Long non-coding RNA MAFG-AS1 promotes proliferation and metastasis of breast cancer by modulating STC2 pathway

Shihao Di
The First Affiliated Hospital of Nanjing Medical University
Die Lu
The First Affiliated Hospital of Nanjing Medical University
Chunni Chen
The First Affiliated Hospital of Nanjing Medical University
Tianshi Ma
The First Affiliated Hospital of Nanjing Medical University
Zigui Zou
The First Affiliated Hospital of Nanjing Medical University
Zhihong Zhang (zhangzh@njmu.edu.cn)
The First Affiliated Hospital of Nanjing Medical Univerity https://orcid.org/0000-0002-5779-9365

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Abstract

Objective Breast cancer is the most common cancer in Chinese women. A number of studies proposed that long non-coding RNA plays an essential role in the regulation of invasion and metastasis of various forms of malignancy, including lung cancer, gastric cancer and bladder cancer. In this study, a long non-coding RNA MAFG-AS1 was explored in detail to understand the significance in the etiology of breast cancer.

Methods Quantitative reverse transcription PCR (qRT-PCR) was used to examine the expression level of LncRNA MAFG-AS1 in tissues and cell lines. The association of LncRNA MAFG-AS1 expression and the postoperative prognosis was analyzed by the Kaplan-Meier method and log-rank test. Cell proliferation was evaluated in vitro and in vivo . Transwell assays were performed to examine the cell migration. Cell cycle and apoptosis was evaluated by flowcytometry analysis. The downstream target gene STC2 of LncRNA MAFG-AS1 was screened using the microarray analysis, which was validated by qRT-PCR, functional analysis, and rescue experiment.

Results Expression of LncRNA MAFG-AS1 in the breast cancer tissues was significantly higher than the precancerous lesions. Elevated expression level of LncRNA MAFG-AS1 was correlated to the larger GTV (gross tumor volume), negative expression of ER, PR, Her2, lymph node metastasis, and poor prognosis. The potency of breast cancer proliferation, invasion and metastasis was inhibited in the absence of LncRNA MAFG-AS1.Tumorigenic capacity of breast cancer cells was inhibited in the absence of LncRNA MAFG-AS1. The downstream target gene regulated by LncRNA MAFG-AS1 was screened out by gene chip technology, GO analysis and QRT-PCR ultimately. Disrupted STC2 suppressed the cell proliferation and metastasis when the level of LncRNA MAFG-AS1 elevated.

Conclusion The LncRNA MAFG-AS1 triggers tumorigenesis in the breast cancer and regulates breast cancer proliferation and metastasis by modulating the downstream target gene STC2. Results from our study indicates that LncRNA MAFG-AS1 can be used.

1. Introduction

Breast cancer is the most common malignancy in China and accounts for the largest proportion of cancer-related morbidity among women[1]. With the advancement of comprehensive treatments such as chemotherapy, endocrine therapy and targeted therapy, the breast cancer related mortality ratehas been significantly reduced but still remains as one of the leading causes of cancer-related death among women, and ranks 5th in cancer-related mortality in female patients[2]. While it is possible to cure breast cancer at its early-stage, negligence in the self-examinations and clinical examinations leading to the diagnosis of advanced breast cancer. Even with the advancement in the comprehensive therapies, the risks of recurrence and metastasis are still high[3].

The vast majority of the human genome is made up of non-coding RNA (ncRNA)[4], apart from about only 2% protein-coding genes, which was found by gene chip technology and whole transcriptome sequencing. Long non-coding RNAs (lncRNAs) belong to a major class of non-coding RNAs (ncRNAs) with more than 200 nucleotides in length playing important roles in widely regulating cellular and biological functions at transcriptional, post-transcriptional and epigenetic levels, whose abnormal expression is relevant with the development and progression of malignancy[5, 6]. Multiple evidences demonstrate that lncRNA plays important roles in development and progression of breast cancer. For example, the poor prognosis of TNBC was related to the elevated expression of LncRNA AFAP1-AS1[7], ARNILA[8], ZNF469[9], DANCR[10] and reduced expression of LncRNA H19[11], GAS5[12]. LncRNA MALAT1 regulated the critical pathway in triple negative breast cancer (TNBC) development and progression. Highly expressing MALAT1 can up-regulate the expression levels of c-MET and SOX4 by competitive binding with targeted mRNAs such as miR-34a/c-5p and miR-449a/b, then promoting proliferation and metastasis of tumor cells[13–15].

The MAF BZIP Transcription Factor G Antisense RNA 1 (MAFG-AS1) is a LncRNA with length of 1914 bp, whose mechanism in breast cancer still remained unclear. Results from this study revealed that the expression of LncRNA MAFG-AS1 in breast cancer tissues was evidently up-regulated compared to the precancerous lesions. Moreover, the high expression of LncRNA MAFG-AS1 was related to the larger GTV (gross tumor volume), negative expression of ER, PR, Her2, lymph node metastasis and poor prognosis. The further research found that LncRNA MAFG-AS1 triggers tumorigenesis in the breast cancer, and regulates breast cancer proliferation and metastasis, potentially via manipulating the downstream target gene stanniocalcin 2 (STC2). Taken together, our study suggest the clinical manifestation of MAFG-AS1, which can be used as a novel biomarker for diagnosis and potential therapeutic target for breast cancer, which participate in regulating the prognosis in breast cancer patients.

2. Materials And Methods

2.1 Collection of breast cancer tissue and clinical data

Fifty-four pairs of breast cancer and nontumorous breast tissues were obtained from the breast cancer patients undergoing surgery at the First Affiliated Hospital of Nanjing Medical University. These patients had not been treated locally or systematically before the surgery. All cases were confirmed by pathological diagnosis as breast cancer. Table 1 summarizes the clinicopathological characteristics of all patients. The study had been approved by the relevant regulatory and independent ethics committees of the First Affiliated Hospital, Nanjing Medical University. Informed consent was obtained from each patient.

2.2 Cell culture

Four human breast cancer cell lines, (T-47D, MDA-MB-231, MDA-MB-468, and BT-474) and the healthy human breast epithelial cell lines (MCF-10A) were purchased from the Chinese Academy of Sciences

Biochemistry and Cell Biology Institute of Technology (Shanghai, China). T-47D cells were cultured in RPMI-1640 (GIBCO-BRL) medium, supplemented with 10% fetal bovine serum (FBS; ScienCell), 0.2 Units / ml insulin, and 100 U / ml penicillin and 100 mg / ml streptomycin (Invitrogen); MDA-MB-231 and MDA-MB-468 cells were cultured in L-15 (GIBCO-BRL) medium, and BT-474 cells were cultured in RPMI-1640 (GIBCO-BRL) medium. Both media supplemented with 10% fetal bovine serum (FBS; ScienCell) and with double antibiotic 100U / ml penicillin and 100 mg / ml Streptomycin (Invitrogen). All cells were cultured at 37 ° C with 5% CO_2. Fresh medium was replaced every 2-3 days and cells were passaged when the ~~cell~~ confluence reached 80%- 90%.

2.3 RNA extraction and qRT-PCR

Total RNA was extracted from BC tissues and cell lines using TRIZOL reagent (Invitrogen). cDNA was synthesized using the PrimeScrip-RT Reagent Kit (Takara). qRT-PCR was performed with the SYBR Green PCR kit (Takara) on an Applied Biosystems 7500 real-time PCR system. The results were normalized to GAPDH expression and analysed using the 2−ΔΔCt method. All of primers used in this study are summarized in Table S1.

2.4 Plasmid preparation

T-47D and MDA-MB-231 cells were transfected with plasmid vector using XtremeGENE HP DNA transfection reagent (Roche, Basel, Switzerland) according to the manufacturer's instructions. The full-length complementary DNA of LncRNA MAFG-AS1 was synthesized and sub-cloned into the pcDNA3.1(+) vector (Invitrogen) by Generay (Shanghai, China). Cells were harvested for qRT-PCR, 48h after transfection.

2.5 RNA interference

T-47D and MDA-MB-231 cells were transfected with siRNAs using the Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. Three individual LncRNA MAFG-AS1 siRNAs (si-LncRNA MAFG-AS1 1#, 2#, 3#), STC2 siRNA, and scrambled negative control siRNA (si-NC) were purchased from Invitrogen. The nucleotide sequences of siRNAs for LncRNA MAFG-AS1, STC2 are listed in Table S1. Cells were harvested for qRT-PCR or western blot analysis 48 h after transfection.

2.6 Cell proliferation assays

Cell vitality was detected using the Cell Counting Kit-8 (CCK-8) assay kit (Bimake). Briefly, transfected cells were seeded into 96-well plates and incubated with CCK-8 reagent every 24 hours according to manufacturer's instructions. For colony formation assay, cells were cultured in 6-well plates, and after 14 days, the cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were photographed. Cell proliferation was identified using Ethynyldeoxyuridine (Edu) assay following the manufacturer's protocol of 5-ethynyl-2-deoxyuridine (Edu) labeling/detection kit (Ribobio, Guangzhou, China). The transfected cells were treated with 50 μM Edu labeling medium and incubated for 2 hours.

Next, the cells were fixed with 4% paraformaldehyde and the cell membrane was permeated withby 0.5% Triton X-100. Subsequently, cells were added with anti-Edu working solution and DAPI staining solution. Edu positive cells were identified and counted under fluorescent microscopy. The percentage of Edu positive cells were calculated from five random fields in three wells.

2.7 Cell migration and invasion assays

For the migration assays, T-47D and MDA-MB-231 cells were transfected with si-LncRNA MAFG-AS1 or pcDNA-LncRNA MAFG-AS1, and were cultured in 24-well plates with an 8-mm pore size polycarbonate membrane (Corning Incorporated). For the invasion assays, cells in serum-free medium were placed into the upper chamber of an insert coated with Matrigel (Sigma-Aldrich). Medium containing 10% FBS was added to the lower chamber. After 24 h, the cells remaining on the upper chamber were wiped with cotton swabs, while cells on the lower membrane surface were fixed with methanol and stained with 0.1% crystal violet after 24 h incubation. Five fields of view were randomly selected in each well for counting. For the wound-healing assays, the cells were seeded in 6-well culture plates at 2×10^5 cells per well. After the cells were adhered, pcDNA-LncRNA MAFG-AS1 (or empty vector) was transfected with the interference sequence or control sequence of MAFG-AS1. Using a 10 microliter pipette tip two to four straight lines were drawn in the six-well plate, the floating cells were washed with PBS. Cell gaps at the starting point were captured under a microscope. The six-well plate was then placed back inside the incubator for 24-48, and the progress on the cell gap closure was closely monitored. Images were captured, and the intercellular space was measured using a software. The treatment group was compared with the control group while analyzing the data.

2.8 Tumor Induction assay in a nude mouse model

Male BALB/c-nude mice (4-weeks-old) were maintained under specific pathogen-free (SPF) conditions and manipulated according to the protocols approved by the Shanghai Medical Experimental Animal Care Commission. T-47D cells were stably transfected with sh-LncRNA MAFG-AS1 and empty vector and harvested from 6-well cell culture plates. Cells were then washed with phosphate-buffered saline (PBS), and re-suspended at a concentration of 1×108 cells/ml. A total of 100 µl of suspended cells was subcutaneously injected into a single side of the armpit of each mouse. Tumor growth was continuously monitored at an interval of every 5 days, and tumor volumes were calculated using the equation V=0.5×D×d2 (V represents volume; D represents longitudinal diameter; d represents latitudinal diameter). At 28 days post-injection, mice were euthanized, and the subcutaneous growth of each tumor was examined. This study was performed strictly in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Animal Ethical and Welfare Committee of Nanjing Medical University.

