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ORIGINAL RESEARCH

Long Intergenic Non-Coding RNA 01121 Promotes Breast Cancer Cell Proliferation, Migration, and Invasion via the *miR-150-5p*/HMGA2 Axis

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Purpose: Long intergenic noncoding RNA 01121 (*LINC01121*) has been reported to be aberrantly expressed and acts as an oncogene in pancreatic cancer. However, the detailed molecular mechanism of *LINC01121* in breast cancer remains largely unclear. In this study, we aimed to investigate the expression and biological function of *LINC01121* in breast cancer.

Methods: *LINC01121* and *miR-150-5p* expression were measured in breast cancer cell lines using quantitative reverse transcription PCR. MTS and flow cytometry assays were performed to determine cell proliferation, the cell cycle, and apoptosis. Cell migration and invasion were assessed by transwell assay. The protein expression of HMGA2 in breast cancer cell lines was measured by Western blotting. A luciferase reporter assay was used to assess the binding of *LINC01121* and *miR-150-5p*.

Results: We found that *LINC01121* was markedly up-regulated in breast cancer cell lines compared with normal breast epithelial cells. *LINC01121* down-regulation markedly suppressed cell proliferation, cell cycle progression, migration, and invasion and promoted apoptosis in breast cancer cells. Further investigation showed that *LINC01121* could serve as a molecular sponge for *miR-150-5p* and indirectly modulate the expression of its target, HMGA2. Moreover, *miR-150-5p* knockdown rescued the effects of *LINC01121* down-regulation on HMGA2 protein expression, cell proliferation, cell cycle progression, apoptosis, migration, and invasion in breast cancer cells.

Conclusion: Knockdown *LINC01121* inhibited breast cancer cell proliferation, migration, and invasion via the *miR-150-5p*/HMGA2 axis.

Keywords: breast cancer, *LINC01121*, growth, migration, invasion, *miR-150-5p*

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Introduction

Breast cancer is the most commonly diagnosed invasive malignancy in females worldwide.[1] In recent years, the incidence and mortality of breast cancer has been increasing.[2] Epidemiological studies indicate that approximately 1.6 million cases of breast cancer are diagnosed and 1.2 million deaths result from breast cancer each year in China.[3] During the past 10 years, many efforts have been made in breast cancer diagnosis and therapy, including combinations of surgery, hormone therapy, and adjuvant therapy.[4] However, the overall prognosis of breast cancer is still unsatisfactory due to recurrence and distant metastasis.[5] The proliferation and metastasis of breast cancer is a multi-step biological process that involves the activation or silencing of genes and epigenetic modifications; however, the details of its molecular mechanism are still largely

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unknown.[6] Therefore, further elucidation of the molecular mechanisms of breast cancer metastasis is urgently needed to provide the theoretical basis for improved breast cancer therapy.

LncRNAs play key regulatory roles in various biological process, such as proliferation, invasion, and metastasis. They have been shown to be aberrantly expressed in many types of cancers, including breast cancer.[7] *HOTAIR* has been shown to have increased levels of expression in breast cancer tissues and cells and may act as oncogene.[8] Low levels of *FENDRR* expression are associated with decreased survival time of breast cancer patients and increased breast cancer cell proliferation and migration.[9] However, the detailed mechanism of lncRNA-mediated tumorigenesis in breast cancer remains largely unclear. Long intergenic non-protein coding RNA 01121 (*LINC01121*) is a 1114 bp non-coding RNA located on human chromosome 2p21. *LINC01121* has been reported to be aberrantly expressed and act as an oncogene in pancreatic cancer.[10] Huang et al[11] reported that pterostilbene could suppress proliferation and epithelial-to-mesenchymal transition and promote apoptosis in breast cancer cells. Further investigation showed that pterostilbene can decrease the expression of *LINC01121*, suggesting that *LINC01121* may act as an oncogene in breast cancer. Therefore, we aimed to further study the function of *LINC01121* in breast cancer.

LncRNAs act as competing endogenous RNAs (ceRNAs) to sponge microRNAs (miRNAs).[12] miRNAs can affect various cellular processes by inhibiting the expression of their target genes.[13,14] *miR-150-5p*, a hematopoiesis-related miRNA, has been frequently reported to be aberrantly expressed in various types of human cancers.[15,16] Tang et al found that *miR-150-5p* has decreased levels of expression in breast cancer tissues and that *miR-150* suppresses metastasis of breast cancer cells by inhibiting high-mobility group protein 2 (HMGA2) expression.[17] However, whether *LINC01121* acts as an *miR-150-5p* sponge to regulate the growth and metastasis of breast cancer cells remains unclear.

In the present study, we first evaluated the expression of *LINC01121* in breast cancer cell lines. Subsequently, we assessed the biological function of *LINC01121* in breast cancer cells. Finally, the interaction between *LINC01121* and *miR-150-5p* and the underlying mechanisms of *LINC01121* in breast cancer cells were investigated.

Materials and Methods

Cell Culture and Transfection

The human breast cancer cell lines, MCF-7, BT-549, MDA-MB-231, and MDA-MB-453 and the normal human breast cell line, MCF-10A, were purchased from the Shanghai Cell Bank of the Chinese Academy of Science (Shanghai, China). Cells were cultured in DMEM (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS, Gibco) and incubated in a humidified incubator with 5% CO_2 at 37 °C. An siRNAs against *LINC01121* (si-*LINC01121*-): si-*LINC01121*-1(5′-CAAAGACAAGGAUGAAAUAAG-3′), si-*LINC01121*-2 (5′-GACUAAACUAAUUAAGCUACA-3′), si-*LINC01121*-3 (5′-GUUCUCAUUUGAUGUUGAAUA-3′), a negative control siRNA (si-NC, 5′-UUCUCCGAACGUGUCACGUGC-3′), *miR-150-5p* mimics (5′-CTGGTACAGGCCTGGGGGACAG-3′), a *miR-150-5p* inhibitor (5′-CTGTCCCCCAGGCCTGTACCAG-3′), a mi RNA mimic negative control (NC mimic, 5′-TTCTCCGAACGTGTCACGTAA-3′), and a mi RNA inhibitor negative control (NC inhibitor, 5′-TTCTCCGAACGTGTCACGTAA-3′) were purchased from GenePharma (Shanghai, China). Cell transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol.

Nuclear and Cytoplasmic RNA Isolation

Cytoplasmic and nuclear RNAs were separated and purified using a PARIS kit (Thermo Fisher Scientific, Inc.). The expression of linc00467 in the nucleus and cytoplasm was measured by qRT-PCR.

