay

An Annexin V FITC/PI Apoptosis Kit (C1062L; Beyotime, Shanghai, China) was used to analyze cell apoptosis. Briefly, after indicated treatment, MDA-MB-231 and MCF-7 cells were digested with 0.25% trypsin (BL006A; Biomiky), and then treated with the reagents provided with the kit according the manufacturer's instructions. Finally, cells were analyzed using a FACSCanto flow cytometer (BD, Franklin Lakes, USA) to measure the apoptotic cells.

Fluorescence *in situ* hybridization (FISH) assay

The localization of ENST00000422059 in MCF-7 and MDA-MB231 cells was verified via FISH. The targeted probe ENST00000422059 was obtained from RiboBio. The FISH assay was conducted according to the manufacturer's instructions and a previous report [16]. Finally, a laser scanning confocal microscope (Zeiss, Oberkochen, Germany) was employed to capture the images.

Double-luciferase reporter assay

The plasmids pmirGLO-KLF5-3UTR-WT, pmirGLO-KLF5-3UTR-MUT, pmirGLO-ENST00000422059-WT, and pmirGLO-ENST00000422059-MUT were purchased from Bioogenetech (Hefei, China). The miR-145-5p mimic or control was purchased from GeneChem (Suzhou, China). Reseeding of MDA-MB-231 and MCF-7 cells (2×10^4 cells/well) was performed in 24-well plates, followed by cell culture at 37°C in a 5% CO_2 incubator until the cell confluency reached approximately 60%. Cells were transfected by mixing 100 μL of opti-MEM with 2 μL of X-tremegene HP Transfection Reagent (C756V59; Roche, Basel, Switzerland) containing 1 μg of plasmid per well. Renilla luciferase and firefly luciferase activities were detected using a Double luciferase reporter gene detection kit (RG027; Beyotime) following the instructions provided by the manufacturer.

Western blot analysis

Quantification of total protein from MDA-MB-231 and MCF-7 cells (1×10^7 cells) was performed using a BCA Protein Concentration Assay Kit (P0010; Beyotime). Total proteins (60 μg) were separated by 8%–12% SDS-PAGE, and then transferred onto a PVDF membrane. Subsequently, the membrane was blocked and incubated with anti-KLF5 antibody (dilution 1:1000; 21017-1-AP; Proteintech), anti-GAPDH antibody (dilution 1:10000; ab181602; Abcam, Cambridge, USA), anti-cleaved caspase 3 antibody (dilution 1:1000; A22869; ABclonal, Wuhan, China) or anti-cyclin D1 antibody (dilution 1:1000; 55506T; CST) at 37°C for 1 h, followed by incubation with goat anti-rabbit IgG secondary antibody (dilution 1:5000; 31210; Thermo Pierce, Rockford, USA) for 1 h at room temperature. After incubation with SuperSignal® West Dura Extended Duration Substrate (34075; Thermo Pierce) for 1 min at room temperature, the protein bands were exposed and analyzed with ImageJ software.

Xenografts

Female nude mice (3–4 weeks old) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd [license number: SCXK (Shanghai) 2017-0005] (Shanghai, China). The animal study was approved by the Experimental Animal Welfare and Ethics Committee of Wannan Medical College. Animals were divided into control, lentivirus-OE-ENST00000422059 + control, lentivirus-OE-miR-145-5p + control, and lentivirus-OE-ENST00000422059 + lentivirus-OE-miR-145-5p groups (6 animals per group). The right forelimb of animals was administered subcutaneous injections of MDA-MB-231 cells (5×10^6 cells/200 μL) expressing relevant genes. Tumor weight and volume were measured based on a previous report [16]. After the tumor was removed and weighed, it was fixed with 4% paraformaldehyde for subsequent experiments.

Immunohistochemistry

After the tumor tissues were removed, an immunohistochemistry assay was conducted according to the method reported previously [16]. In brief, tumor tissues were fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 4-μm sections. Then further processed for Hematoxylin and eosin (H&E) staining and immunohistochemical staining for Ki-67 (1:500; ab92742; Abcam). Finally, the images were visualized using a CX43 microscope (Olympus).

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). GraphPad Prism software was used for data analysis and graphing. Student's *t* test was employed for comparative analysis between two

groups, while one-way ANOVAs were used to conduct multiple-group comparisons. A P value of <0.05 indicated statistical significance.

Results

Promotion of cell proliferation and inhibition of cell apoptosis in BC by KLF5

Overall survival analysis revealed that, in contrast to BC patients with low KLF5 expression levels, patients with high KLF5 expression levels had a worse prognosis [HR = 1.26 (1.14–1.39), log-rank $P = 9.5 \times 10^{-6}$] (Figure 1A). To reveal the pro/antiproliferative effects of KLF5 on BC cells, KLF5 overexpression plasmids or siRNA were transfected into MDA-MB-231 cells. As indicated in Figure 1, KLF5 was significantly overexpressed or reduced after overexpression and silencing, as confirmed by western blot analysis and qPCR (Figure 1B–E). KLF5 overexpression enhanced cell proliferation in MDA-MB-231 and MCF-7 cells, according to the CCK-8 assay, followed by a decrease in the apoptosis rate (Figure 1E–G). In contrast, when *KLF5* was silenced, cell proliferation was inhibited, and the apoptosis rate was increased (Figure 1E–G). These data indicate that KLF5 promotes BC cell proliferation and inhibits cell apoptosis.