2.9 Immunohistochemical (IHC)analysis

The primary tumors were immunostained for Ki-67 as previously described. Flow Cytometric analysis T-47D and MDA-MB-231 cells were transfected with si-LncRNA MAFG-AS1 or scrambled, and were

harvested 48 h after transfection by trypsinization. Cells were stained with FITC-Annexin V, and propidium iodide (PI) by using the FITC Annexin V Apoptosis Detection Kit (BD Biosciences) following the manufacturer's protocol. The cells were analyzed by flow cytometry (FACScan®; BDBiosciences) with CellQuest software (BD Biosciences). The cells were classified into viable, dead, early apoptotic,and apoptotic cells, and then the ratio of early apoptoticcells was compared with the control for each experiment.

2.10 Subcellular fractionation location

The separation of nuclear and cytosolic fractions was performed using the PARIS Kit (Life Technologies) according to the manufacturer's protocol.

2.11 Statistical analysis

The Students t-test (two-tailed), one-way ANOVA, and the Mann-Whitney U test were conducted to analyze in vitro and in vivo data by SPSS 17.0 software. P values less than 0.05 were considered as significant.

3. Results

3.1 LncRNA MAFG-AS1 is up-regulated in breast cancer tissues and associated with poor prognosis

In this study 105 patients with breast cancer were analyzed using bioinformatic tool "TANRIC" (http://ibl.mdanderson.org/tanric/_design/basic/index.html) (Data comes from TCGA database), the expression level of MAFG-AS1 in breast cancer tissues was significantly higher than the precancerous lesions (Figure 1A). To further confirm this finding, we conducted a qRT-PCR analysis and found 54 breast cancer samples positive for LncRNA MAFG-AS1, and the expression level was significantly higher compared to the precancerous lesions(Figure 1D). Analytical results from TCGA database, qRT-PCR combindly indicate a possible link between the elevated level of MAFG-AS1, and development and progression of breast cancer.

In order tofurther explore the relationship between expression level of LncRNA MAFG-AS1 and the GTV (gross tumor volume), TNM stages, lymph node metastasis and prognosis, based on qRT-PCR results, 54 breast cancer samples were categorized into upregulated group (n=27) and downregulated group (n=27) (Figure 1E). The analysis of correlation between the clinical samples and the clinical data was used to explore the relationship between expression level of LncRNA MAFG-AS1 and clinicopathologic characteristics of breast cancer patients. We found that the upregulation of LncRNA MAFG-AS1 was related to the larger GTV (gross tumor volume) (p=0.033), negative expression of ER(p=0.040), PR(p=0.024), Her2(p=0.036),and lymph node metastasis(p=0.028).We could not find any effect of MAFG-AS1with the age (p=0.780), histological classification (p=0.413), and smoking habit (p=0.313) (Table 1).

Moreover, the analytical results of TCGA database suggested that LncRNA MAFG-AS1is highly expressed in breast cancer tissues of patients with poor prognosis, including PR negative and Luminal B subtype breast cancer patients. All together our results indicated that LncRNA MAFG-AS1 is closely related to the

prognosis of breast cancer patients, which can potentially be used as a diagnostic marker for the breast cancer and metastasis.

Table 1 Correlation between LncRNA MAFG-AS1 expression and clinicopathological characteristics of BC patients (n=54).

Characteristics	N (%)	LncRNA MAFG-AS1		P
		Upregulated group (27)	Downregulated group (27)	Chi-squared test P-value
Age(years)				
≤50	21 (38.9)	10	11	0.780
>50	33 (61.1%)	17	16	
Tumor size				
< 2cm	25 (46.3%)	8	17	0.033*
2-5cm	27 (50.0%)	18	9	
> 5cm	2 (3.7%)	1	1	
ER status				
Negative	17 (31.5%)	12	5	0.040*
Positive	37 (68.5%)	15	22	
PR status				
Negative	20 (37.0%)	14	6	0.024*
Positive	34 (63.0%)	13	21	
Her2 status				
Negative	10 (18.5%)	8	2	0.036*
Positive	44 (81.5%)	19	25	
Lymph node metastasis				
No	24 (44.4%)	8	16	0.028*
Yes	30 (55.6%)	19	11	
Histologicalgrade				
I-II	29 (53.7%)	13	16	0.413
III	25 (46.3%)	14	11	
Smoking History				
Smokers	1 (1.9%)	1	0	0.313
Never Smokers	53 (98.1%)	26	27	

*P<0.05 was considered significant.

3.2 Functional measurement of LncRNA MAFG-AS1 in breast cancer cell lines

3.2.1 LncRNA MAFG-AS1 promotes proliferation of the breast cancer cells in vitro

In order to evaluate the biological function of LncRNA MAFG-AS1 in breast cancer cell lines, the expression levels of LncRNA MAFG-AS1was examined in fourdifferentbreast cancer cell lines (MDA-MB-468, MDA-MB-231, T-47D, BT-474) by qRT-PCR. Healthy breast cell line MCF-10A was used as a control. The quantitative results demonstrated that expression levels of LncRNA MAFG-AS1 elevated evidently in all four breast cancer cell lines compared to the healthy control cell line. Relatively, MAFG-AS1 expression was more evident in MDA-MB-231,and T-47D lines . Therefore, MDA-MB-231, and T-47D lines were selected as the experimental subjects in following studies (Figure 2A). At first, three siRNAs targeted LncRNA MAFG-AS1 were designed and transfected into cell lines MDA-MB-231 and T-47D to silence lncRNA expression. The knock down of LncRNA MAFG-AS1 was confirmed by qRT-PCR. We found all three siRNAs were able to reduce the expression levels of MAFG-AS1 in the experimental cell lines compared control group. Moreover, since the transfection efficiency of si-LncRNA lncRNA MAFG-AS1 #2, and #3 were relatively higher, they were chosen for next confirmatory test. Next eukaryotic expression plasmids of LncRNA MAFG-AS1 were constructed and the following transfection assay demonstrated that the expression levels of LncRNA MAFG-AS1 was elevated distinctly in MDA-MB-231, and T-47D cell lines compared to the control transfection (Figure 2B). CCK-8, colony formation assays andEdU assays were used to verify the regulating effect of LncRNA MAFG-AS1 for breast cancer cell proliferation.Results from these assays revealed that the absence of LncRNA MAFG-AS1 in MDA-MB-231 and T-47D could suppress the breast cancer proliferation activity dramatically(Figure 2C), and over-expressing LncRNA MAFG-AS1 could promote the breast cancer cells proliferation activity clearly(Figure 2D) ; as the results of colony formation assays demonstrated that knockdown of LncRNA MAFG-AS1 in MDA-MB-231 and T-47D could suppress the breast cancer colony viability visibly(Figure 2E), while over-expressing LncRNA MAFG-AS1 could promote the breast cancer cells proliferation activity overtly(Figure 2F).Similarly, results from EdU assays demonstrated, inhibition of MDA-MB-231 and T-47D proliferation in the absence of MAFG-AS1 (Figure 2G), while the proliferation rate was accelerated when MAFG-AS1 was over-expressed (Figure 2H). All together, our results demonstrated that LncRNA lncRNA MAFG-AS1 can modulate proliferation in breast cancer cells.

3.2.2 Downregulation of LncRNA MAFG-AS1 triggers phase G1 stasis, and apoptosis of BC cells

Cell cycle and apoptosis are the critical factors in the regulation of cell-proliferation. In order to explore the mechanistic link between the level of LncRNA lncRNA MAFG-AS1 and suppression of the breast cancer cells proliferation, we evaluated the cell cycle events, and apoptosis by the flow cytometry(FCM).We observed that the cell cycle was ceased at phase G1-G0, in MDA-MB-231 and T-47D, cells in the absence of MAFG-AS1 (Figure 3A, 3B). Moreover, qRT-PCR result demonstrated that expression levels of G1phase-related genes (Cyclin D3, CyclinD1, CDK6, CDK4, CDK2) declined after knocking down of LncRNA lncRNA MAFG-AS1 in MDA-MB-231 and T-47D (Figure 3C). Apoptosis assay

with flow cytometry suggested that the ratio of apoptotic cells elevated evidently in the absence of LncRNA lncRNA MAFG-AS1 in MDA-MB-231 and T-47D (Figure 3D,3E). All together, we conclude that the knockdown of LncRNA lncRNA MAFG-AS1 suppressed breast cancer cell proliferation by triggering cell cycle arrest at G1 phase and augment of cell apoptosis.

3.2.3 knockdown of LncRNA MAFG-AS1 suppresses breast cancer invasion and metastasis

Cancer cells migration and invasion is a critical step for tumor infiltration and metastasis. Therefore, the wound-healing assays were utilized to explore the impact of LncRNA MAFG-AS1 on the migration ability of breast cancer cells. We observed that over-expression of lncRNA MAFG-AS1 promotes the migration ability of breast cancer cells (Figure 4A,4B). Next, we explored the migration and invasion abilities of breast cancer cell through a transwell migration, and invasion assays. We found an increased number of cells transmitting the basement membrane compared to the control group, indicating that lncRNA MAFG-AS1 promotes the migration and invasion of breast cancer cell, and hence suppression of the expression of MAFG-AS1 has important clinical application in the etiology of tumorigenesis. Our results suggest that knockdown of LncRNA lncRNA MAFG-AS1 suppressed migration and invasion abilities of breast cancer cell.

3.2.4 The LncRNA MAFG-AS1 promotes breast cancer tumorigenesis in vivo

In order to further evaluate the impact of LncRNA MAFG-AS on breast cancer cells tumorigenic, a xenograft model in nude mice was designed. We transfected T-47D cells with sh-LncRNA lncRNA MAG-AS1 and empty vector which were then inoculated into 4-week-old female nude mice either subcutaneously, or under the axilla separately. We recorded the size of GTV, immediately after the tumor formation. Finally, we euthanized the animals, and the tumor images were collected, The size and weight of the tumor were measured. The changes of GTV suggested that knockdown of LncRNA lncRNA MAFG-AS1 suppressed the tumorigenic capacity of breast cancer cells compared to the control group (Figures 6A-C). Tumor weight indicated that knockdown of LncRNA MAFG-AS1 dramatically influenced in reducing the mean weigh of tumors compared to the control group (Figure 6D). Furthermore, qRT-PCR result suggested that knockdown of LncRNA MAFG-AS1 suppressed the expression level of LncRNA MAFG-AS1 in tumor compared to the control group (Figures 6E). Immunohistochemistry result indicated that Ki67 expression in the absence of LncRNA MAFG-AS1 were much weaker compared to the control group. This observation further supports our hypothesis that MAFG-AS1 is a negative regulator of breast cancer tumorigenesis in vivo.