Quantitative Reverse Transcription PCR (qRT-PCR)

Total RNA was extracted from cells using TRIzol reagent (Invitrogen). For *LINC01121*, first-strand cDNA was reverse transcribed from total RNA using the ImProm-IITM Reverse Transcription System (Promega, Madison, WI, USA). For *miR-150-5p*, cDNA was reverse transcribed using the ImProm-IITM Reverse Transcription System and stem-loop primer (Promega). The conditions of reverse transcription were 42°C for 60 min, followed by 85°C for 10 min. All PCRs were performed using an ABI 7500 RT-PCR system (Applied Biosystems) with SYBR® Premix Ex Taq™ Kit (TaKaRa, Kusatsu, Japan). PCR primers were purchased from GenePharma with the following sequences: *LINC01121* forward, 5′-TGGATGGATGGGTTGTGGTCTT-3′ and reverse, 5′-TCCTTGTCTTTGTTACGCCTGT-3′; *GAPDH* forward, 5′-GCTCATTTGCAGGGGGGAG-3′ and reverse, 5′- GTTGGTGGTGCAGGAGGCA-3′; *miR-*

150-5p forward, 5′-ACACTCCAGCTGGG TCTCCCAA CCCTTGTA-3′ and reverse, 5′-CTCAACTGGTGTCGTGG A-3′; miR-1193 forward, 5′- ACACTCCAGCTGGGAT AGACCGGTGACGTGC-3′ and reverse, 5′- CTCA ACTGGTGTCGTGGA-3′; miR-520h forward, 5′- ACACTCCAGCTGGGAGTGCTTCCCTTTAGAG-3′ and reverse, 5′- CTCAACTGGTGTCGTGGA-3′; miR-520g-3p forward, 5′- ACACTCCAGCTGGGATTCCCTTTAGAG TGT-3′ and reverse, 5′- CTCAACTGGTGTCGTGGA-3′; and *U6* forward, 5′-CTCGCTTCGGCAGCACA-3′ and reverse, 5′-AACGCTTCACGAATTTGCGT-3′. *GAPDH* and *U6* were used as endogenous controls for *LINC01121* and *miR-150-5p* expression, respectively. Fold change in expression was calculated using the $2^{-\Delta\Delta CT}$ method.[18] All experiments were repeated in independent triplicate.

MTS Assay

Cell proliferation was evaluated using a CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS, Promega). In brief, after transfection, 96-well plates were seeded with 1×10^4 cells/100 μL/well in triplicate and incubated in a humidified incubator with 5% CO_2 at 37 °C. The AQueous One Solution reagent (10 μL) was then added to the wells at 0, 24, 48, and 72 h. After cultured for 4 h at 37 °C, the absorbance was measured at 490 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). All experiments were repeated in independent triplicate.

Cell Cycle and Apoptosis Assay

A Cell Cycle Detection Kit (Keygentec, Nanjing, China) was used to assess the cell cycle and an Annexin V-FITC Apoptosis Detection Kit (Keygentec) was used to detect apoptosis. The percentage of the cell population in different phases of the cell cycle and the percentage of the cells undergoing apoptosis were measured using flow cytometry (BD Biosciences, San Jose, CA, USA). All experiments were repeated in independent triplicate.

Transwell Migration and Invasion Assays

A BD 24-well transwell chamber (BD Biosciences) was used to assess cell migration and invasion. Briefly, 2×10^5 transfected MCF-7 or MD-MBA-231 cells, in 200 μL of serum-free medium, were seeded to the top chamber. For invasion assays, the upper chamber was coated in Matrigel (BD Biosciences), but for migration assays, Matrigel was not added. Medium containing 10% FBS was loaded into the lower transwell chamber. After incubation at 37 °C with 5% CO_2 for 24 h, cells on the transwell membrane were stained with 0.5% crystal violet for 15 min. Migrant or invasive cells were counted under a microscope (Olympus, Tokyo, Japan). All experiments were repeated in independent triplicate.

Western Blotting

RIPA lysis buffer with protease inhibitor (Beyotime, Shanghai, China) was used to extract total protein. Equal amounts of denatured protein (30 μg) were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with Tris-buffered saline (TBS), containing 5% non-fat milk powder, at 25 °C for 2 h. The membranes were then incubated at 4 °C overnight with the following diluted primary antibodies: anti-HMGA2 (dilution 1:1000; ab97276, Abcam; Danvers, MA, USA) and anti-β-actin (dilution 1:1000; ab8227; Abcam). After washing with TBS containing Tween 20 (TBST), membranes were incubated with an HRP-labeled secondary antibody (dilution 1:5000) for 2 h at 25 °C. After washing three times with TBST, the protein bands were detected using an enhanced chemiluminescence detection kit (Beyotime) and a ChemiDoc™ XRS imaging system (Bio-Rad). All experiments were repeated in independent triplicate.

Luciferase Reporter Assays

Wild-type (WT) *LINC01121* containing putative *miR-150-5p* binding sites and *LINC01121* containing mutated *miR-150-5p* binding sites (MUT) were synthesized and then inserted into the luciferase reporter vector, psi-CHECK-2 (Promega). For luciferase assays, MCF-7 cells were co-transfected with luciferase reporter plasmids and an *miR-150-5p* mimic, an *miR-150-5p* inhibitor, or a negative control miRNA. Forty-eight hours after transfection, relative luciferase activity was determined using a Dual-Luciferase Reporter Assay System (Promega), according to the manufacturer's instructions. All experiments were repeated in independent triplicate.

Statistical Analysis

Statistical analyses were performed using SPSS 19.0 statistical software (IBM Inc., Chicago, IL, USA). Data are presented as means ± standard deviation (SD). A *t*-test was performed to detect significant differences between two groups. An ANOVA was performed to detect significant differences between three groups. A *p*-value less than 0.05 was regarded as statistically significant.