Inhibition of cell proliferation and promotion of apoptosis in BC by miR-145-5p

Bioinformatics analysis revealed a binding site with the miRNA in the 3′UTR region of KLF5 mRNA, which suggested that KLF5 might be the target gene of the miRNA (Figure 2A). With overexpression of miR-145-5p, dual-luciferase reporter analysis indicated a remarkable decrease in luciferase activity of wild-type KLF5. However, no change was observed in mut-type KLF5 (Figure 2B). Then, the expression of the miRNA in BC samples was analyzed using TCGA data, and the results indicated attenuated expression in metastatic and primary BC samples in contrast to that in normal samples (Figure 2C). Overall survival analysis showed that BC-affected individuals with an increased expression level of the miRNA had an improved prognosis [HR = 0.81 (0.67–1.00), log-rank $P = 0.047$] (Figure 2D). To verify the role of the miRNA in BC, miR-145-5p mimic/inhibitor was transfected into BC cells, cell proliferation was detected by CCK8 assay and apoptosis was detected by flow cytometry. The results indicated that miR-145-5p attenuated cell proliferation and enhanced cell apoptosis in BC cells (Figure 2E–H).

miR-145-5p functions by targeting KLF5

To explore whether miR-145-5p exerts its biological function by targeting KLF5, KLF5 expression was detected after overexpressing or silencing miR-145-5p in BC cells. Western blot analysis and qPCR assay showed that the mRNA and protein levels of KLF5 were remarkably lowered following miRNA overexpression (Figure 3A–D). However, following miRNA silencing, the levels of KLF were significantly increased in the two BC cell lines (Figure 3A–D). Subsequently, a rescue experiment was conducted to verify whether the miRNA directly acts on KLF5 to exert its biological function in BC cells. Proliferation and apoptosis were detected in BC cells after cotransfection with miR-145-5p mimic or negative control and plasmid-KLF5 or negative control. The CCK-8 assay and EdU assay showed that BC cell proliferation was markedly enhanced with cotransfection of miRNA and KLF5 compared with cotransfection with the KLF5 negative control group, and KLF5 attenuated the