3.3 LncRNA MAFG-AS1 modulates the breast cancer cells proliferation and metastasis

3.3.1 The lncRNA MAFG-AS1 modulates its downstream target STC2

The altered gene expression profile after silencing the lncRNA MAFG-AS was evaluated using the Gene chip technology to screen targeted gene regulated by LncRNA lncRNA MAFG-AS1(Figures 7A). The screening result revealed the change in the expression pattern of 2237 genes (Fold Change>2, P

Value<0.05) after silencing lncRNA MAFG-AS1 in T-47D cells. This included the 1387 up-regulated genes, and 850 down-regulated genes. Gene enrichment analysis for Gene Ontology (GO) annotations was used, and the results of GO analysis suggested that MAFG-AS1 induced changes in the gene expression profile were correlated to the cell apoptosis, differentiation and metastasis, and consistent with the previous results (Figures 7B). In order to further examine the correlation between LncRNA the lncRNA MAFG-AS and breast cancer proliferation and metastasis, we detected the differentially expressed gene network related to apoptosis, differentiation and metastasis by using qRT-PCR. Based on the gene chip analysis, which presented that expression levels of STC2, TUBA1A, TOP2A, SLC25A6, SERPINA6, CBX5, CDK1 reduced apparently in T-47D cells after knockdown of LncRNA MAFG-AS1, conforming to the observations of gene chip, while over-expressing LncRNA MAFG-AS1 upregulated the expression of genes mentioned above. Also, we found that down-regulation of STC2 was most prominent in T-47D cells after knocking down of LncRNA MAFG-AS1 (Figures 7C, 7D). All-together, these results suggested that STC2 might be an important downstream gene regulated by LncRNA MAFG-AS1.

3.3.2 Tumor promotion of STC2 participating in the process of LncRNA MAFG-AS1 working

In order to study the specific function of STC2 in breast cancer, we studied the expression profiles of STC2 in 54 breast cancer tissues by using qRT-PCR. Result from qRT-PCR revealed that the expression level of STC2 in breast cancer tissues was distinctly higher compared to the precancerous lesions (Figure 8A). We detected STC2 expression in four different breast cancer tissues and normal breast cell lines MCF-10A proceeded, whose results showed that the expression level of STC2 in 4 breast cancer tissues were overtly higher compared to the normal breast cell lines MCF-10A (Figure 8B). Then we designed and synthesized the siRNA of STC2 to verify the function of STC2 in breast cancer (Figure 8C). We utilized CCK8 and colony formation assays to dissect the knockdown of STC2 and found to be suppressed the breast cancer proliferation dramatically (Figures 8D,8E). It was found that knockdown of STC2 suppressed the migration of breast cancer cells by transwell migration assays, and that knockdown of STC2 suppressed breast cancer cells proliferation by inducing cells into cell cycle arrest at G1 phase and augment of cell apoptosis (Figures 8G,8H). Our results confirmed that suppressing the expression of either STC2 or lncRNA MAFG-AS1 had same impact on breast cancer proliferation and metastasis that were drammatically reduced.

We further evaluated the connection between LncRNA lncRNA MAFG-AS1, and STC2 using qRT-PCR for detecting the expression levels of LncRNA lncRNA MAFG-AS1 and STC2 in breast cancer tissues and in precancerous lesions. We found a positive correlation between the expression levels (Figure 8I). For testing whether STC2 participated in promoting the breast cancer cells proliferation and metastasis mediated by LncRNA MAFG-AS1, rescue experiments were carried out. pcDNA-LncRNA MAFG-AS1 and si-STC2 were co-transfected into MDA-MB-231 and T-47D cells. The results of CCK-8 and colony formation assays demonstrated that knockdown of STC2 in MDA-MB-231 and T-47D cells could partially rescue the increased cell proliferating activities induced by over-expressing LncRNA MAFG-AS1 (Figures 9A,9C). Furthermore, the results of transwell migration assays displayed that knockdown of STC2 in MDA-MB-231 and T-47D cells could rescue the increased cell migrating viabilities promoted by over-expressing

LncRNA MAFG-AS1 partially (Figure 9B). Hence, all the results proved that LncRNA MAFG-AS1 promote breast cancer proliferation and metastasis may partially by up-regulating the expression of STC2.

3.3.3 Evaluation of cancer- promoting mechanism of lncRNA MAFG-AS1

To further understand the difference between cancer- promoting mechanism of LncRNA MAFG-AS1 in nucleus and cytoplasm, and in order to study the functional mechanism of lncRNA MAFG-AS1, nuclear extract was exploited to detect its location in cells, which revealed that LncRNA lncRNA MAFG-AS1 located in cytoplasm primarily in MDA-MB-231 and T-47D cells (Figures 10A,10B). LncRNA MAFG-AS1 located in cytoplasm participated in post-transcriptional regulation of genome. A recent study found that lncRNAs played a crucial role in tumor cells invasion and metastasis as a regulator in epithelial-mesenchymal transition (EMT). We detected the expression profile of EMT related molecular markers to explore: whether LncRNA MAFG-AS1 regulated the invasion and metastasis viabilities of breast cancer cells by influencing EMT. The result revealed that knockdown of LncRNA MAFG-AS1 induced the expression of E-cadherin and downregulated the expression of N-cadherin and Vimentin (Figures 10C,10D). To sum up, results from this study demonstrated that knockdown of LncRNA lncRNA MAFG-AS1 suppressed breast cancer migration and invasion by modulating EMT.

4. Discussion

Recently, number of studied have demonstrated that lncRNAs may play an important role in cancer development and progression, which caught increasing attention. LncRNAs expressed abnormally in breast cancer cells, and participates in important biological process including proliferation, apoptosis, invasion and metastasis[16–19]. In spite that the function and mechanism of LncRNAs in breast cancer development and progression still remain unclear. Therefore, more studies to understand the relation of lncRNAs in the etiology of breast cancer is required, and more comprehensive and systematic studies for their regulatory network are under intense investigation, with clinical significance for early diagnosis, treatment, and improved prognosis in breast cancer.

In this study, bioinformatic tool was utilized to reveal that expression level of LncRNA MAFG-AS1 in breast cancer tissues and found to be evidently elevated. Following qRT-PCR for detection in collected fifty-four samples also demonstrated that expression of lncRNA MAFG-AS1 in breast cancer tissues were high, and that higher expressions were connected to the higher malignant grade in clinic, which indicated the possibility that LncRNA MAFG-AS1 can be a potential molecular marker for diagnosis and prognosis. We studied the function of lncRNA MAFG-AS1 in breast cancer proliferation, metastasis and apoptosis by knockdown and over-expression of it. Results from in vivo, and in vitro studies manifested that low-expressing LncRNA MAFG-AS1 could suppress the breast cancer cells proliferation, invasion, metastasis and tumorigenic capacity and could induce its apoptosis. On the contrary, over-expressing LncRNA MAFG-AS1 promoted the breast cancer cells proliferation, invasion and metastasis. All the results exhibited that LncRNA MAFG-AS1 played a critical role in the process of breast cancer as a carcinogen.

Then gene chip was used to scree targeted genes regulated by LncRNA MAFG-AS1, which suggested that after knockdown of LncRNA MAFG-AS1 in T-47D cells expression of 2237 genes had changed (Fold Change > 2, P Value < 0.05), including upregulated expression in 1387 genes and downregulated expression in 850 genes. Finally, we screened for the STC2 targeted gene regulated by lncRNA MAFG-AS1 for the following study of mechanism by GO analysis and QRT-PCR.

STC2 is a sort of human glycoprotein hormone, which was found in CS (corpuscles of Stannius) of bony fish by Stannius, made of cysteine[20], histidine and other amino acid residues. STC2 is involved in the paracrine and autocrine regulation by expressing in tissues and participating in phosphate-regulating physiology, metabolism, regeneration, stress response and development[21, 22]. Moreover, it was discovered that expression of STC2 changed overtly in solid cancers such as colorectal cancer, nasopharyngeal carcinoma, endometrial carcinoma, gastric carcinoma, hepatocellular carcinoma, SCCHN, which indicated that STC2 took effect in cancer development and progression, including promoting cancer cells invasion and metastasis, suppressing the cells apoptosis and so on[23–28]. Our finding of confirmatory test also demonstrated that STC2 may take part in regulatory network of LncRNA MAFG-AS1. To summarize, it can be concluded that LncRNA MAFG-AS1 could promote breast cancer cells proliferation and metastasis by up-regulating targeted gene STC2.

To further explore the specific mechanism how LncRNA MAFG-AS1 impacted breast cancer cells invasion and metastasis, we detected molecular expressions related to cells invasion and metastasis. It was found that EMT had significant effect in process of cancer cells proliferation, invasion and metastasis, and the significant markers of EMT occurrence is the deficient expression of E-cadherin and elevated expression of N-cadherin and Vimentin[29, 30]. Hence, we further investigated the marker proteins relevant to cells invasion and metastasis in the breast cancer cells with knockdown of LncRNA MAFG-AS1, which showed that the suppression for breast cancer invasion and metastasis in the absence of LncRNA MAFG-AS1 may be associated with the EMT. Previous reports suggest that MMP plays a role in mediating cancer cells invasion and metastasis[31]. Our results from the qRT-PCR study revealed that knockdown of expression of LncRNA MAFG-AS1 in breast cancer cells could suppress the expression of MMP2 protein. Our hypothesis is supported by previous studies that confirmed that knockdown of STC2 in AKT-ERK signaling pathway that could potentially suppress the process of EMT[32], and that up-regulating STC2 suppressed autophagy by downregulating ratio of LC3 II / LC I and Beclin-1. Moreover, up-regulating STC2 could suppress cell apoptosis by activating AKT-ERK signaling pathway to suppress PARP, Bax and caspase 3 lysis proteins[33](Fig. 11). Results from our study indicated that knockdown of lncRNA MAFG-AS1 suppressed breast cancer cells migration, invasion and proliferation might by downregulating STC2 in AKT-ERK signaling pathway in breast cancer. However, the specific molecular mechanisms how LncRNA MAFG-AS1 regulates autophagy, EMT and cell apoptosis still need further investigation.

5. Conclusions

The LncRNA MAFG-AS1 was found to be upregulated in breast cancer tissues and cells, and the up-regulation of LncRNA MAFG-AS1 is related to some clinical parameters, which predicted the poor prognosis. Our study demonstrated that LncRNA MAFG-AS1 might promote breast cancer cells proliferation and metastasis by regulating the expression of STC2. To summarize, lncRNA MAFG-AS1 could be a diagnostic markers and therapeutic target in the breast cancer, and it modulates the prognosis of breast cancer patients. However, the specific molecular mechanisms of LncRNA MAFG-AS1 in breast cancer development and progression still requires additional evidence.

Abbreviations

Abbreviations	Full name
lncRNAs	Long noncoding RNAs
GEO	Gene Expression Omnibus
TCGA	The Cancer Genome Atlas
siRNA	Small interference RNA
BC	Breast cancer
miRNAs	MicroRNAs
PBS	Phosphate buffered saline
QRT-PCR	Quantitative Real time-Polymerase Chain Reaction
EDTA	Ethylene Diamine Tetraacetic Acid
STC2	Stanniocalcin 2
EMT	Epithelial-to-mesenchymal transition

Declarations

Acknowledgments

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Authors' contributions

Shihao Di, Die Lu, Chunni Chen and Zhihong Zhang performed the experiments. Tianshi Ma and Zigui Zou provided expertise in method development. Shihao Di and Zhihong Zhang conceived the experiments and wrote the manuscript. All authors edited the manuscript. All authors approved the final manuscript.

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Availability of data and materials

Not applicable

Ethics approval and consent to participate

This study was performed strictly in accordance with the recommendatons in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocal was approved by the Animal Ethical and Welfare Committee of Nanjing Medical University.

Consent for publication

Not applicable

Competing Interests

All authors have declared no conflict of interests.

References

[1] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin,2018,68(6):394-424.