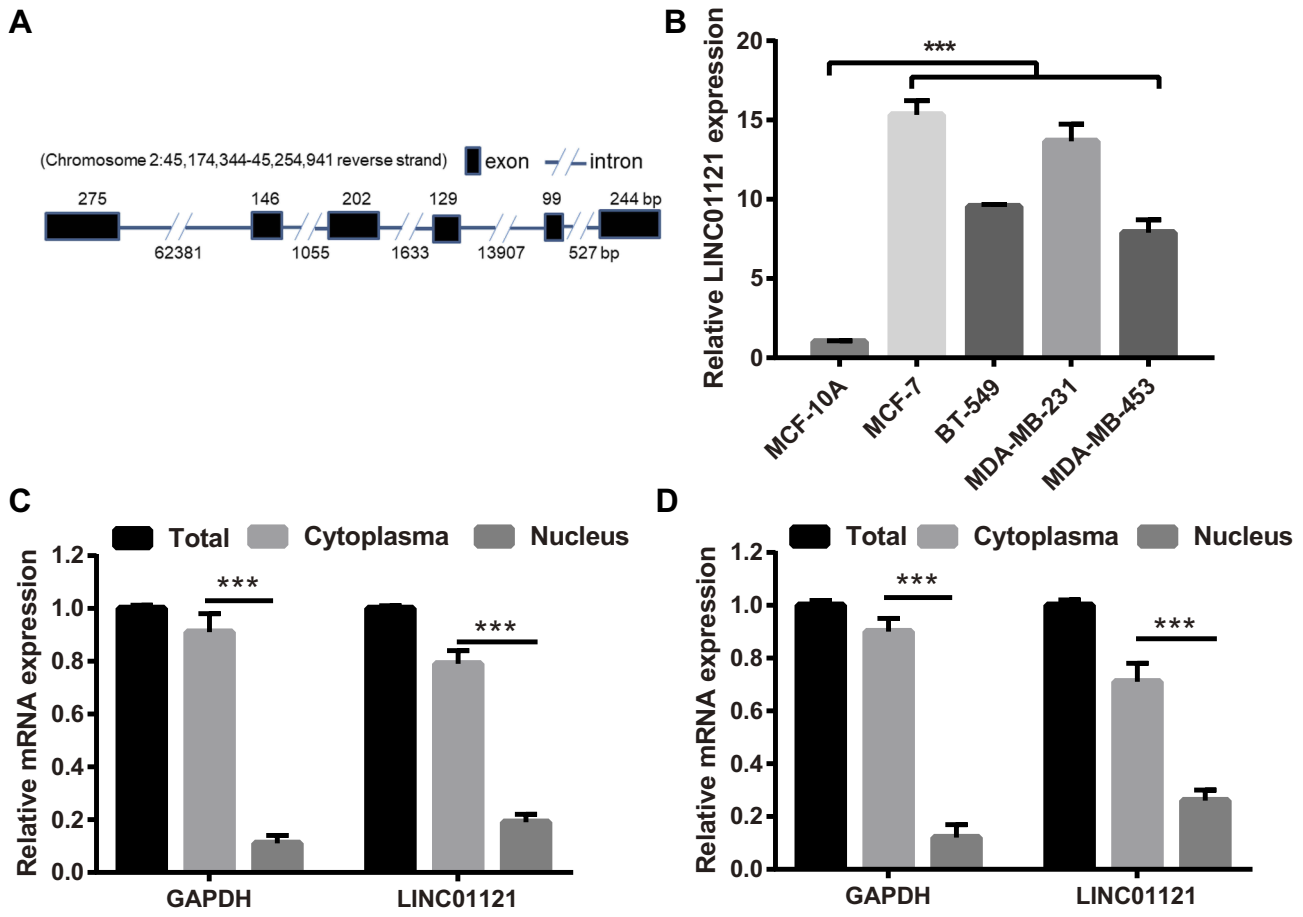

Figure 1 *LINC01121* expression levels were significantly in breast cancer cell lines compared with MCF-10A. (**A**) The structure of *LINC01121* (1095bp), such as chromosomal locations and exons, was diagrammed. (**B**) *LINC01121* expression levels in breast cancer cell lines (MCF-7, BT-549, MDA-MB-231, and MDA-MB-453) and the normal human breast cell line (MCF-10A) were measured by qRT-PCR after cultured at 24 h. ***$p < 0.001$, vs MCF-10A cells. (**C**) *LINC01121* expression was significantly higher in cytoplasm than that in nuclear in MCF-7 cell. (**D**) *LINC01121* expression was significantly higher in cytoplasm than that in nuclear in MDA-MB-231 cell.

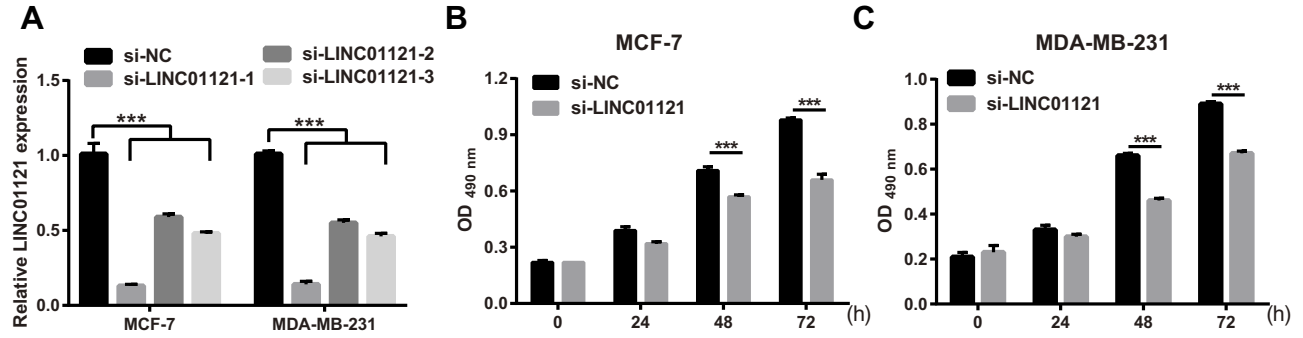

Figure 2 *LINC01121* down-regulation significantly suppressed proliferation in MCF-7 and MDA-MB-231 cells (**A**) *LINC01121* expression levels in MCF-7 and MDA-MB-231 cells after transfected at 48 h si-*LINC01121*-1/2/3 was measured by qRT-PCR. (**B** and **C**) Proliferation of MCF-7 and MDA-MB-231 cells was determined by MTS assay after transfected si-*LINC01121* at 48 h (si-*LINC01121*-1) (***$p < 0.001$).

Results

LINC01121 Was Markedly Up-Regulated in Breast Cancer Cell Lines

The structure of *LINC01121* (1095bp) such as chromosomal locations and exons were diagrammed in Figure 1A. First, we investigated the relative expression levels of *LINC01121* in breast cancer cell lines by qRT-PCR. The expression of *LINC01121* in breast cancer cell lines (including MCF-7, BT-549, MDA-MB-231, and MDA-MB-453) was markedly up-regulated, especially

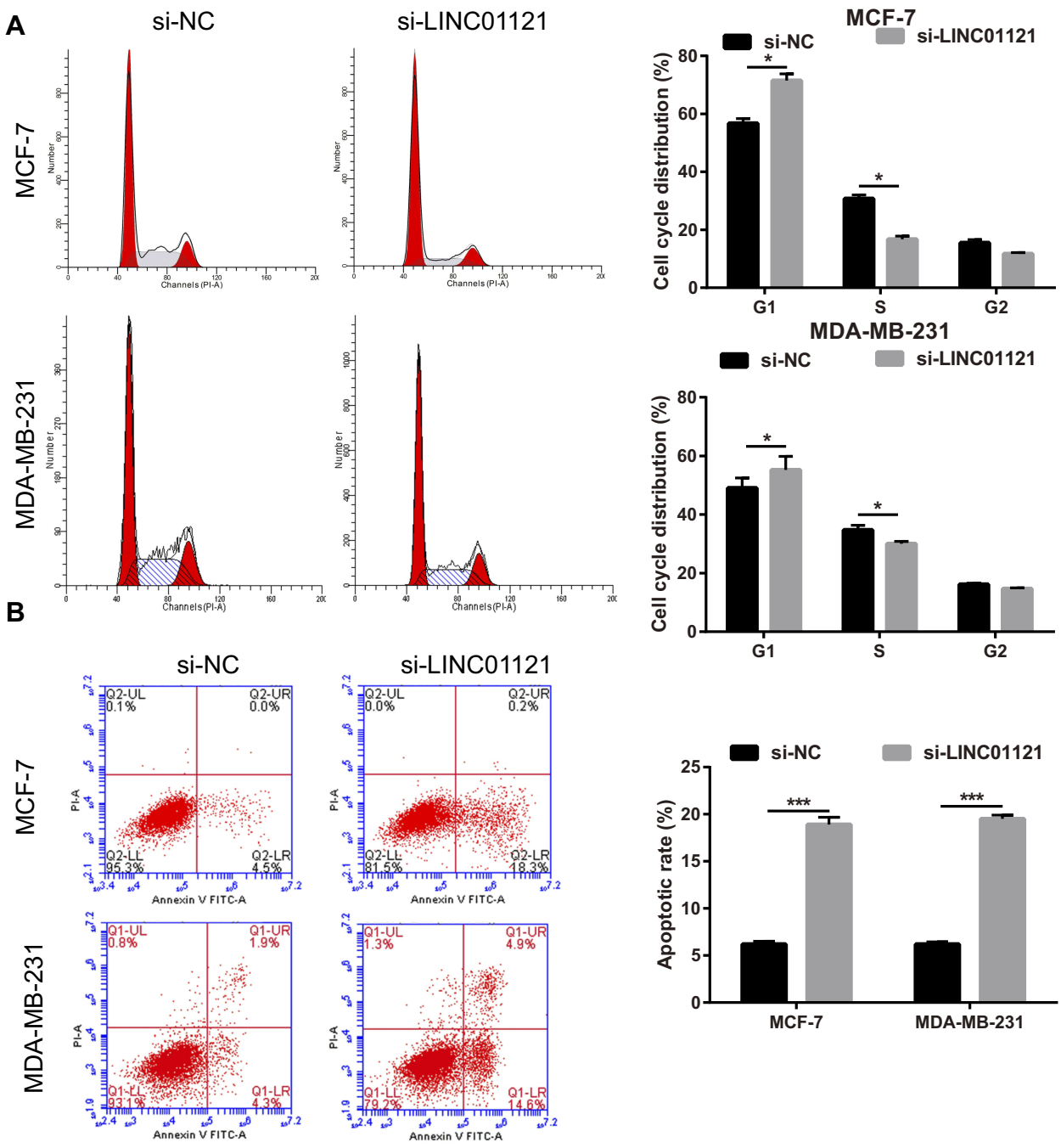

Figure 3 *LINC01121* down-regulation significantly suppressed cell cycle progression and promoted apoptosis in MCF-7 and MDA-MB-231 cells. (**A** and **B**) Cell cycle progression and apoptosis in MCF-7 and MDA-MB-231 cells were assessed using flow cytometry after transfected si-*LINC01121* at 48 h (*P<0.05, ***$p < 0.001$).