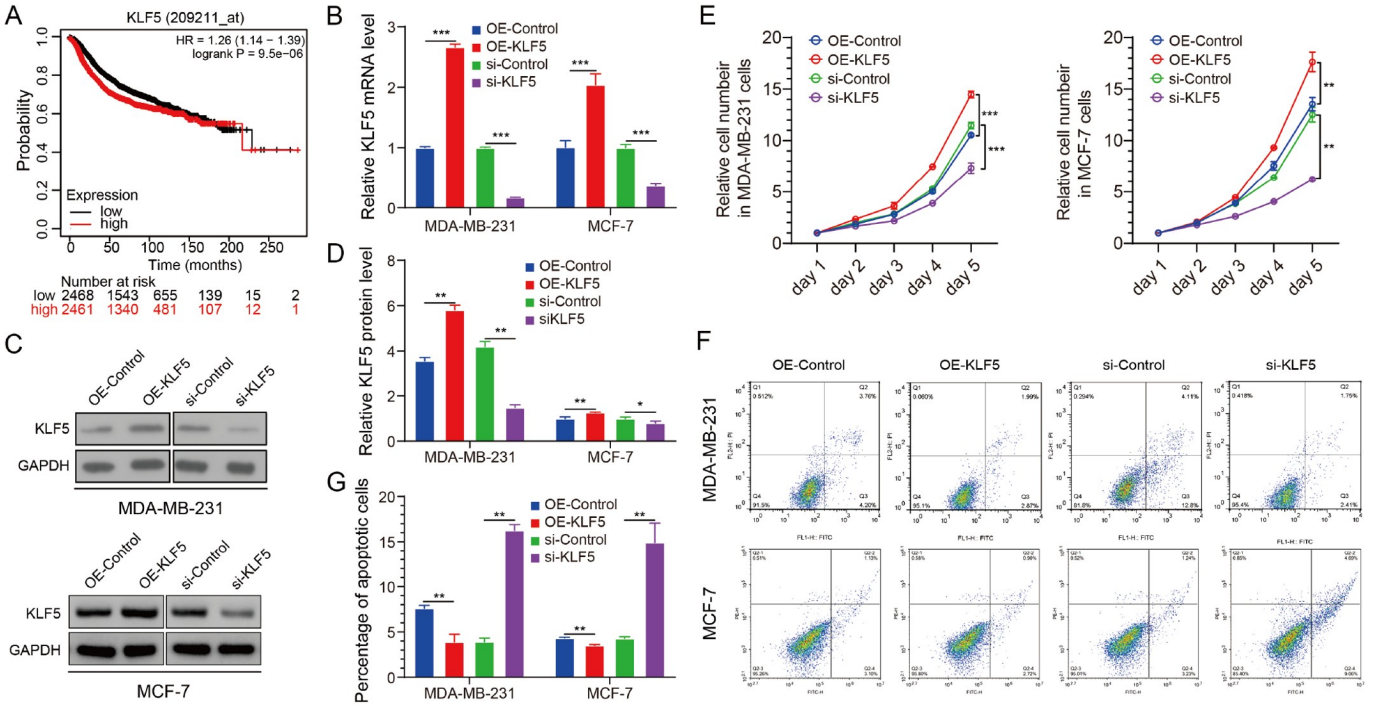


Figure 1. Enhancement of cell proliferation and inhibition of apoptosis by KLF in BC (A) Overall survival and KLF5 expression in BC patients. (B) Detection of the efficiency of KLF5 overexpression/silencing. (C) Analysis of the impact of KLF5 on BC cell proliferation. (D,E) Representative images (D) and quantitative analysis (E) verifying the efficiency of KLF5 overexpression/silencing. (F,G) Impact of KLF5 on BC cell apoptosis. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

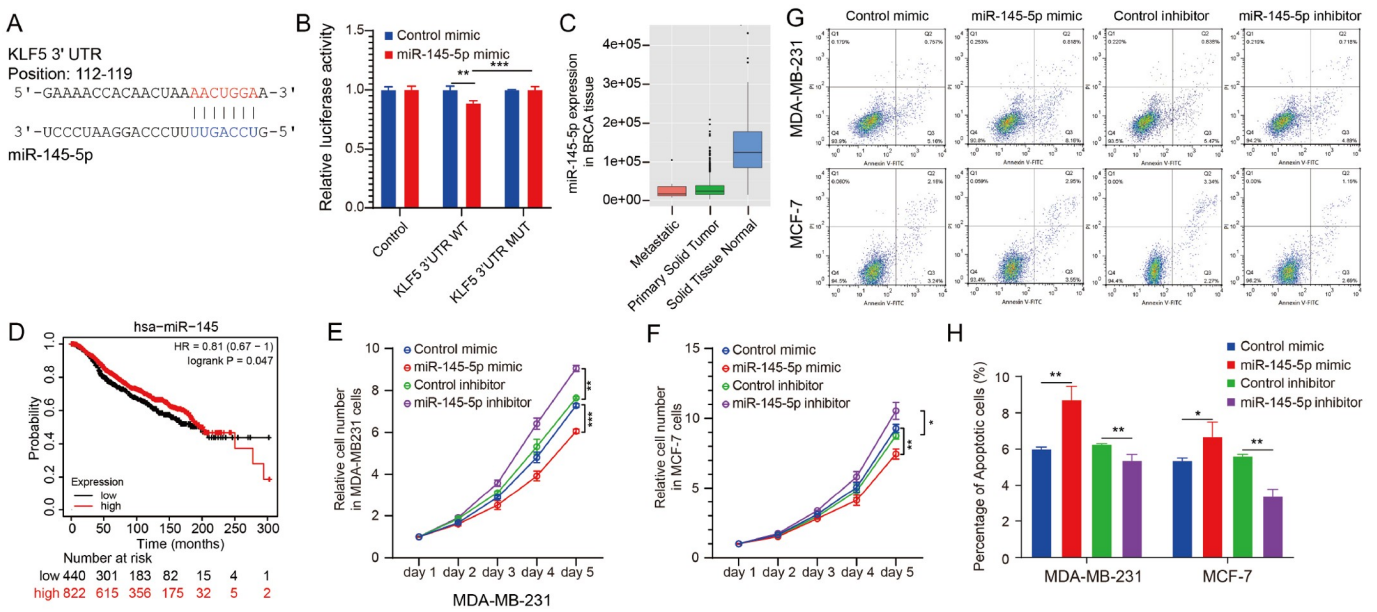


Figure 2. miR-145-5p is a targeted miRNA of KLF5 and is contrary to the effect of KLF5 on cell proliferation and apoptosis (A) Schematic representation of the miR-145-5p seed sequence complementary to KLF5 3′UTR sequences. (B) Analysis of the luciferase activity of miRNA binding to the KLF5 3′UTR. (C) Bioinformatics analysis of miRNA expression in BC samples from the TCGA public dataset. (D) Overall survival of miRNA expression in BC patients. (E) Analysis of the impact of the miRNA on BC cell proliferation. (F,G) Analysis of the impact of the miRNA on BC cell apoptosis. $*P<0.05$, $**P<0.01$, $***P<0.001$.

inhibitory effect of miR-145-5p on BC cell proliferation (Figure 3E,H,I). Apoptosis assay revealed that miRNA overexpression promoted apoptosis in BC cells (Figure 3F,G). In contrast, KLF5 overexpression weakened the proapoptotic action of the miRNA (Figure 3F–I). These findings indicated that the miRNA decreases proliferation and promotes apoptosis by negatively regulating KLF5 expression.

Promotion of cell proliferation and inhibition of cell apoptosis by ENST00000422059

By means of bioinformatics analysis, a binding site was found between ENST00000422059 and miR-145-5p (Figure 4A). To further explore the function, the cellular localization of ENST00000422059 was detected. The FISH assay showed that ENST00000422059 is located in the cytoplasm (Figure 4B). Subsequently, a double luciferase reporter assay was performed to detect the binding site between the miRNA and ENST00000422059. The results indicated a remarkable reduction in the luciferase activity of wild-type ENST00000422059 following overexpression of the miRNA, and no significant difference was observed in mut-type ENST00000422059 (Figure 4C).