[2] Chen W, Sun K, Zheng R, et al. Cancer incidence and mortality in China, 2014. Chin J Cancer Res,2018,30(1):1-12.

[3] Wang Z, Chen M, Pan J, et al. Pattern of distant metastases in inflammatory breast cancer - A large-cohort retrospective study. J Cancer,2020,11(2):292-300.

[4] Santosh B, Varshney A, Yadava P K. Non-coding RNAs: biological functions and applications. Cell Biochem Funct,2015,33(1):14-22.

[5] Ren X. Genome-wide analysis reveals the emerging roles of long non-coding RNAs in cancer. Oncol Lett,2020,19(1):588-594.

[6] Huang Y, Guo Q, Ding X P, et al. Mechanism of long noncoding RNAs as transcriptional regulators in cancer. RNA Biol,2019.

[7] Zhang K, Liu P, Tang H, et al. AFAP1-AS1 promotes epithelial-mesenchymal transition and tumorigenesis through Wnt/β-catenin signaling pathway in triple-negative breast cancer. Frontiers in Pharmacology,2018,9(NOV).

[8] Yang F, Shen Y, Zhang W, et al. An androgen receptor negatively induced long non-coding RNA ARNILA binding to miR-204 promotes the invasion and metastasis of triple-negative breast cancer. Cell death and differentiation,2018.

[9] Wang P, Chou C, Lin C, et al. A novel long non-coding RNA linc-ZNF469-3 promotes lung metastasis through miR-574-5p-ZEB1 axis in triple negative breast cancer. ONCOGENE,2018,37(34):4662-4678.

[10] Sha S, Yuan D, Liu Y, et al. Targeting long non-coding RNA DANCR inhibits triple negative breast cancer progression. BIOLOGY OPEN,2017,6(9):1310-1316.

[11] Shima H, Kida K, Adachi S, et al. Lnc RNA H19 is associated with poor prognosis in breast cancer patients and promotes cancer stemness. Breast Cancer Research and Treatment,2018,170(3):507-516.

[12] Li S, Zhou J, Wang Z, et al. Long noncoding RNA GAS5 suppresses triple negative breast cancer progression through inhibition of proliferation and invasion by competitively binding miR-196a-5p. BIOMEDICINE & PHARMACOTHERAPY,2018,104:451-457.

[13] Zuo Y, Li Y, Zhou Z, et al. Long non-coding RNA MALAT1 promotes proliferation and invasion via targeting miR-129-5p in triple-negative breast cancer. BIOMEDICINE & PHARMACOTHERAPY,2017,95:922-928.

[14] Li L, Yang Z, Wang Y, et al. Long non-coding RNA MALAT1 promote triple-negative breast cancer progression by regulating miR-204 expression. INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL PATHOLOGY,2016,9(2):969.

[15] Sun Z, Zhang T, Chen B. Long Non-Coding RNA Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1) Promotes Proliferation and Metastasis of Osteosarcoma Cells by Targeting c-Met and SOX4 via miR-34a/c-5p and miR-449a/b. Med Sci Monit,2019,25:1410-1422.

[16] Liu Y, Wei G, Ma Q, et al. Knockdown of long noncoding RNA TP73-AS1 suppresses the malignant progression of breast cancer cells in vitro through targeting miRNA-125a-3p/metadherin axis. Thorac Cancer,2020.

[17] Niu Y, Bao L, Chen Y, et al. HIF-2-induced long non-coding RNA RAB11B-AS1 promotes hypoxia-mediated angiogenesis and breast cancer metastasis. Cancer Res,2020.

[18] Cui Y, Lu C, Zhang Z, et al. A Long Non-coding RNA Lnc712 Regulates Breast Cancer Cell Proliferation. Int J Biol Sci,2020,16(1):162-171.

[19] Naorem L D, Prakash V S, Muthaiyan M, et al. Comprehensive analysis of dysregulated lncRNAs and their competing endogenous RNA network in triple-negative breast cancer. Int J Biol Macromol,2019.

[20] Zhao J, Jiao Y, Song Y, et al. Stanniocalcin 2 Ameliorates Hepatosteatosis Through Activation of STAT3 Signaling. Front Physiol,2018,9:873.

[21] Joshi A D, Hossain E, Elferink C J. Epigenetic Regulation by Agonist-Specific Aryl Hydrocarbon Receptor Recruitment of Metastasis-Associated Protein 2 Selectively Induces Stanniocalcin 2 Expression. Mol Pharmacol,2017,92(3):366-374.

[22] Roche F P, Pietila I, Kaito H, et al. Leukocyte Differentiation by Histidine-Rich Glycoprotein/Stanniocalcin-2 Complex Regulates Murine Glioma Growth through Modulation of Antitumor Immunity. Mol Cancer Ther,2018,17(9):1961-1972.

[23] Li J B, Liu Z X, Zhang R, et al. Sp1 contributes to overexpression of stanniocalcin 2 through regulation of promoter activity in colon adenocarcinoma. World J Gastroenterol,2019,25(22):2776-2787.

[24] He H, Qie S, Guo Q, et al. Stanniocalcin 2 (STC2) expression promotes post-radiation survival, migration and invasion of nasopharyngeal carcinoma cells. Cancer Manag Res,2019,11:6411-6424.

[25] Aydin H A, Toptas T, Bozkurt S, et al. Stanniocalcin-2 May Be a Potentially Valuable Prognostic Marker in Endometrial Cancer: a Preliminary Study. Pathol Oncol Res,2019,25(2):751-757.

[26] Ke J, Zhang B H, Li Y Y, et al. MiR-1-3p suppresses cell proliferation and invasion and targets STC2 in gastric cancer. Eur Rev Med Pharmacol Sci,2019,23(20):8870-8877.

[27] Wang Y, Wu J, Xu J, et al. Clinical significance of high expression of stanniocalcin-2 in hepatocellular carcinoma. Biosci Rep,2019,39(4).

[28] Li T, Qin Y, Zhen Z, et al. Long non-coding RNA HOTAIR/microRNA-206 sponge regulates STC2 and further influences cell biological functions in head and neck squamous cell carcinoma. Cell Prolif,2019,52(5):e12651.

[29] Zakaria N, Yahaya B H. Adipose-Derived Mesenchymal Stem Cells Promote Growth and Migration of Lung Adenocarcinoma Cancer Cells. Adv Exp Med Biol,2020.

[30] Tripathi S, Levine H, Jolly M K. The Physics of Cellular Decision Making During Epithelial-Mesenchymal Transition. Annu Rev Biophys,2020.

[31] Xue K, Li J, Nan S, et al. Downregulation of LINC00460 decreases STC2 and promotes autophagy of head and neck squamous cell carcinoma by up-regulating microRNA-206. Life Sci,2019,231:116459.

[32] Sun G, Liu M, Han H. Overexpression of microRNA-190 inhibits migration, invasion, epithelial-mesenchymal transition, and angiogenesis through suppression of protein kinase B-extracellular signal-regulated kinase signaling pathway via binding to stanniocalicin 2 in breast cancer. J Cell Physiol,2019,234(10):17824-17838.

[33] Xue K, Li J, Nan S, et al. Downregulation of LINC00460 decreases STC2 and promotes autophagy of head and neck squamous cell carcinoma by up-regulating microRNA-206. Life Sci,2019,231:116459.

Figures

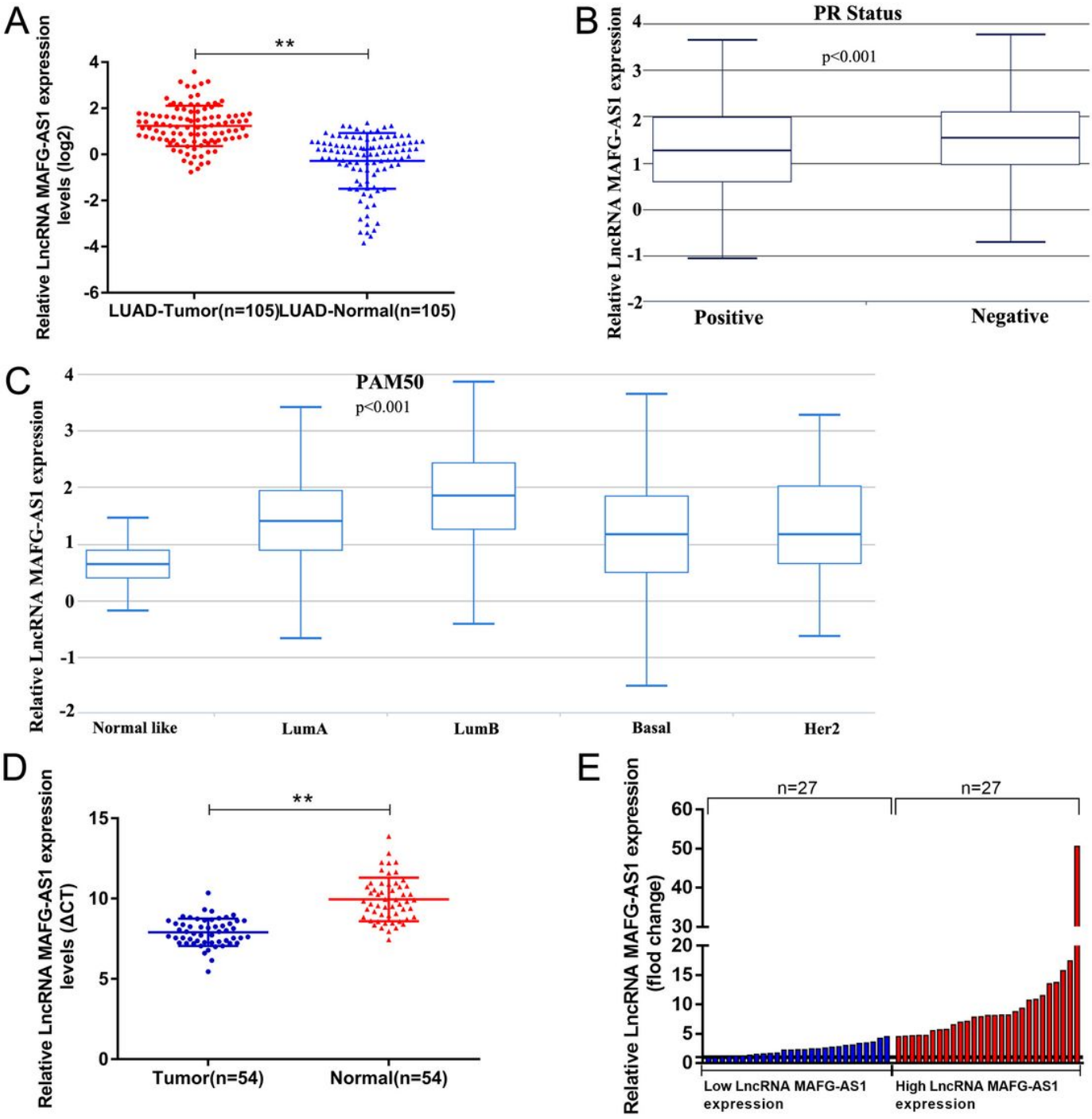

Figure 1

Relative expression of LncRNA MAFG-AS1 in BC tissues and its clinical significance. (A)Data collected from TCGA database showed relative expression of LncRNA MAFG-AS1 in BC tissues (n=105) and their corresponding nontumorous tissues (n=105). (B and C)Data collected from TCGA database showed relative expression of LncRNA MAFG-AS1 with PR Station and PAM50.(E) Relative LncRNA MAFG-AS1 expression in BC tissues (n=54) compared with their adjacent non-cancerous tissues analyzed by qRT-PCR and tissues were divided into two groups according to the fold-change of LncRNA MAFG-AS1 expression.*P<0.05,**P <0.01.