in MCF-7 and MDA-MB-231 cell lines, compared with its expression in the normal breast epithelial cell line, MCF-10A (Figure 1B). Therefore, MCF-7 and MDA-MB-231 cell lines were selected for subsequent experiments. Additionally, we found that *LINC01121* expression was significantly higher in cytoplasm than that in nuclear in MCF-7 and MDA-MB-231 cell lines (Figure 1C and D).

LINC01121 Down-Regulation Significantly Suppressed Breast Cancer Cell Proliferation and Cell Cycle Progression and Promoted Apoptosis

To investigate the biological function of *LINC01121* in breast cancer cells, we transfected MCF-7 and MDA-MB-231 cells with si-*LINC01121* to down-regulate

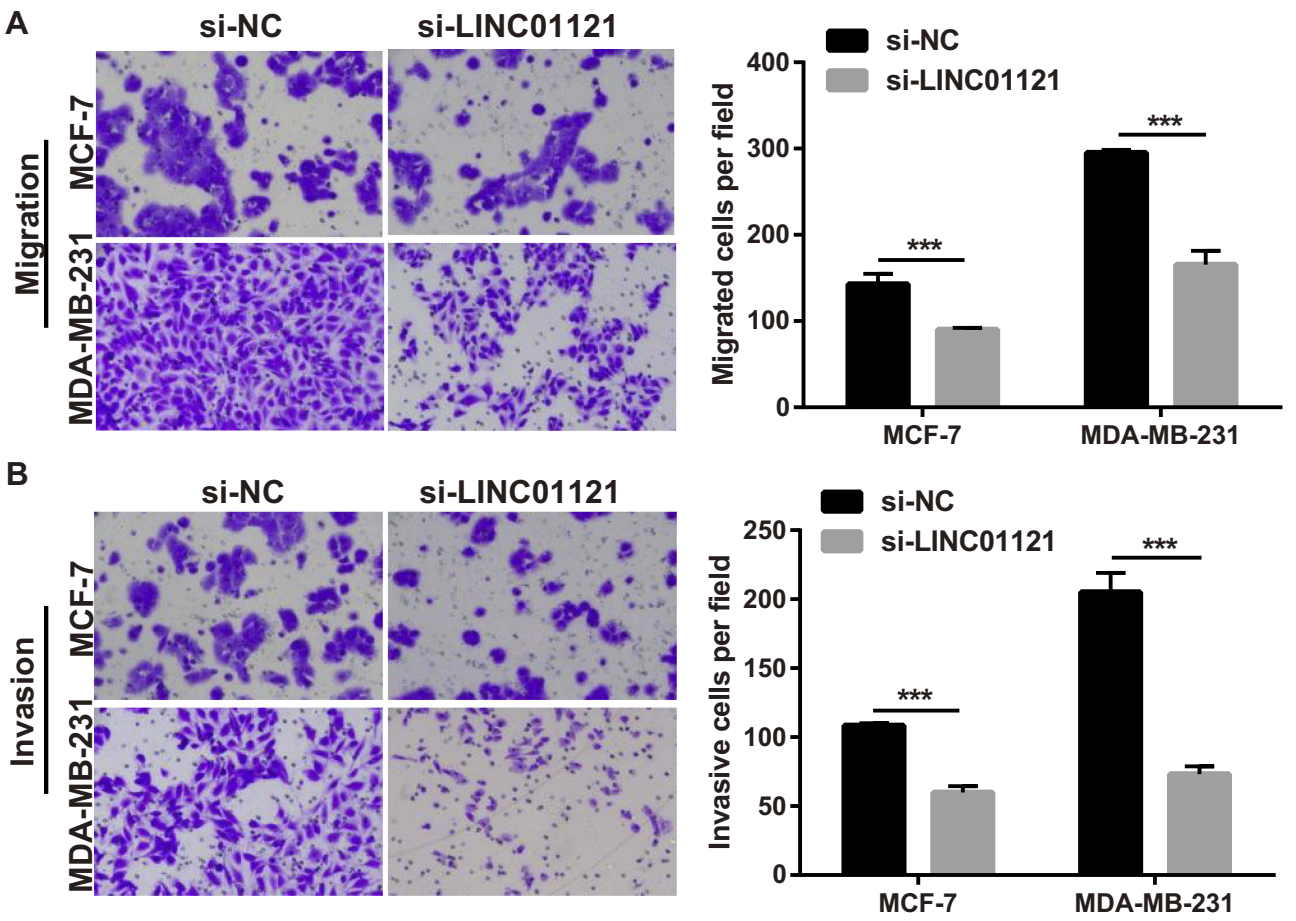

Figure 4 *LINC01121* down-regulation suppressed MCF-7 and MDA-MB-231 cell migration and invasion. (**A** and **B**) Migration and invasion of MCF-7 and MDA-MB-231 cells was determined by transwell experiments after transfected si-LINC01121 at 48 h (***$p < 0.001$).

LINC01121 expression. qRT-PCR results showed that the expression of *LINC01121* was markedly decreased in both MCF-7 and MDA-MB-231 cells after transfected si- *LINC01121*-1/2/3, particularly si-*LINC01121* -1 which chose for further study (Figure 2A). MTS results showed that *LINC01121* down-regulation significantly suppressed the proliferation of MCF-7 and MDA-MB-231 cells compared with cells transfected with si-NC (Figure 2B and C). Cell cycle analysis revealed that *LINC01121* down-regulation increased the fraction of MCF-7 and MDA-MB-231 cells in G1 phase and decreased the fraction of MCF-7 and MDA-MB-231 cells in S phase compared with cells transfected with si-NC (Figure 3A). *LINC01121* down-regulation significantly promoted the apoptosis of MCF-7 and MDA-MB-231 cells compared with cells transfected with si-NC (Figure 3B). These data suggested that *LINC01121* down-regulation suppressed breast cancer cell proliferation and cell cycle progression and promoted apoptosis.

LINC01121 Down-Regulation Significantly Suppressed Breast Cancer Cell Migration and Invasion

Next, transwell experiments showed that *LINC01121* down-regulation significantly suppressed cell migration and invasion in both MCF-7 and MDA-MB-231 cells compared with cells transfected with si-NC (Figure 4).