To explore the function of ENST00000422059, KLF5 expression, cell proliferation, and apoptosis were detected in BC cells after overexpression or silencing of ENST00000422059. The overexpression plasmid and siRNA were used to regulate the expression of ENST00000422059 (Figure 4D). Western blot analysis and qPCR assay showed that with ENST00000422059 overexpression, KLF5 expression was increased and miRNA expression was decreased, while KLF5 expression was decreased and miRNA expression was increased when ENST00000422059 was silenced (Figure 4E,F).

The CCK-8 assay revealed that BC cell proliferative activity was increased when ENST00000422059 was overexpressed, while the BC cell proliferative potential was remarkably weakened after silencing of ENST00000422059 (Figure 4G). Flow cytometric analysis indicated that with ENST00000422059 overexpression, BC cell apoptosis was remarkably inhibited. However, when ENST00000422059 was silenced, BC cell apoptosis was markedly enhanced (Figure 4H). All these results indicated that ENST00000422059 was capable of promoting cell proliferation and inhibiting cell apoptosis. When ENST00000422059 was inhibited, the protein level of cleaved caspase 3 was increased compared with that in the control siRNA group in two BC cell lines (Figure 4I). When ENST00000422059 was inhibited, the protein level of cyclin D1 was decreased compared with that in the control siRNA group in two BC cell lines (Figure 4I). These data indicate that ENST00000422059 promotes cell proliferation and inhibits cell apoptosis.

ENST00000422059 functions via regulation of the miR-145-5p/KLF5 axis in BC cells

To verify the ability of ENST00000422059 to inhibit BC cell apoptosis and promote BC cell proliferation by regulating the miR-145-5p/KLF5 axis, the miRNA mimics or negative control was cotransfected with the plasmid-KLF5 or negative control into MCF-7 and MDA-MB231 cells. CCK-8 assay showed that overexpression of miRNA alone inhibited the cell proliferation rate and that overexpression of KLF5 alone promoted cell proliferation, in contrast to that of the miR-145-5p and ENST00000422059 co-overexpression group (Figure 5A). The EdU results also confirmed the CCK-8 results mentioned above (Figure 5D,E). Flow cytometric analysis showed that ENST00000422059 could reverse the proapoptotic effect of the miRNA in BC cells (Figure 5B,C). The rescue assay indicated that ENST00000422059 overexpression could restore the impact of miRNA overexpression on cell function. All these results demonstrated that ENST00000422059 inhibited cell apoptosis and improved cell proliferation in BC cells via regulation of the miR-145-5p/KLF5 axis.