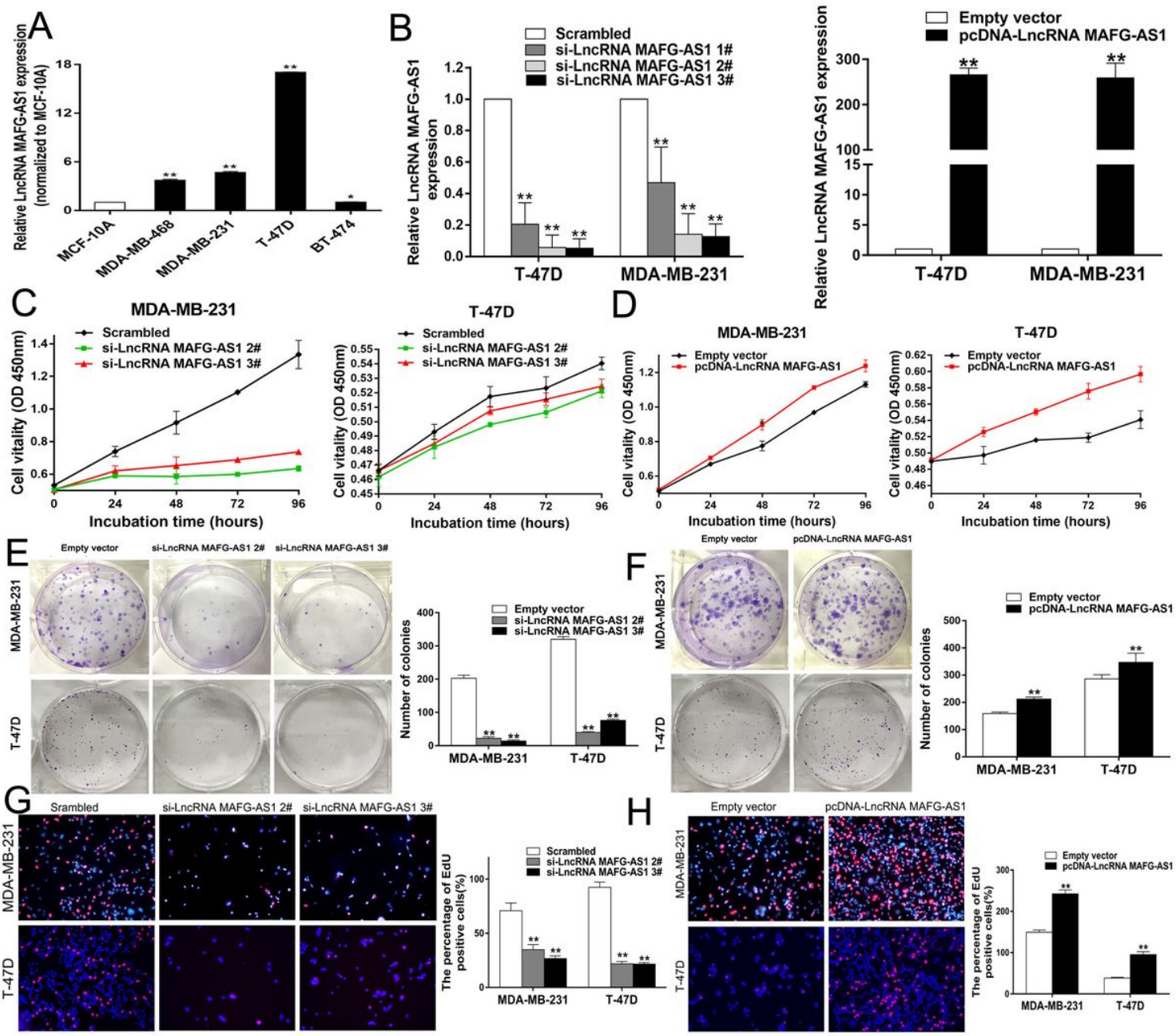

Figure 2

LncRNA MAFG-AS1 promotes BC cell proliferation in vitro. (A) QRT-PCR assay examined LncRNA MAFG-AS1 expression in normal breast epithelial cell line (MCF-10A) and BC cell lines. (B) Left: QRT-PCR analysis of LncRNA MAFG-AS1 expression in BC cells transfected with control (scrambled), si-LncRNA MAFG-AS11#, si-LncRNA MAFG-AS1 2# and si-LncRNA MAFG-AS1 3#. Right: Relative expression of LncRNA MAFG-AS1 in BC cells transfected with empty vector and pcDNA-LncRNA MAFG-AS1. (C and D) CCK8 assays were performed to determine the viability of BC cells treated with si-LncRNA MAFG-AS1 or pcDNA-LncRNA MAFG-AS1. (E and F) Colony formation assays were used to detect the proliferation of si-LncRNA MAFG-AS1-transfected or pcDNA-LncRNA MAFG-AS1-transfected BC cells. Colonies were counted and captured. (G and H) Proliferous BC cells were displayed by EdU immunostaining assays.

EdU positive cells were counted and captured. Values are shown as the mean±s.d in three independent experiments. *P < 0.05, **P< 0.01.

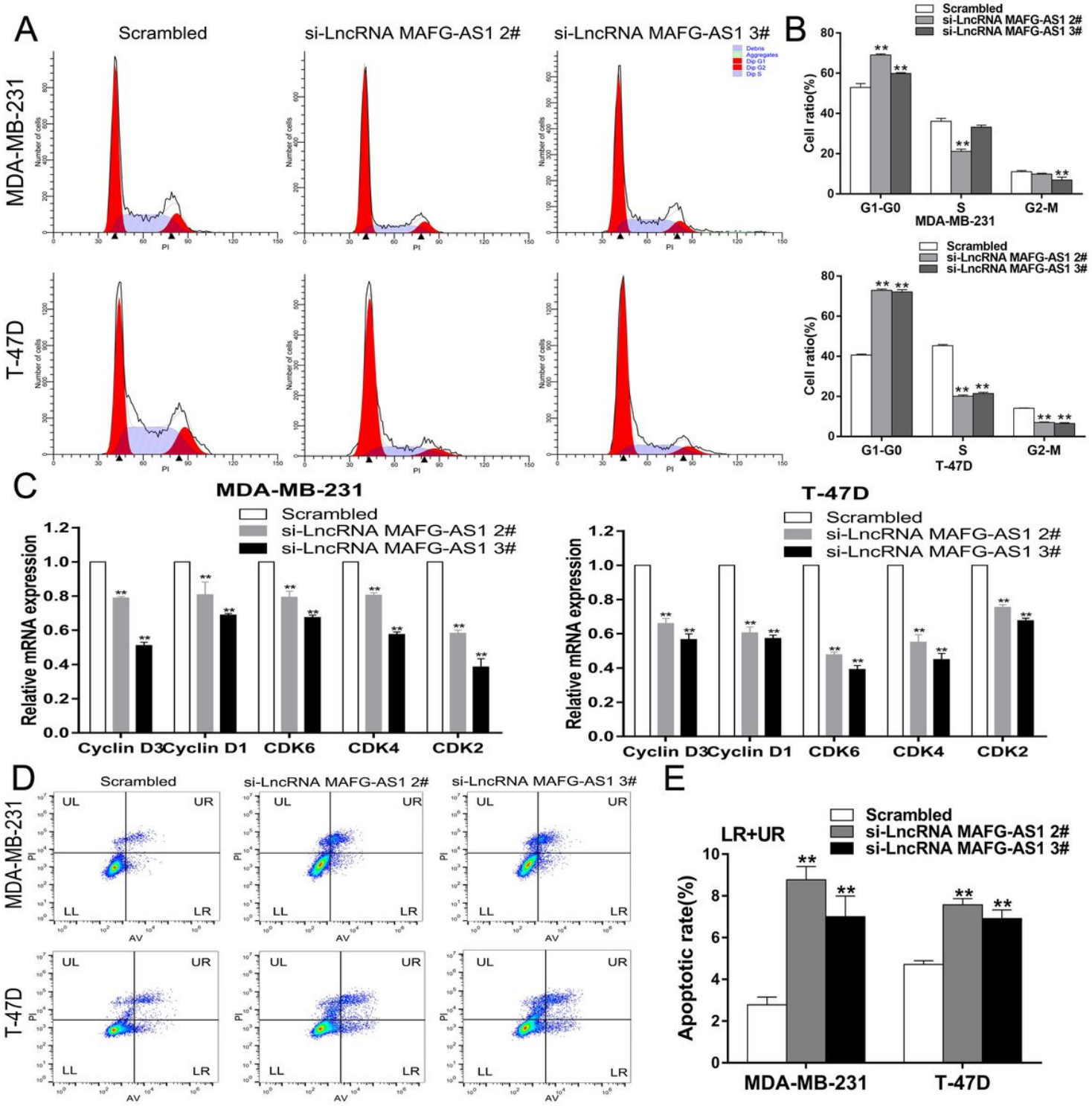

Figure 3

Inhibition of LncRNA MAFG-AS1 induces G1 arrest and apoptosis in BC cells. MDA-MB-231 and T-47D cells were transfected with si-LncRNA MAFG-AS12#, 3# or scrambled. (A and B) Cell cycle wasdetermined in BC cells. (C) QRT-PCR analysis of CyclinD3, CyclinD1, CDK6, CDK4, CDK2 in BC cells. (D and

E)Apoptotic rates of cells were tested by flow cytometry.Values are shown as the mean±s.d in three independent experiments. *P < 0.05,**P <0.01.