LINC01121 Directly Bounds to miR-150-5p

Next, we investigated whether *LINC01121* acted as a ceRNA of miRNAs in breast cancer. Bioinformatics prediction analysis showed that *LINC01121* has

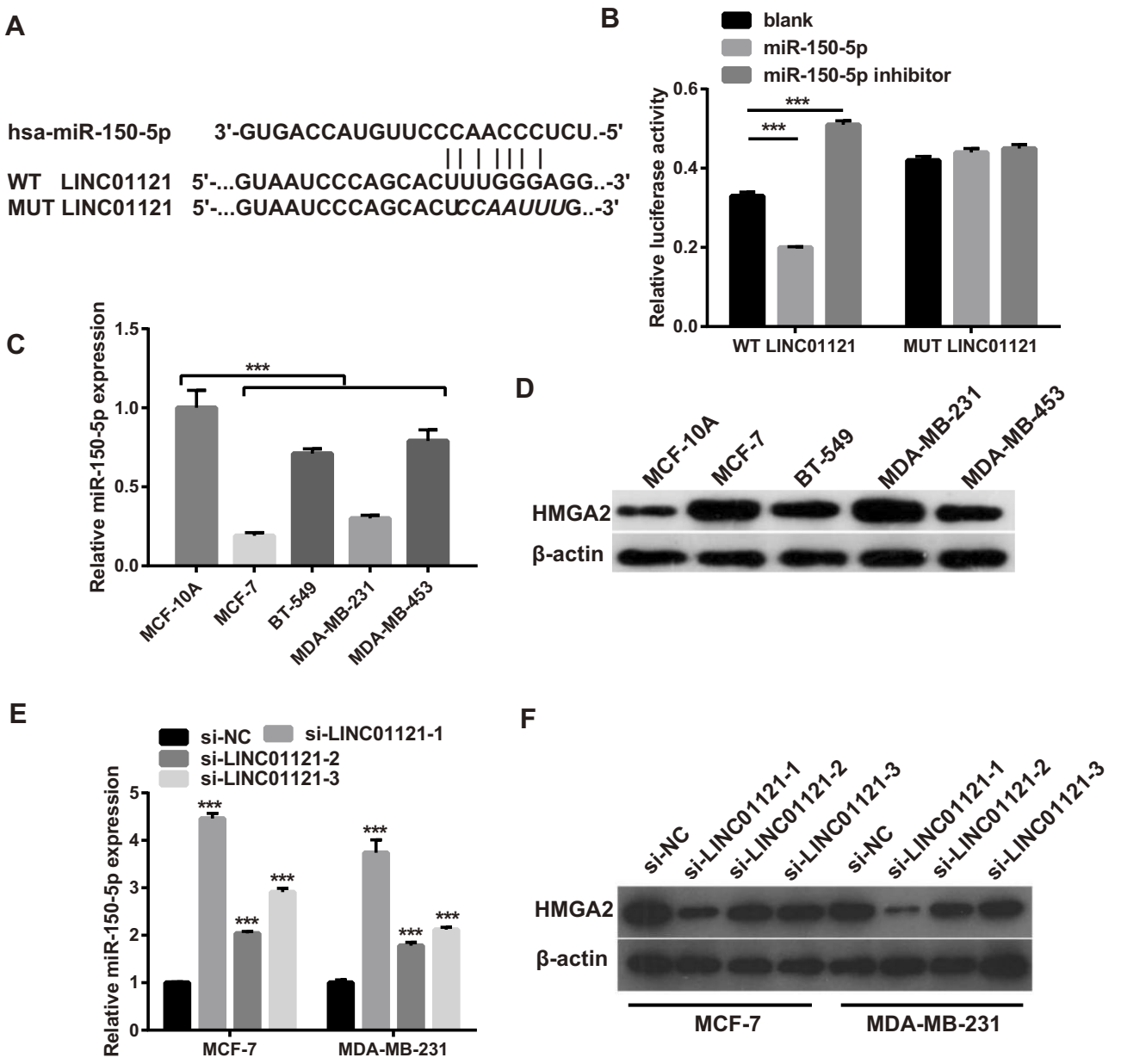

Figure 5 *LINC01121* directly bound to *miR-150-5p* and indirectly affected HMGA2 expression (**A**) The predicted binding site between *LINC01121* and *miR-150-5p*. (**B**) Luciferase reporter assays demonstrated that *LINC01121* directly bound to *miR-150-5p*. (**C**) The relative expression levels of *miR-150-5p* in breast cancer cells (MCF-7, BT-549, MDA-MB-231, and MDA-MB-453) and the normal breast epithelial cell line (MCF-10A) were measured by qRT-PCR. (**D**) The expression of HMGA2 protein in MCF-7, BT-549, MDA-MB-231, MDA-MB -453 and MCF-10A cells was measured by Western blotting. (**E**) The expression of *miR-150-5p* in MCF-7 and MDA-MB-231 cells was measured by qRT-PCR after transfected si-*LINC01121* at 48 h. (**F**) The expression of HMGA2 in MCF-7 and MDA-MB-231 cells was measured by Western blotting after transfected si-*LINC01121* at 48 h (***$p < 0.001$).

theoretical binding site for *miR-1193, miR-520h, miR-520g-3p*, and *miR-150-5p*. However, previous studies reported that only *miR-150-5p* is an anti-cancer gene.[17] Thus, *miR-150-5p* was selected for further investigation. The theoretical binding site between *LINC01121* and *miR-150-5p* was shown in Figure 5A. To verify that *LINC01121* can directly bind to *miR-150-5p*, luciferase reporter assays were performed. Compared with the blank+ WT *LINC01121* group, relative luciferase activity was inhibited in *miR-150-5p*+ WT *LINC01121* group while it was promoted in *miR-150-5p* inhibitor+ WT *LINC01121* group (Figure 5B). However, the relative luciferase activity was not affected between MUT *LINC01121* +blank, MUT *LINC01121* +*miR-150-5p*, and MUT *LINC01121* +*miR-150-5p* inhibitor groups (Figure 5B). Next, *miR-150-5p* expression levels were