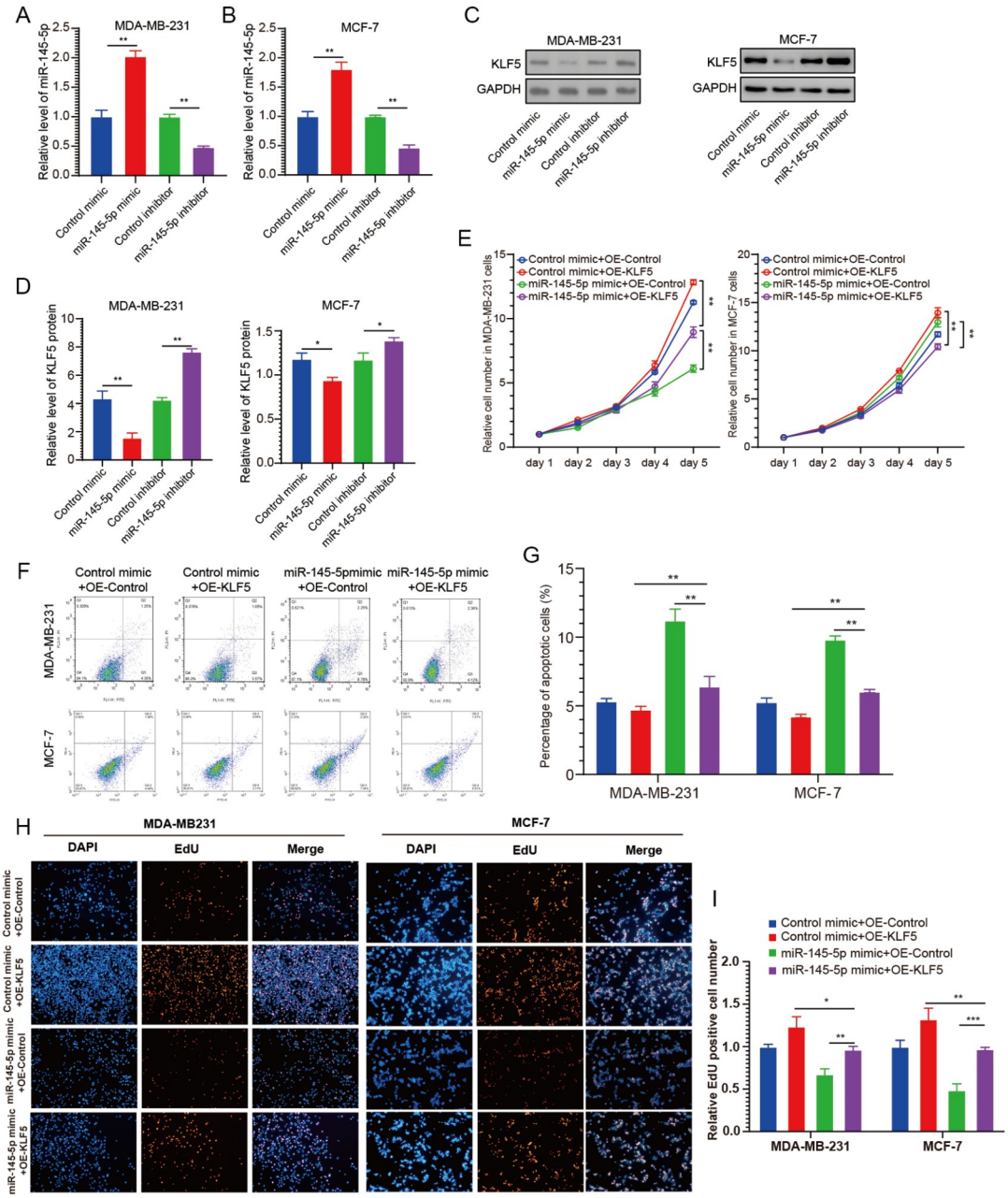


Figure 3. Inhibition of cell proliferation and promotion of cell apoptosis by miR-145-5p via regulation of KLF5 gene expression (A,B) Detection of miRNA overexpression/silencing. (C,D) Impact of the miRNA on KLF5 expression in MCF-7 and MDA-MB231 cells. (E) CCK8 assay of the group treated with control mimic plus control vector, control mimic plus OE-KLF5, miR-145-5p mimic plus OE-control, miR-145-5p mimic plus OE-KLF5. (F,G) The cell apoptosis of the group described in (E) was analyzed by flow cytometry. (H,I) EdU assay of the group described in (E). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Promotion of tumor growth by ENST00000422059 via regulation of the miR-145-5p/KLF5 axis *in vivo*

The function of ENST00000422059 was further evaluated in a nude mouse BC xenograft model. A lentivirus overexpressing ENST00000422059 was constructed, and another lentivirus overexpressing miR-145-5p was also constructed. MDA-MB-231 cells (1×10^6) stably expressing the target gene were subcutaneously administered to nude mice. On the 25th day, the tumor volume of the ENST00000422059 overexpression group was markedly enhanced compared to that of the control + miR-NC group ($P<0.05$; Figure 6A–C). However, the tumor volume of the lentivirus-miR-145-5p group shrank remarkably ($P<0.01$; Figure 6A–C). In the group cotransfected with ENST00000422059 and miR-145-5p, the tumor volume was similar to that of the control + miR NC group (Figure 6A–C). This was compensated by overexpression of ENST00000422059 (lentivirus-OE-ENST00000422059 + lentivirus-OE-miR-145-5p group) (Figure 6A–C). A markedly enhanced expression of Ki-67 was observed in the ENST00000422059 overexpression group in contrast to the control + miR NC group. Furthermore, it was markedly reduced in the miRNA overexpres-