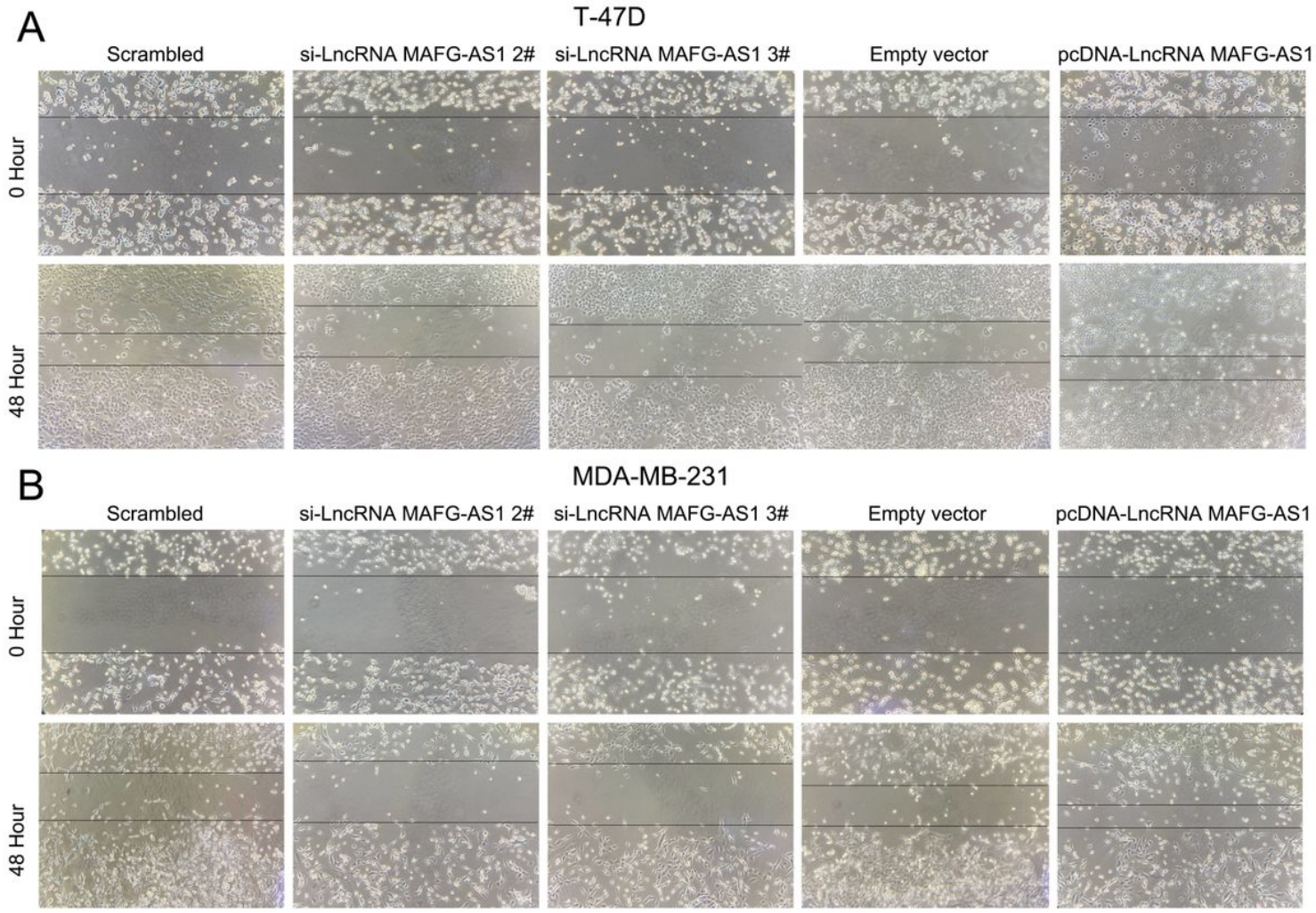

Figure 4

Effects of LncRNA MAFG-AS1 on BC migration in vitro.MDA-MB-231 and T-47D cells were transfected with control (scrambled), si-LncRNA MAFG-AS11#, si-LncRNA MAFG-AS1 2#, si-LncRNA MAFG-AS1 3#, empty vector and pcDNA-LncRNA MAFG-AS1. (A, B) Wound-healing assays were used to investigate the migratory ability of BC cells. *P < 0.05 and **P < 0.01.

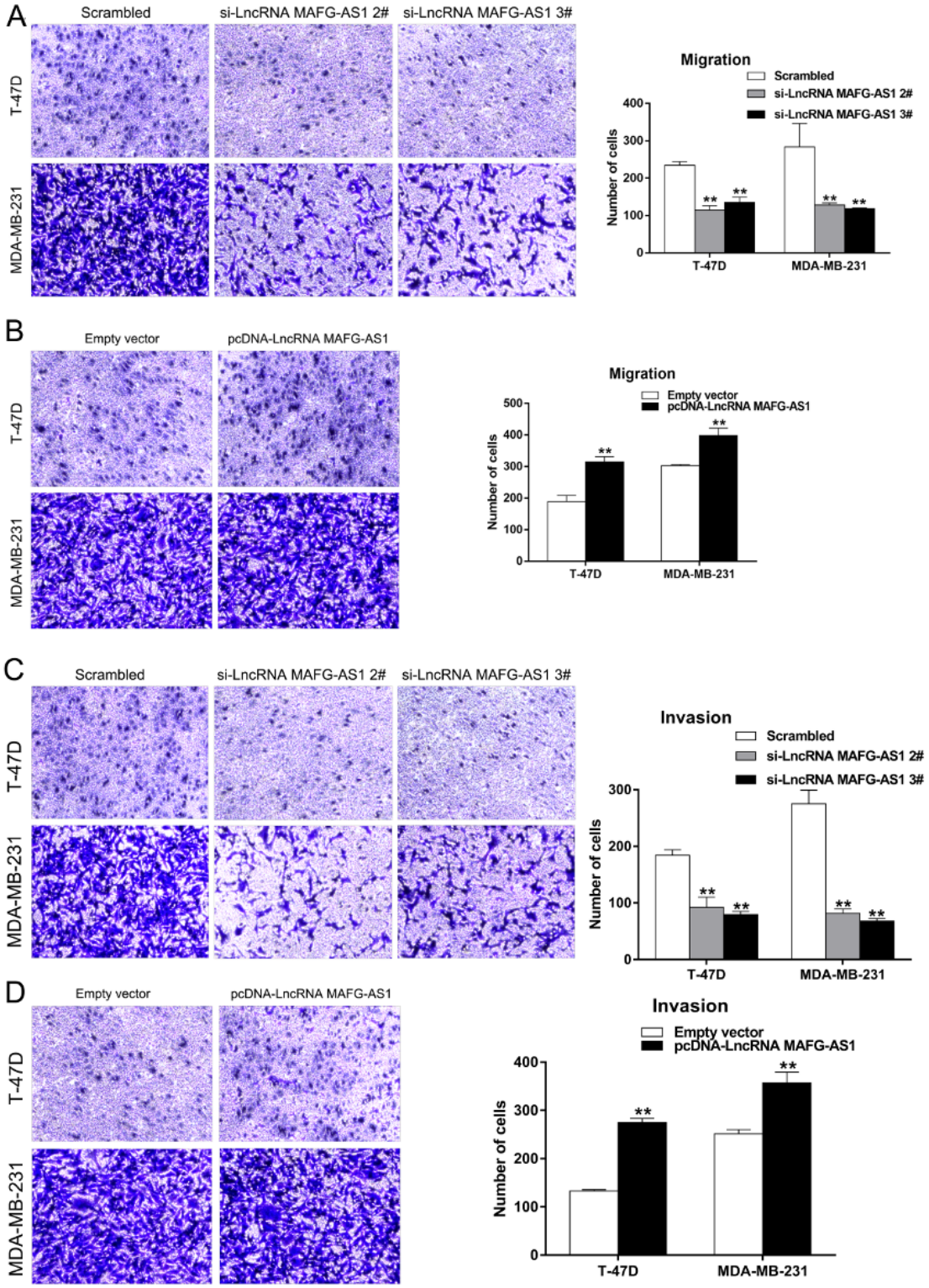

Figure 5

Effects of LncRNA MAFG-AS1 on BC migration and invasion in vitro. MDA-MB-231 and T-47D cells were transfected with control (scrambled), si-LncRNA MAFG-AS11#, si-LncRNA MAFG-AS1 2#, si-LncRNA MAFG-AS1 3#, empty vector and pcDNA-LncRNA MAFG-AS1. (A, B, C and D) Transwell assays were used to investigate the changes in migratory and invasive abilities of BC cells. *P < 0.05 and **P < 0.01.

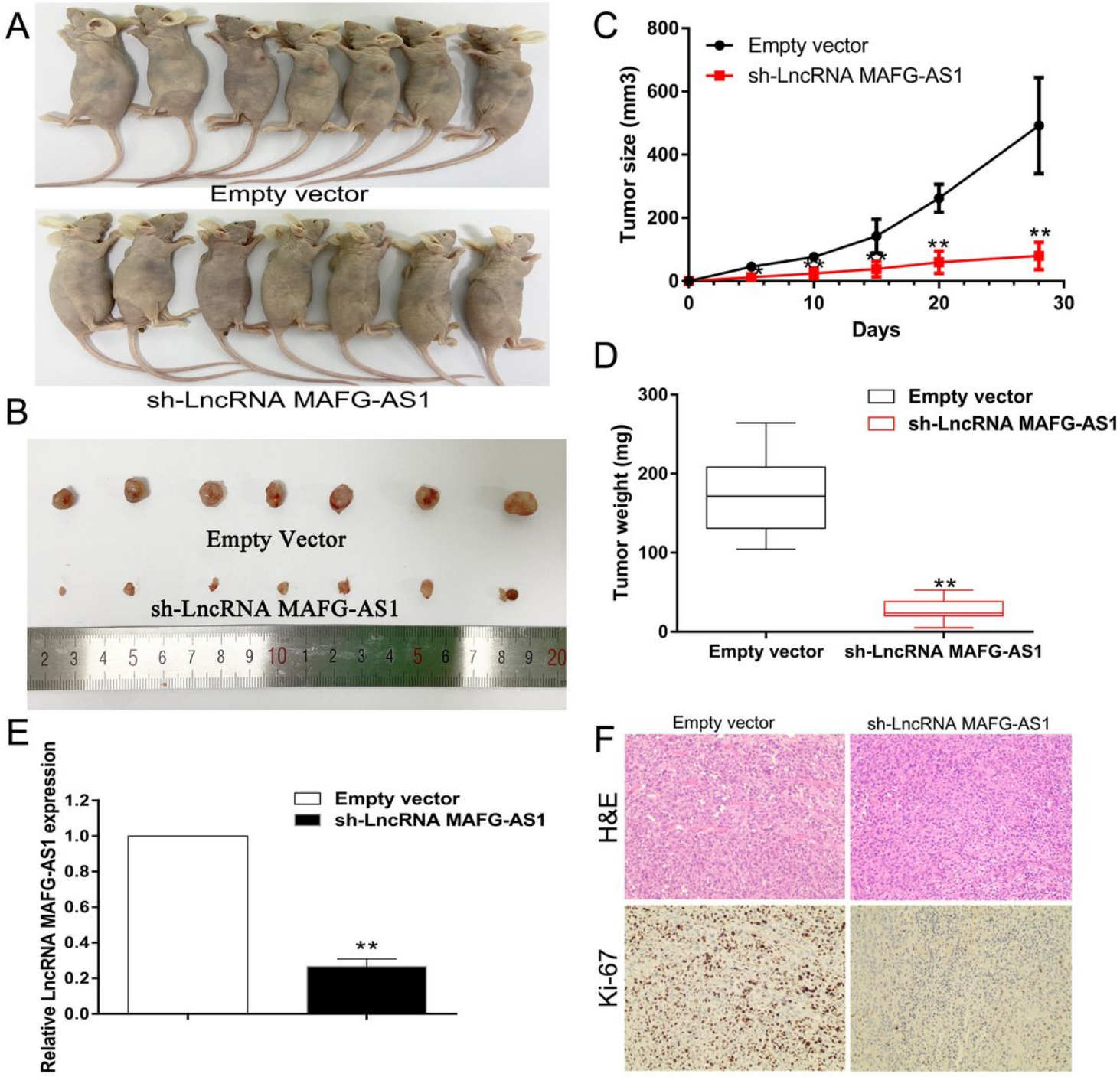

Figure 6

LncRNA MAFG-AS1 knockdown inhibits tumorigenesis of BC cells in vivo. (A and B) Emptyvector or sh-LncRNA MAFG-AS1 was transfected into T-47D cells, which were injectedin the BALB/c-nude mice (n=7), respectively.Tumors before and after carrying from the nude mice. (C)Tumor volumes were calculated after injection every five days. Points, mean (n = 7); bars indicate SD.(D) Tumor weightswere represented as means of tumor weights ± SD.(E) QRT-PCR was used to detect the average expression of LncRNA MAFG-AS1 in xenografttumors (n=7). (F) The tumor sections were under H&E staining and IHC staining using antibodies against ki-67. *P < 0.05, **P< 0.01.