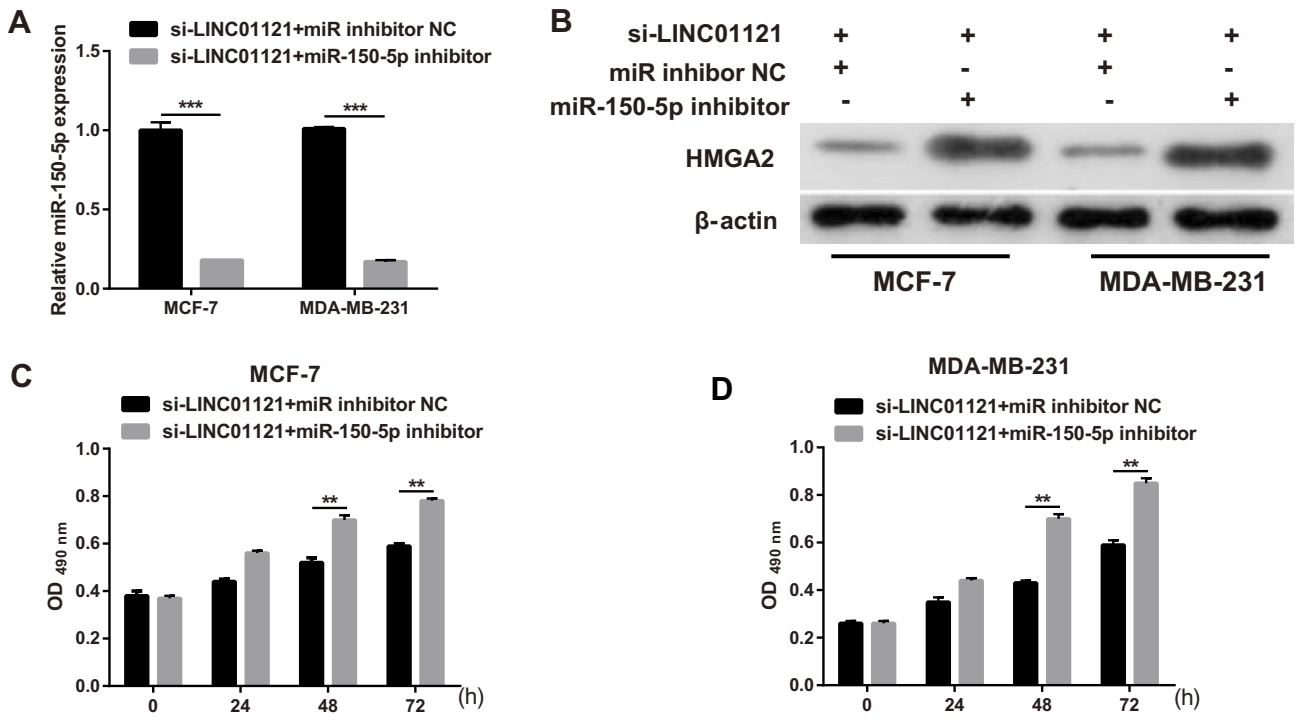

Figure 6 *miR-150-5p* knockdown significantly attenuated the effects of *LINC01121* silencing on HMGA2 protein expression and cell proliferation in breast cancer cells (**A**) The relative expression levels of *miR-150-5p* in MCF-7 and MDA-MB-231 cells were measured by qRT-PCR after co-transfected *miR-150-5p* inhibitor and si-*LINC01121* or co-transfected with an NC inhibitor and si-*LINC01121* at 48 h. (**B**) HMGA2 protein expression in MCF-7 and MDA-MB-231 cells was measured by Western blotting after co-transfected *miR-150-5p* inhibitor and si-*LINC01121* or co-transfected with an NC inhibitor and si-*LINC01121* at 48 h. (**C** and **D**) Proliferation of MCF-7 and MDA-MB-231 cells was measured by MTS assay after co-transfected *miR-150-5p* inhibitor and si-*LINC01121* or co-transfected with an NC inhibitor and si-*LINC01121* at 48 h (**$p < 0.01$, ***$p < 0.001$).

significantly lower in the four breast cancer cell lines than that in the normal breast epithelial cell line, MCF-10A (Figure 5C). Previous studies have shown that *miR-150-5p* modulates breast cancer metastasis by targeting HMGA2.[17] We found that HMGA2 expression was up-regulated in four breast cancer cell lines compared with its expression in MCF-10A cells (Figure 5D). Moreover, we found that *miR-150-5p* expression was promoted while HMGA2 expression was inhibited in MCF-7 and MDA-MB-231 cells after transfected si-*LINC01121* -1/2/3, particularly si-*LINC01121*-1 (Figure 5E–F). Additionally, other three predict miRNA (miR-1193, miR-520h, miR-520g-3p) expression after *LINC01121* knockdown at 48 h were measured by qRT-PCR. The results showed that *LINC01121* knockdown promoted miR-1193, miR-520h, and miR-520g-3p expression (Supplement Figure 1). But the change of *miR-150-5p* expression significantly higher than the change of other three miRNA (miR-1193, miR-520h, miR-520g-3p). These data suggested that *LINC01121* may directly bound to *miR-150-5p* to regulate HMGA2 expression.

Knockdown *miR-150-5p* Significantly Attenuated the Effects of *LINC01121* Down-Regulation on HMGA2 Protein Expression, Cell Proliferation, Cell Cycle Progression, and Apoptosis in Breast Cancer Cells

To further investigate the relationship between *LINC01121* and *miR-150-5p*, we transfected MCF-7 and MDA-MB-231 cells that had previously been transfected with si-*LINC01121*, with an *miR-150-5p* inhibitor. *miR-150-5p* expression significantly decreased, while HMGA2 expression significantly increased, in MCF-7 and MDA-MB-231 cells co-transfected an *miR-150-5p* inhibitor and si-*LINC01121* compared with cells co-transfected with an NC inhibitor and si-*LINC01121* (Figure 6A and B). Moreover, *miR-150-5p* knockdown promoted cell proliferation (Figure 6C and D), increased the fraction of cells in S phase, decreased the fraction of cells in G1 phase (Figure 7A), and suppressed apoptosis (Figure 7B) in MCF-7 and MDA-MB-231 cells co-transfected with an *miR-150-5p* inhibitor and si-

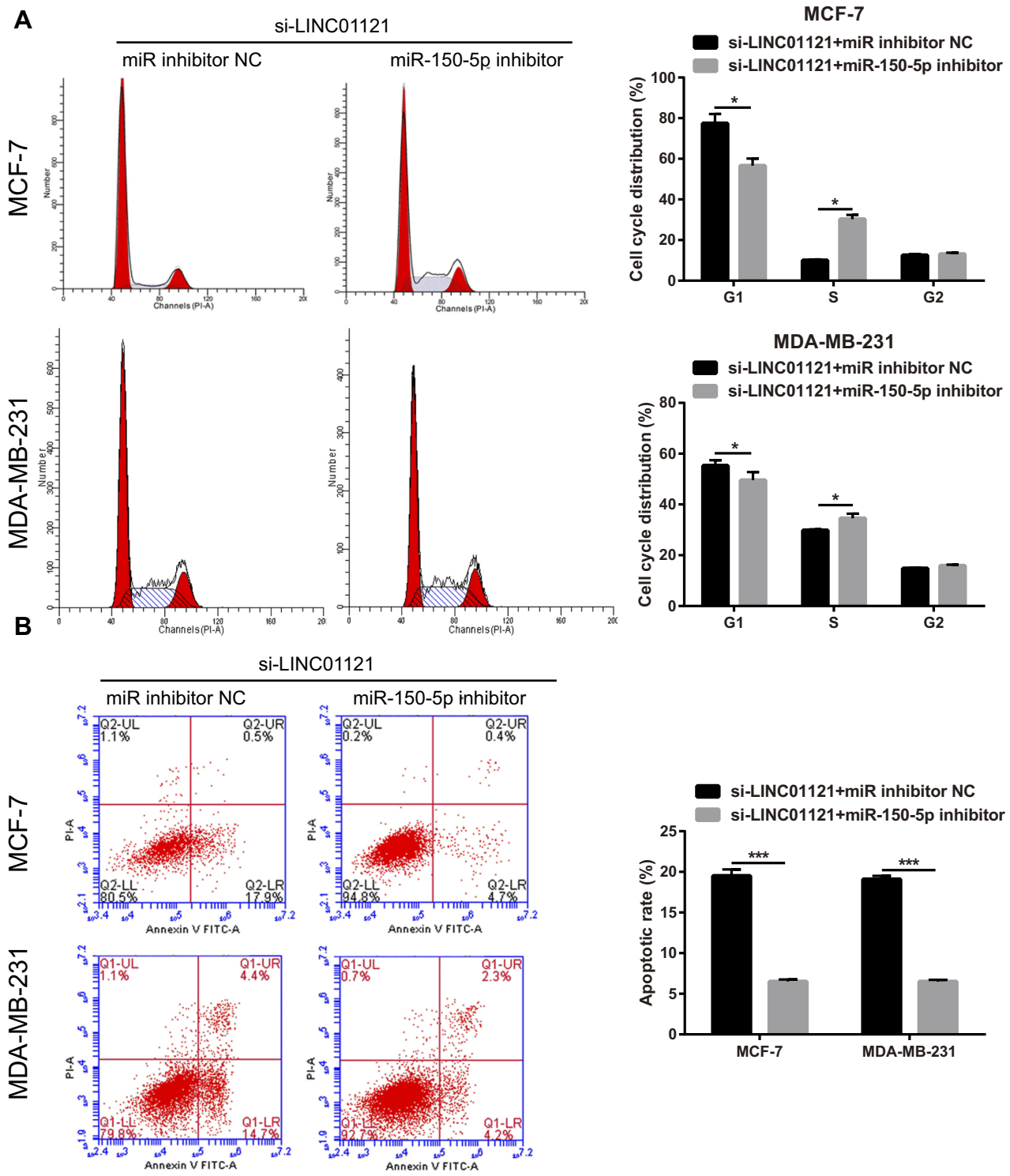

Figure 7 *miR-150-5p* knockdown significantly attenuated the effects of *LINC01121* silencing on cell cycle progression and apoptosis in breast cancer cells. (**A** and **B**) The effect of *miR-150-5p* knockdown on cell cycle progression and apoptosis in MCF-7 and MDA-MB-231 cells was assessed by flow cytometry after co-transfected *miR-150-5p* inhibitor and si-*LINC01121* or co-transfected with an NC inhibitor and si-*LINC01121* at 48 h (*P<0.05, ***$p < 0.001$).