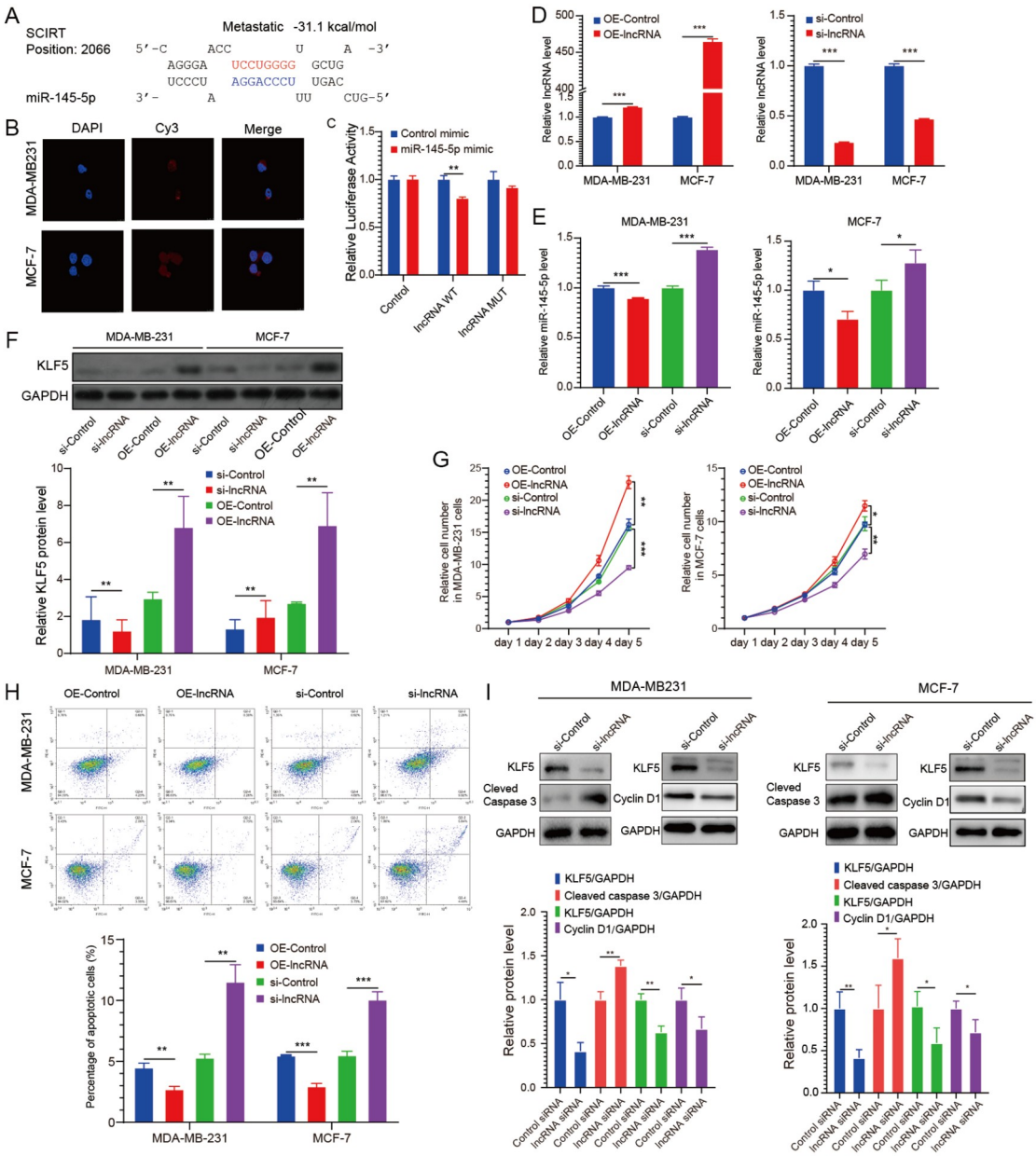


Figure 4. Sponging of miR-145-5p by ENST00000422059 is contrary to the effect of miR-145-5p on cell proliferation and apoptosis (A) Schematic representation of the miRNA seed sequence complementary to ENST00000422059. (B) The location of ENST00000422059 was determined in BC cells via FISH. (C) Analysis of the luciferase activity of the miRNA bound to ENST00000422059. (D) Detection of the efficiency of ENST00000422059 overexpression/silencing. (E) Detection of the miR-145-5p level following overexpression/silencing of ENST00000422059 in BC cells. (F) The KLF5 protein level was detected when ENST00000422059 was overexpressed/silenced in BC cells. (G) Promotion of cell proliferation by ENST00000422059. (H) Inhibition of cell apoptosis by ENST00000422059. (I) Cleaved caspase 3 and cyclin D1 protein levels were detected when ENST00000422059 was silenced in BC cells. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

sion group (Figure 6D). Western blot analysis showed that the miRNA could lower the expression of the KLF5 protein in the transplanted tumor. At the same time, cotransfection with ENST00000422059 resulted in a marked decrease in the inhibitory effect of the miRNA on KLF5 (Figure 6E,F).

In addition, miRNA expression was also detected. qPCR assays showed that ENST00000422059 cotransfection significantly reduced miRNA expression in transplanted tumors (Figure 6G). Altogether, these findings suggested that ENST00000422059 enhanced the growth of tumors via regulation of the miR-145-5p/KLF5 axis *in vivo*.

Discussion

KLF5 (also known as IKLF/BTEB2) belongs to the Krupel-like transcription factor family and broadly regulates several functions, including cell proliferation, differentiation, and self-renewal of embryonic stem cells [17]. Kyriazis *et al.* [18] reported that KLF5 could be activated by FOXO1 transcription, which increased oxidative stress, ceramide accumulation, and mitochondrial damage, thus triggering diabetes cardiomyopathy. Shieh *et al.* [19] found that loss of KLF5 in intestinal epithelial cells could cause intestinal epithelial barrier damage and regulate the cellular