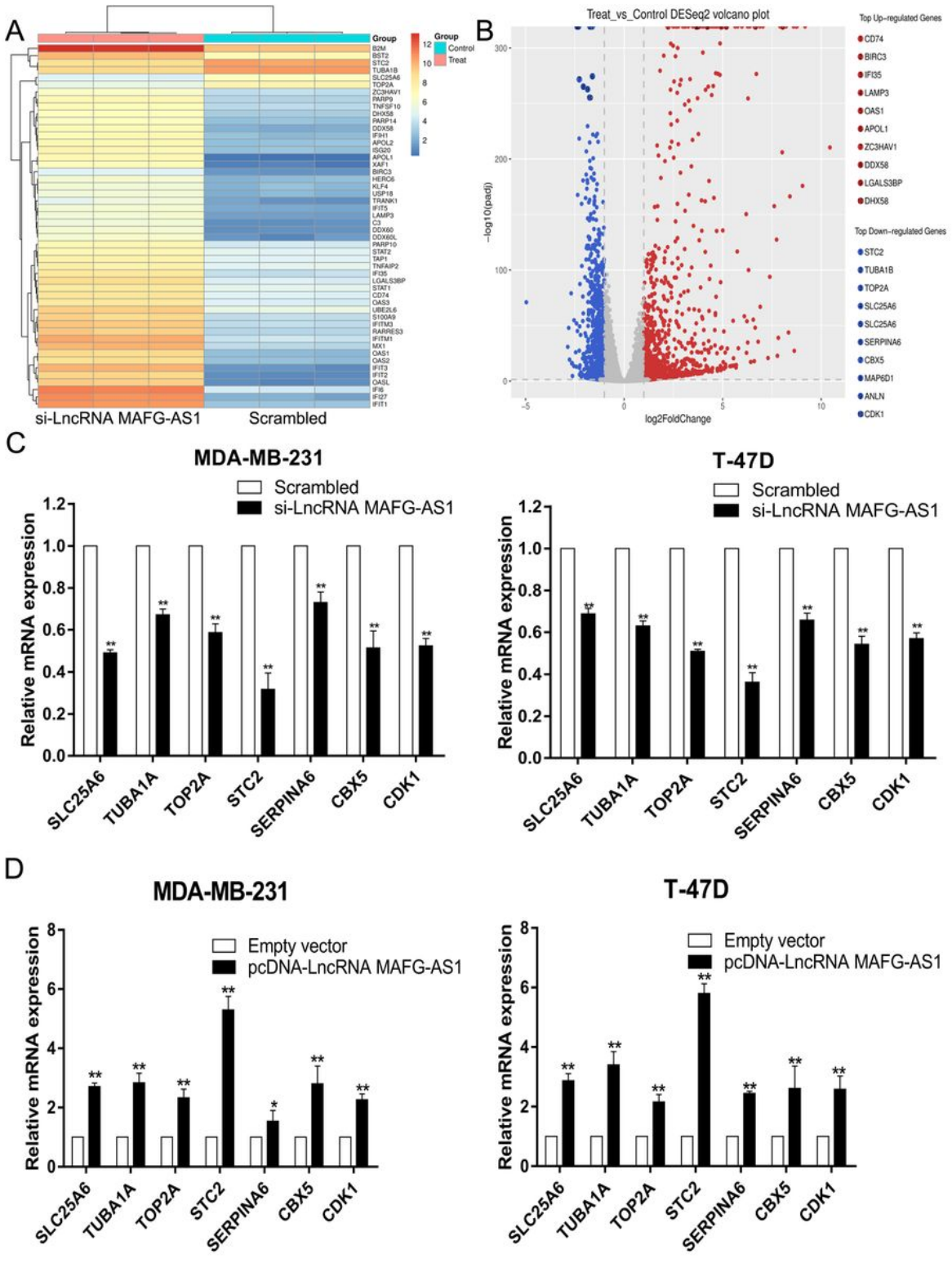

Figure 7

STC2 is a downstream target gene regulated by LncRNA MAFG-AS1. (A) Meancentered, hierarchical clustering of transcripts altered in scrambled siRNA-treated cells and si-LncRNA MAFG-AS1-treated cells, with three repeats. (B) Gene Ontology (GO) analysis for genes with altered expressions between the scrambled siRNA-treated and si-LncRNA MAFG-AS1-treated cells in vitro. (C and D) QRT-PCR analysis in si-LncRNA MAFG-AS1-treated or pcDNA-LncRNA MAFG-AS1-treated BC cells showed altered mRNA level

of genes involved in cell apoptosis and migration upon LncRNA MAFG-AS1 depletion. Values are shown as the mean±s.d in three independent experiments. *P < 0.05, **P < 0.01.

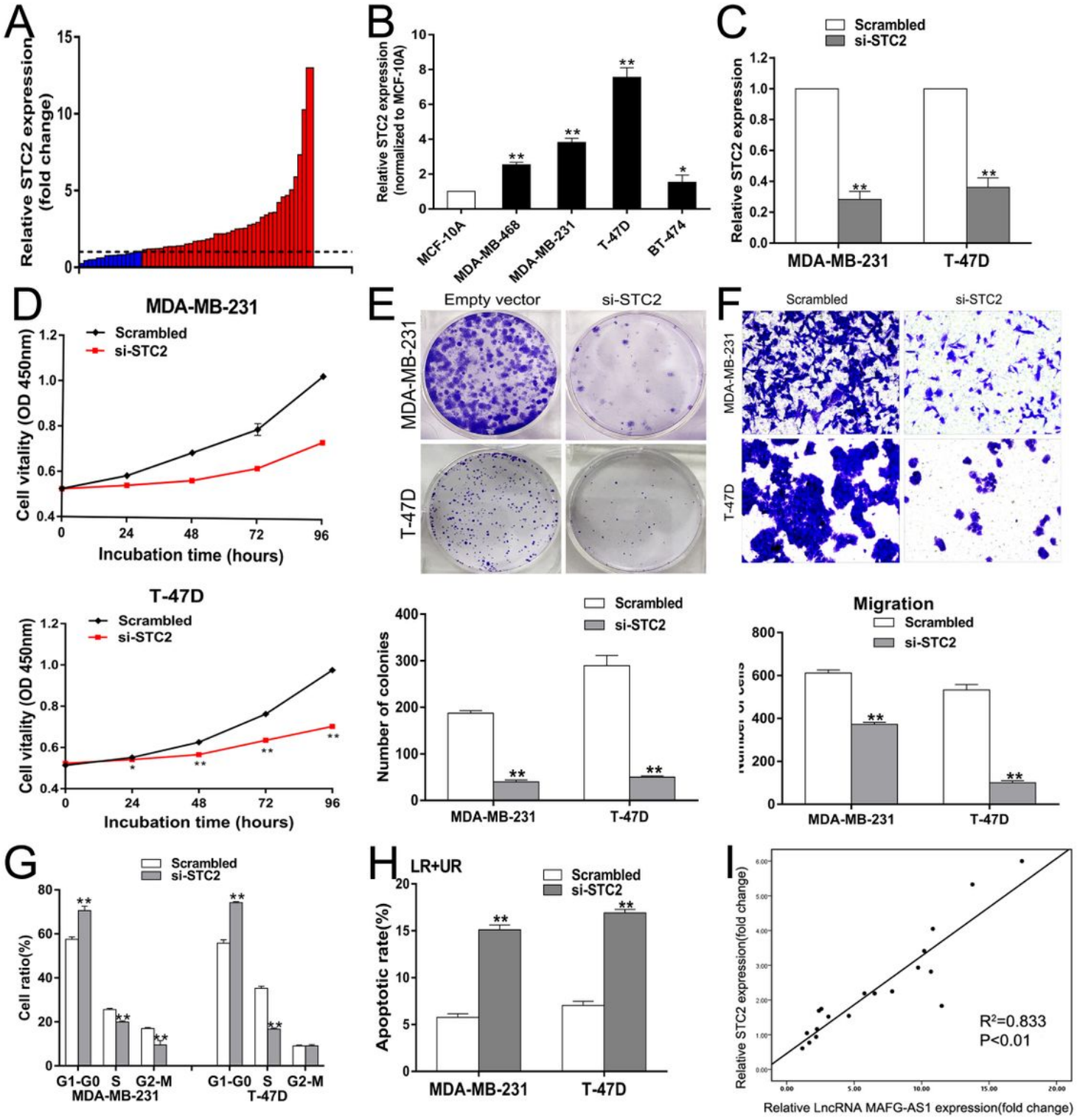

Figure 8

Downregulation of STC2 inhibits BC cells proliferation and is involved in the oncogene function of LncRNA MAFG-AS1. (A) Relative expression of STC2 in BC tissues (n=54) and the paired noncanceroustissues(n=54)analyzedbyqRT-PCRandnormalizedtoGAPDH.(B)QRT-PCRassay examined

STC2 expression in normal breast epithelial cell line (MCF-10A) and BC celllines. (C) QRT-PCR analysis of STC2 expression in BC cells transfected with control (scrambled) and si-STC2. (D) CCK8 assays were performed to determine viability of BC cells treated with the control and si-STC2. (E) Colony formation assays were used to detect the proliferation of si-STC2-transfected BC cells. Colonies were counted and captured. (F) Transwell assays were conducted to evaluate migration of BC cells.(G and H) Cell cycle and apoptosis of BC cells was investigated by flow-cytometry. (I) Analysis of the relationship between STC2 and LncRNA MAFG-AS1 expression. *P < 0.05, **P < 0.01.