LINC01121 compared with cells co-transfected with an NC inhibitor and si-*LINC01121*. These data suggested that *miR-150-5p* knockdown attenuated the effects of si-*LINC01121* on HMGA2 protein expression, cell proliferation, cell cycle progression, and apoptosis in MCF-7 and MDA-MB-231 cells.

miR-150-5p Knockdown Significantly Attenuated the Repressive Effects of *LINC01121* Down-Regulation on the Migration and Invasion of Breast Cancer Cells

Transwell migration and invasion assays showed that *miR-150-5p* knockdown significantly promoted the migration and invasion of MCF-7 and MDA-MB-231 cells co-transfected an *miR-150-5p* inhibitor and si-*LINC01121*, compared with cells co-transfected with an NC inhibitor and si-*LINC01121* (Figure 8).

Discussion

LncRNAs function as regulators of gene expression and have a great impact on the development and progression of various human cancers, including breast cancer.[19] In the present study, we found that *LINC01121* was markedly up-regulated in breast cancer cell lines compared with normal breast epithelial cells. *LINC01121* down-regulation significantly suppressed cell proliferation, cell cycle progression, migration, and invasion and promoted apoptosis in MCF-7 and MDA-MB-231 cells. These results indicated that *LINC01121* acts as an oncogene and is involved in the process of breast cancer proliferation, migration, and invasion. Qian et al[10] reported that *LINC01121* is overexpressed and acts as an oncogene in pancreatic cancer tissues, which is similar to our results.

One of the functions of lncRNAs is to act as miRNA sponges to regulate the expression of their miRNA targets. Herein, we used bioinformatics tools to predict that *miR-150-5p* was a target of *LINC01121*. The interaction between *LINC01121* and *miR-150-5p* was confirmed using luciferase reporter assays, which showed that *LINC01121* could directly bind to *miR-150-5p*. Moreover, *LINC01121*

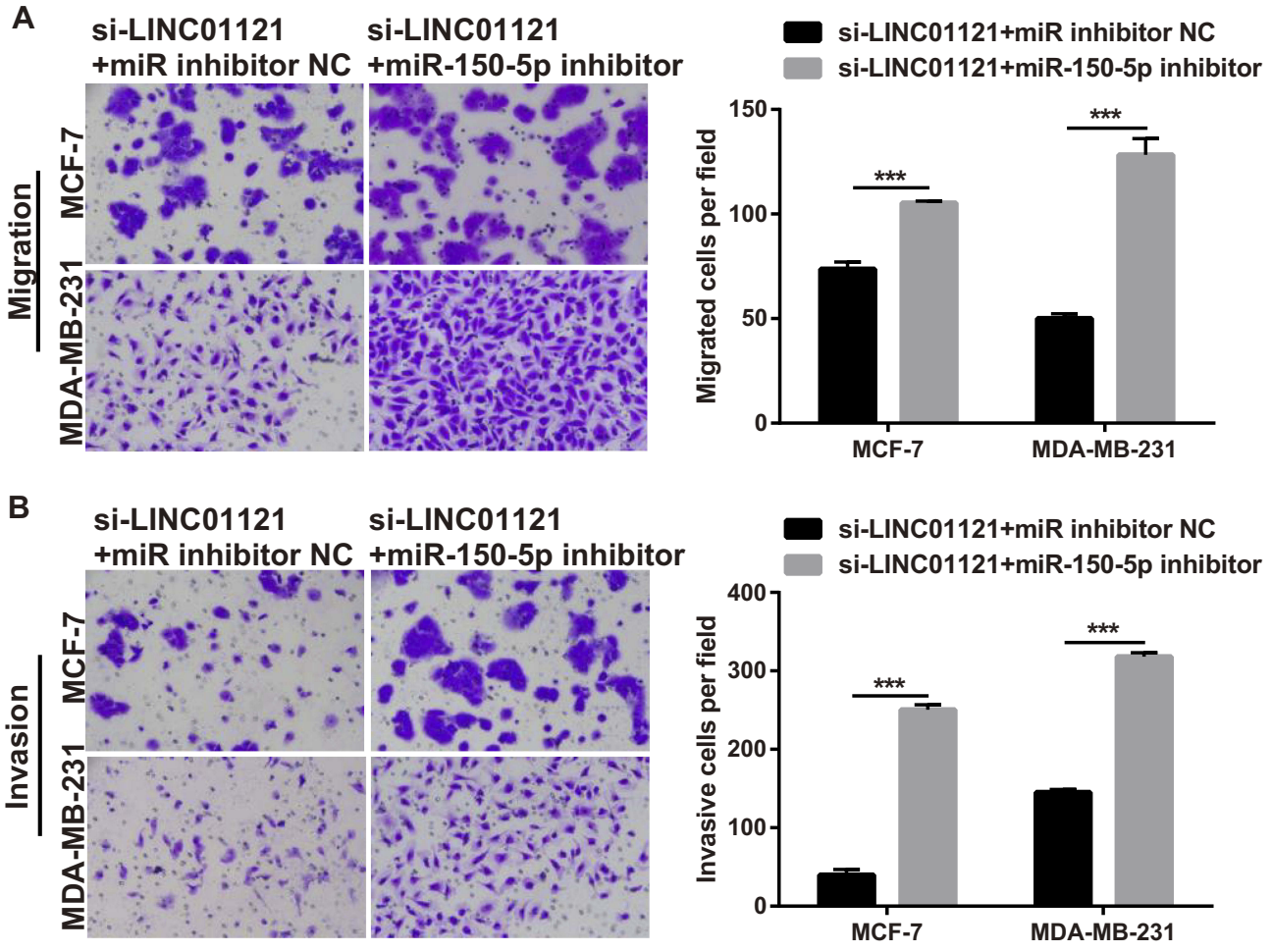

Figure 8 *miR-150-5p* knockdown significantly attenuated the repressive effects of *LINC01121* down-regulation on the migration and invasion of breast cancer cells (**A** and **B**) Migration and invasion of MCF-7 and MDA-MB-231 cells were measured by transwell after co-transfected *miR-150-5p* inhibitor and si-*LINC01121* or co-transfected with an NC inhibitor and si-*LINC01121* at 48 h (***$p < 0.001$).