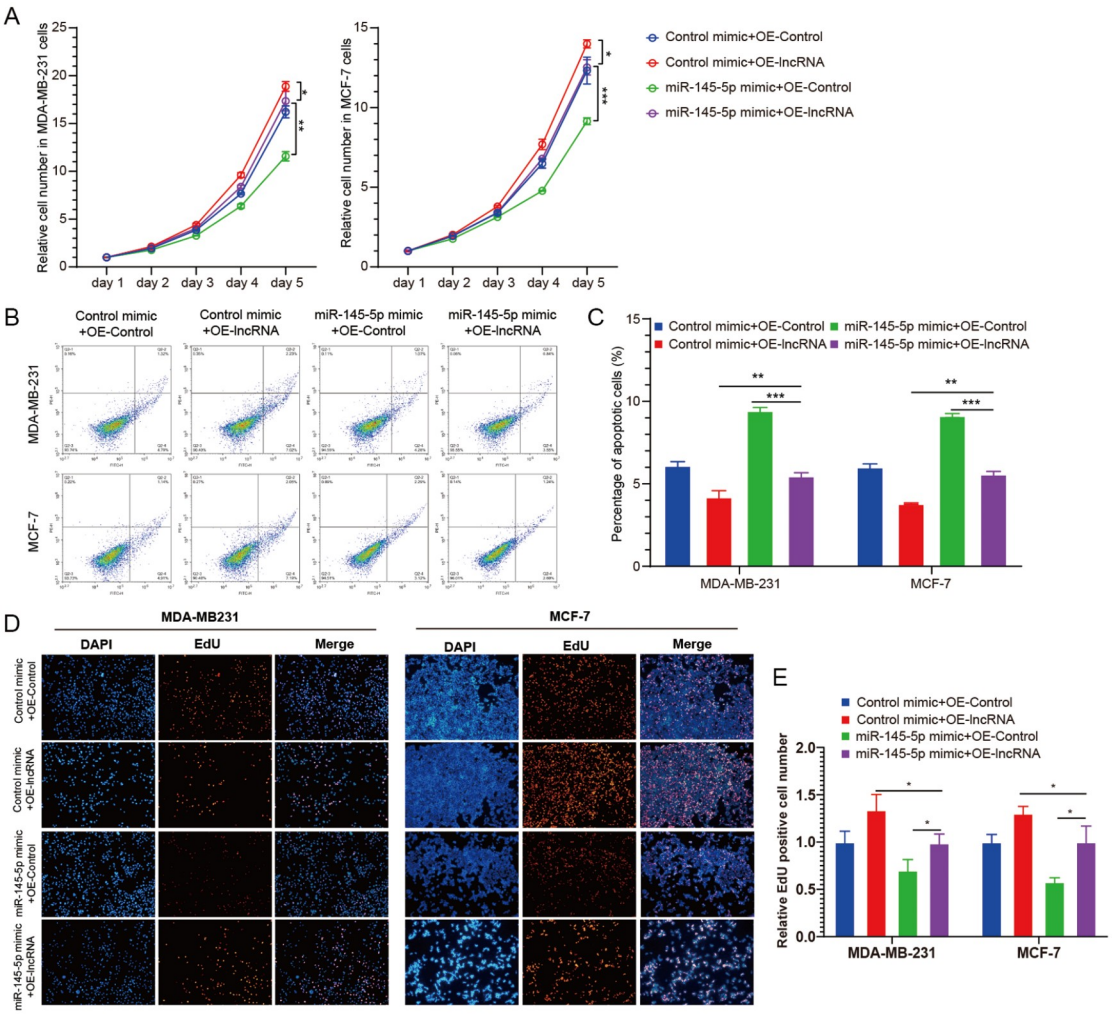


Figure 5. Inhibition of cell apoptosis and promotion of cell proliferation by ENST00000422059 by sponging of miR-145-5p (A) CCK-8 assay of the group treated with control mimic plus control vector, control mimic plus OE-ENST00000422059, miR-145-5p mimic plus OE-control, or miR-145-5p mimic plus OE-ENST00000422059. (B,C) Analysis of cell apoptosis of the group described in (A). (D,E) EdU assay of the group described in (A). $*P<0.01$, $**P<0.01$, $***P<0.001$.

localization of p-STAT3 in these cells through the Th17 immune response, thus affecting the onset and progression of inflammatory bowel disease (IBD). Moreover, several studies have demonstrated the involvement of KLF5 in the onset and progression of various tumors. Xu *et al.* [20] revealed that KLF5 and MYC could bind to the promoter of LINC00346, thus enhancing LINC00346 expression and promoting growth, migration, and invasion in gastric cancer. Ma *et al.* [21] reported that KLF5 expression in prostate cancer was downregulated, which enhanced the phosphorylation of IGF1 and STAT3, thus promoting the invasive ability of prostate cancer cells both *in vivo* and *in vitro*. Recent research indicated that KLF5 mediated cisplatin chemoresistance in triple-negative BC and increased the transcription level of CXCR4 to accelerate epithelial and mesenchymal transformation [22,23]. Our research confirmed that KLF5 has enhanced expression in BC, and patients with increased KLF5 expression levels exhibit a poor prognosis. CCK-8 and apoptosis assays demonstrated that KLF5 promoted proliferation and attenuated apoptosis in BC cells.

Some studies have verified that KLF5 can be regulated by miRNAs in many types of cancer, such as oral squamous cell cancer [24], gastric cancer [25], nasopharyngeal carcinoma [26], and hepatocellular carcinoma [27]. Based on bioinformatics analysis, a binding site was found between miR-145-5p and KLF5. Previous studies have shown that miR-145-5p regulates KLF5 to inhibit the progression of gastric cancer and liver cancer [25,27], but it has not been reported in BC. In our research, we found that miR-145-5p can inhibit cell proliferation and promote cell apoptosis by regulating KLF5 in BC.