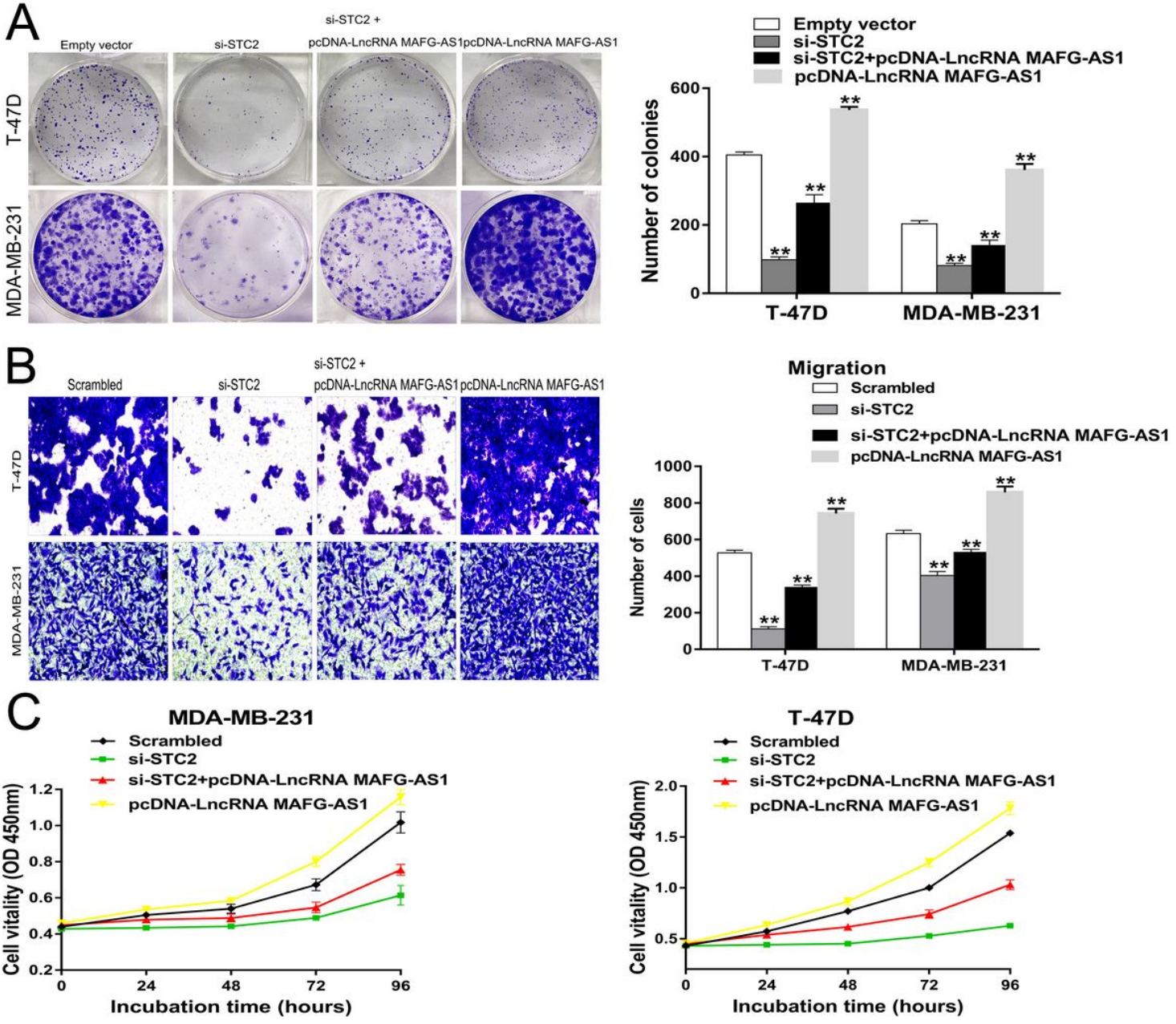

Figure 9

Downregulation of STC2 inhibits BC cells proliferation and is involved in the oncogene function of LncRNA MAFG-AS1. (A) Colony formation assays were used to determine the proliferation for si-STC2 and pcDNA-LncRNA MAFG-AS1 co-transfected BC cells.(B) Transwell assays were used to evaluate the

migration for si-STC2 and pcDNA-LncRNA MAFG-AS1 co-transfected BC cells. (K) CCK8 assays were used to determine the cell viability for si-STC2 and pcDNA-LncRNA MAFG-AS1 co-transfected BC cells. Values are shown as the mean±s.d in three independent experiments. *P < 0.05, **P < 0.01.

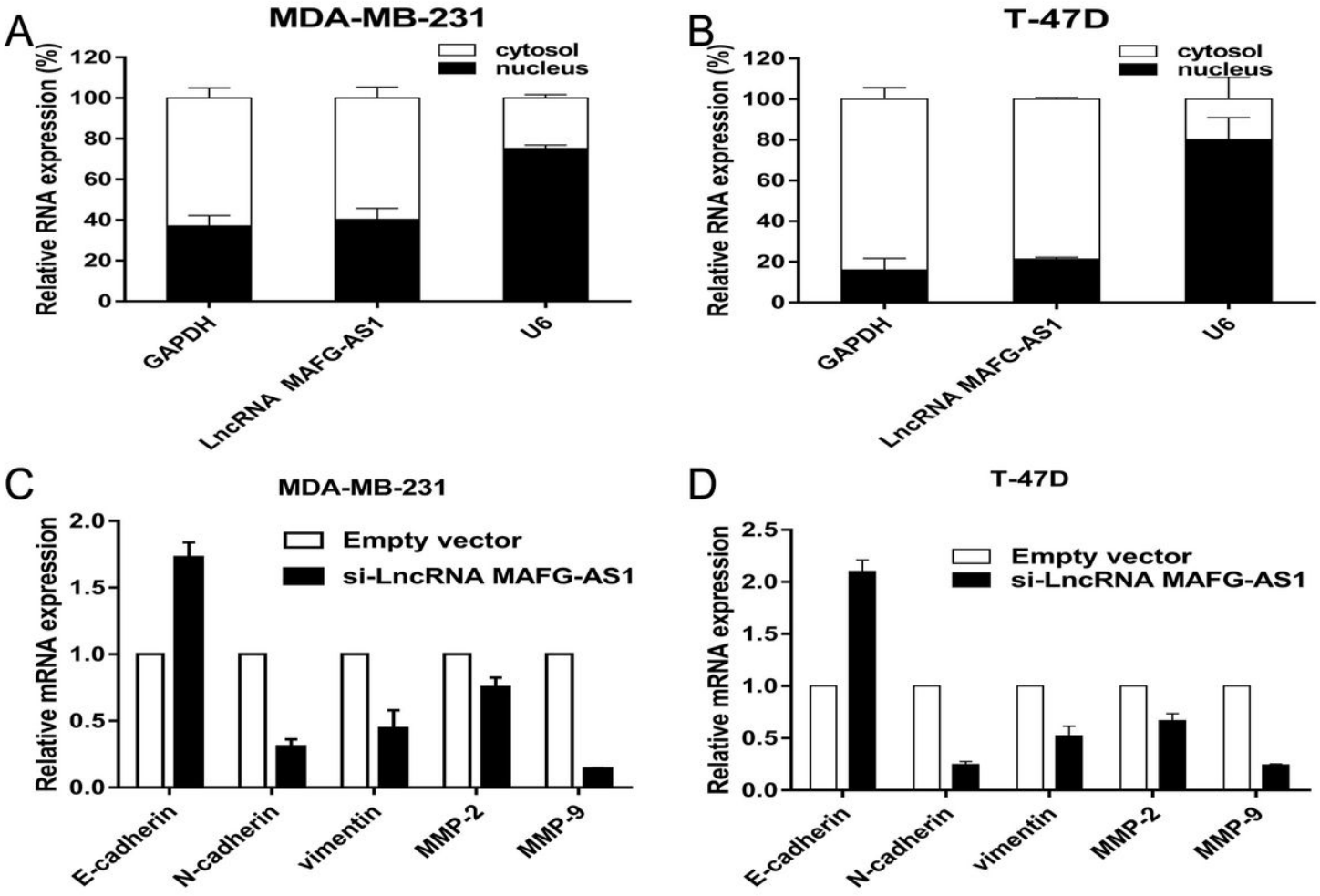

Figure 10

Prediction of potential mechanism of oncogenic role of LncRNA MAFG-AS1(A and B) LncRNA MAFG-AS1 expression levels in cell nucleus or cytoplasm of MDA-MB-231 and T-47D cells were detected by qRT-PCR. GAPDH was used as cytoplasm control and U6 was used as nucleus control. (C) Analysis of E-cadherin, N-cadherin, Vimentin, MMP-2, MMP-9 expression in MDA-MB-231 and T-47D cells treated with si-LncRNA MAFG-AS1.

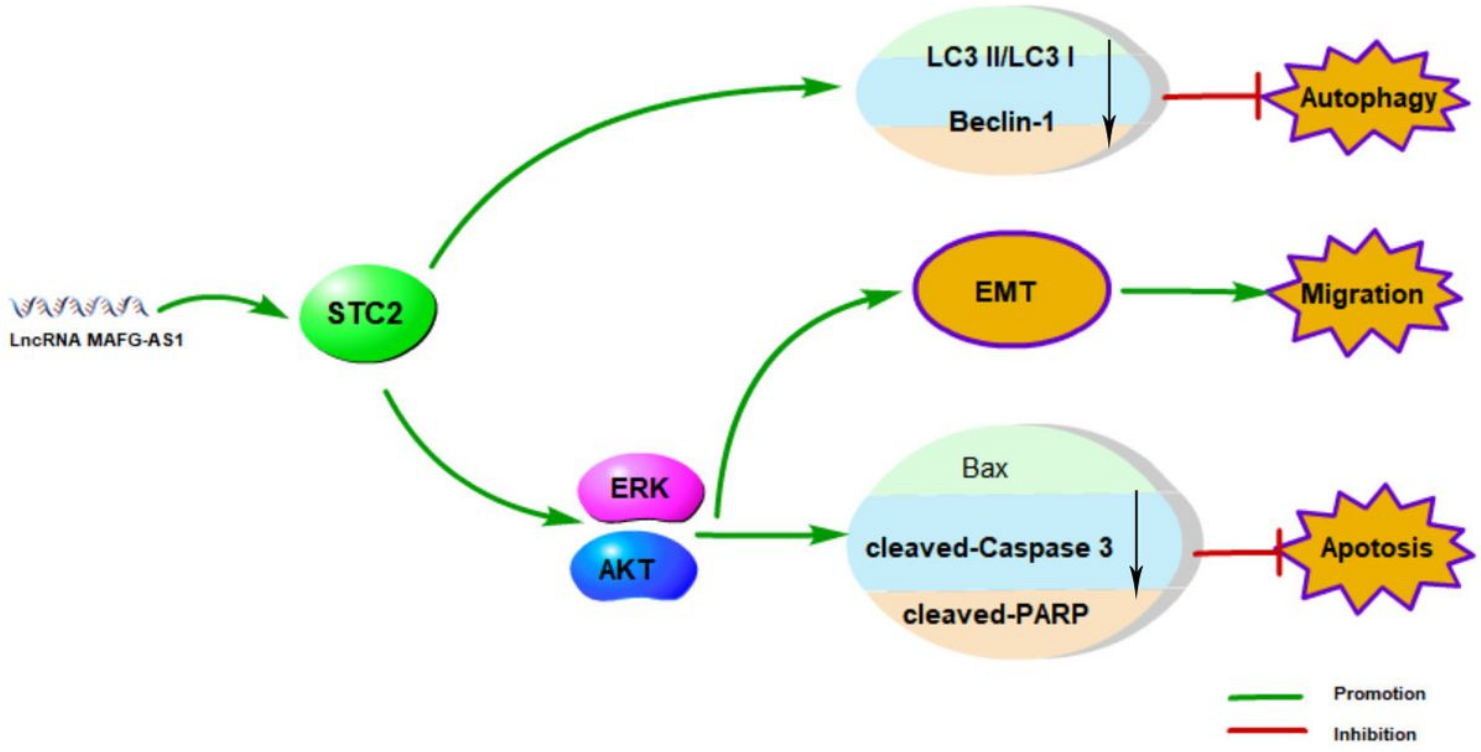

Figure 11

Schematic showing the mechanism of action of LncRNA MAFG-AS1. The upregulation of LncRNA MAFG-AS1 promoted the migration and inhibited the apoptosis and autophagy of BC cells. Upregulated STC2 inhibited autophagy by downregulating LC3 II/LC3 I ratio and Beclin-1 (autophagy related genes). Upregulated STC2 inhibited the expression of cleaved-PARP, Bax and cleaved-caspase 3 proteins by activating ERK and AKT signaling pathways, and ultimately inhibits cell apoptosis. Upregulated STC2 promoted the migration by activating ERK and AKT signaling pathways.BC, breast cancer ; STC2, Stanniocalcin-2; Bax, Bcl-2-associated X protein; AKT,Protein kinase B; ERK: extracellular signal-regulated kinase; EMT, Epithelial-Mesenchymal Transition.

Supplementary Files

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