knockdown promoted *miR-150-5p* expression in both MCF-7 and MDA-MB-231 cells. These results suggested that *LINC01121* directly bound to *miR-150-5p*. Tang et al[17] found that *miR-150-5p* suppresses the migration of breast cancer cells and its expression is decreased in triple-negative breast cancer tumor tissues, indicating that *miR-150-5p* may play an inhibitory role in triple-negative breast cancer. Hu et al[20] found that *BLACAT1* promotes breast cancer cell growth and metastasis by sponging *miR-150-5p*. Alipoor et al also found that *MIAT* knockdown suppresses MCF7 cell growth and migration by sponging *miR-150-5p*.[21] Further rescue experiments demonstrated that *miR-150-5p* knockdown significantly reversed the effects of *LINC01121* silencing on cell proliferation, cell cycle progression, apoptosis, migration, and invasion in MCF-7 and MDA-MB-231 cells. Taken together, these data indicated that *LINC01121* down-regulation suppressed breast cancer cell proliferation and metastasis through the *miR-150-5p* axis, which is similar to the results of Hu and Alipoor.[20,21]

Previous studies have demonstrated that HMGA2 is highly overexpressed in breast cancer and promotes breast cancer cell proliferation and metastasis.[22,23] *miR-150-5p* suppresses triple-negative breast cancer metastasis by inhibiting HMGA2 expression.[17] In the present study, we found that HMGA2 was overexpressed in breast cancer cell lines, which is similar to the results of previous studies.[17,22,23] Additionally, we found that *LINC01121* knockdown inhibited HMGA2 expression in MCF-7 and MDA-MB-231 cells and that inhibiting *miR-150-5p* expression promoted HMGA2 expression and reversed the effect of *LINC01121* silencing on HMGA2 expression. These data indicated that *HMGA2*, a target gene of *miR-150-5p*, is involved in the *LINC01121*-mediated regulation of breast cancer cell proliferation migration, and invasion.

Conclusion

LINC01121 knockdown suppressed breast cancer cell proliferation, migration, and invasion through the *miR-150-5p*/HMGA2 axis. These findings suggested that *LINC01121* may serve as a novel molecular target for breast cancer therapy. We will confirm the effect of *LINC01121* on breast cancer cell growth and metastasis in vitro in future experiments.

Abbreviations

LINC01121, Long intergenic noncoding RNA 01121; ceRNAs, competing endogenous RNAs; miRNAs, micro RNAs; HMGA2, high-mobility group protein 2; qRT-PCR, quantitative reverse transcription PCR; SD, standard deviation.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66(1):7–30. doi:10.3322/caac.21332
2. DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA Cancer J Clin*. 2017;67(6):439–448. doi:10.3322/caac.21412
3. Fan L, Strasser-Weippl K, Li JJ, et al. Breast cancer in China. *Lancet Oncol*. 2014;15(7):e279–289. doi:10.1016/S1470-2045(13)70567-9
4. Ribeiro Pereira ACP, Koifman RJ, Bergmann A. Incidence and risk factors of lymphedema after breast cancer treatment: 10 years of follow-up. *Breast*. 2017;36:67–73. doi:10.1016/j.breast.2017.09.006
5. Lemler DJ, Lynch ML, Tesfay L, et al. DCYTB is a predictor of outcome in breast cancer that functions via iron-independent mechanisms. *Breast Cancer Res*. 2017;19(1):25. doi:10.1186/s13058-017-0814-9
6. Golse N, Adam R. Liver metastases from breast cancer: what role for surgery? Indications and results. *Clin Breast Cancer*. 2017;17(4):256–265. doi:10.1016/j.clbc.2016.12.012
7. Bai J, Zhao WY, Li WJ, Ying ZW, Jiang DQ. Long noncoding RNA LINC00473 indicates a poor prognosis of breast cancer and accelerates tumor carcinogenesis by competing endogenous sponging miR-497. *Eur Rev Med Pharmacol Sci*. 2019;23(8):3410–3420. doi:10.26355/eurrev_201904_17705
8. Zhao W, Geng D, Li S, Chen Z, Sun M. LncRNA HOTAIR influences cell growth, migration, invasion, and apoptosis via the miR-20a-5p/HMGA2 axis in breast cancer. *Cancer Med*. 2018;7(3):842–855. doi:10.1002/cam4.2018.7.issue-3
9. Li Y, Zhang W, Liu P, et al. Long non-coding RNA FENDRR inhibits cell proliferation and is associated with good prognosis in breast cancer. *Onco Targets Ther*. 2018;11:1403–1412. doi:10.2147/OTT
10. Qian YG, Ye Z, Chen HY, et al. *LINC01121* inhibits cell apoptosis while facilitating proliferation, migration, and invasion though negative regulation of the Camp/PKA signaling pathway via GLP1R. *Cell Physiol Biochem*. 2018;47(3):1007–1024. doi:10.1159/000490167
11. Huang Y, Du J, Mi Y, et al. Long non-coding RNAs contribute to the inhibition of proliferation and EMT by pterostilbene in human breast cancer. *Front Oncol*. 2018;8:629. doi:10.3389/fonc.2018.00629
12. Wang XB, Wang H, Long HQ, Li DY, Zheng X. LINC00641 regulates autophagy and intervertebral disc degeneration by acting as a competitive endogenous RNA of miR-153-3p under nutrition deprivation stress. *J Cell Physiol*. 2019;234(5):7115–7127. doi:10.1002/jcp.27466
13. Cui J, Zhou B, Ross SA, Zempleni J. Nutrition, microRNAs, and human health. *Adv Nutr*. 2017;8(1):105–112. doi:10.3945/an.116.013839
14. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009;136(2):215–233. doi:10.1016/j.cell.2009.01.002
15. Jiang X, Huang H, Li Z, et al. Blockade of miR-150 maturation by MLL-fusion/MYC/LIN-28 is required for MLL-associated leukemia. *Cancer Cell*. 2012;22(4):524–535. doi:10.1016/j.ccr.2012.08.028
16. Cao M, Hou D, Liang H, et al. miR-150 promotes the proliferation and migration of lung cancer cells by targeting SRC kinase signalling inhibitor 1. *Eur J Cancer*. 2014;50(5):1013–1024. doi:10.1016/j.ejca.2013.12.024

17. Tang W, Xu P, Wang H, et al. MicroRNA-150 suppresses triple-negative breast cancer metastasis through targeting HMGA2. *Onco Targets Ther*. 2018;11:2319–2332. doi:10.2147/OTT.S161996
18. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc*. 2008;3(6):1101–1108. doi:10.1038/nprot.2008.73
19. Liu Y, Sharma S, Watabe K. Roles of lncRNA in breast cancer. *Front Biosci (Schol Ed)*. 2015;7:94–108. doi:10.2741/s427
20. Hu X, Liu Y, Du Y, Cheng T, Xia W. Long non-coding RNA BLACAT1 promotes breast cancer cell proliferation and metastasis by *miR-150-5p*/CCR2. *Cell Biosci*. 2019;9:14. doi:10.1186/s13578-019-0274-2
21. Alipoor FJ, Asadi MH, Torkzadeh-Mahani M. MIAT lncRNA is overexpressed in breast cancer and its inhibition triggers senescence and G1 arrest in MCF7 cell line. *J Cell Biochem*. 2018;119(8):6470–6481. doi:10.1002/jcb.v119.8
22. Sun M, Song CX, Huang H, et al. HMGA2/TET1/HOXA9 signaling pathway regulates breast cancer growth and metastasis. *Proc Natl Acad Sci U S A*. 2013;110(24):9920–9925. doi:10.1073/pnas.1305172110
23. Yang E, Cisowski J, Nguyen N, et al. Dysregulated protease activated receptor 1 (PAR1) promotes metastatic phenotype in breast cancer through HMGA2. *Oncogene*. 2016;35(12):1529–1540. doi:10.1038/onc.2015.217