The class of lncRNAs is commonly expressed in mammals and has a length of more than 200 nucleotides. Several lncRNAs are vital regulators of tumorigenesis, development, and metastasis through different mechanisms. For example, continual overexpression of HOTAIR can affect the degree of chromatin openness of tumor cells, thereby regulating gene expression and ultimately promoting the metastasis of breast tumors to the lung in mice [28]. lncRNA BCAR4 accelerates cell migration and infiltration through the miR-644a/CCR7 axis and the MAPK pathway and upregulates ABCB1 to induce chemoresistance in BC [29]. The mechanism of action of lncRNAs is primarily based on their different cellular localizations. In general, cytoplasmic-localized lncRNAs affect tumor progression

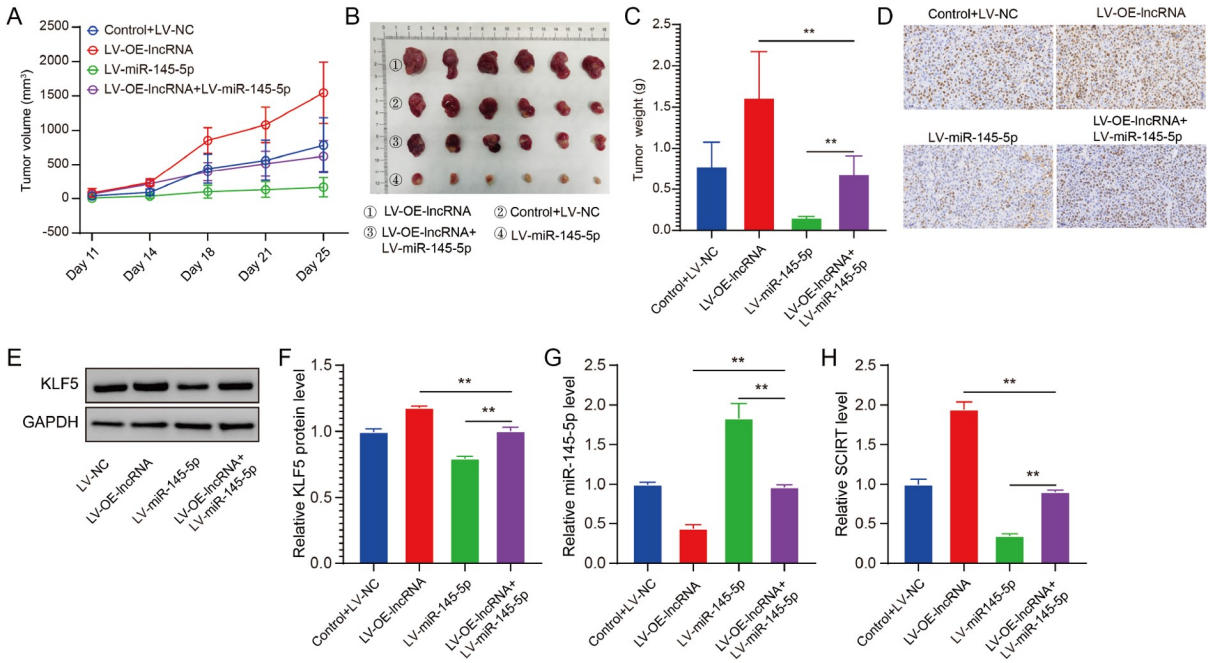


Figure 6. ENST00000422059 promotes tumor growth via regulation of the miR-145-5p/KLF5 axis (A) Impact of ENST00000422059/miR-145-5p on BC cell-derived tumor growth. (B) Overall appearance of xenograft tumor size. (C) Analysis of tumor weight. (D) Representative images and statistics of Ki-67-positive cells. (E,F) Detection of KLF5 protein levels in tumor tissues. (G) Detection of the miRNA level in tumor tissues. (H) Detection of the ENST00000422059 level in tumor tissues. $*P<0.05$, $**P<0.01$.

through the ceRNA mechanism in various tumors [30–32]. To elucidate the upstream regulatory mechanism of miR-145-5p, we performed bioinformatics analysis. We found that Enst00000422059 and miR-145-5p have a binding site. FISH assays confirmed that ENST00000422059 is located in the cytoplasm. Thus, we hypothesized that ENST00000422059 could regulate KLF5 by sponging the miRNA.

Next, the ability of the miRNA to bind with ENST00000422059 was demonstrated by dual luciferase reporter experiments. Cell function and western blot analysis experiments showed that ENST00000422059 alleviated miRNA inhibition of cell proliferation and reversed the proapoptotic effect in BC cells by regulating the miR-145-5p/KLF5 axis. Thus, ENST00000422059 acts as a competitive endogenous RNA for the purpose of regulating KLF5 function. We also found that the protein level of cleaved caspase 3 was increased when ENST00000422059 was inhibited in the two BC cell lines. Finally, xenograft experiments confirmed the tumor-promoting effect of the ENST00000422059/miR-145-5p/KLF5 axis *in vivo*. These results reveal a new mechanism by which ENST00000422059 promotes the development of BC.

In summary, the biological function of ENST00000422059 in BC was revealed in this study. Furthermore, ENST00000422059 was confirmed to sponge miR-145-5p to enhance KLF5 expression both *in vitro* and *in vivo*, which in turn promotes the deterioration of BC. Nevertheless, there are still some limitations in this research. The effect of the ENST00000422059/miR-145-5p/KLF5 axis on other cell functions of BC (such as the cell cycle and cell migration) and the expression of cleaved Caspase 3 in tumor tissue were not investigated in animal experiments. There was a lack of verification of ENST00000422059 and miR-145-5p expression in BC and corresponding paracancerous tissue samples. These issues need to be clarified in future research.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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