

LncRNA MAFG-AS1 promotes the aggressiveness of breast carcinoma through regulating miR-339-5p/MMP15

H. LI¹, G.-Y. ZHANG², C.-H. PAN³, X.-Y. ZHANG¹, X.-Y. SU⁴

¹Department of Obstetrics and Gynecology, Shandong Jiyang Public Hospital, Ji'nan, Shandong, China

²Department of Anesthesiology, Shandong Jiyang Public Hospital, Ji'nan, Shandong, China

³Department of Obstetrics and Gynecology, LanCun central hospital, Jimo, Shandong, China

⁴Department of Critical Care Medicine, Tai'an Central Hospital, Tai'an, Shandong, China

Abstract. – OBJECTIVE: The main purposes of this study are to investigate the possible effects of long noncoding RNAs (lncRNAs) MAFG-AS1 on the growth and metastasis of breast carcinoma.

PATIENTS AND METHODS: The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) assay was used to assess the MAFG-AS1 level in breast cancer tissues and cells. The wound healing and transwell invasion analysis were applied to explore the invasion and migration of breast cancer cell *in vitro*. The expressions of epithelial-mesenchymal transition (EMT) related markers were determined by Western blotting. Xenograft model and lung metastasis model were used to assess the progression of breast carcinoma cell *in vivo*.

RESULTS: The level of lncRNA MAFG-AS1 is higher in breast carcinoma, and the aggressive phenotypes of breast carcinoma cell are enhanced by MAFG-AS1 transfection. Moreover, we identify that MAFG-AS1 overexpression reduces the expression of miR-339-5p and miR-339-5p is the target of MAFG-AS1 in breast carcinoma. In addition, matrix metalloproteinase 15 (MMP15) is the functional regulated gene of miR-339-5p in breast carcinoma. The aggressiveness of breast carcinoma induced by lncRNA MAFG-AS1 is weakened by the miR-339-5p. Finally, we demonstrated that the development of breast carcinoma cell is enhanced by MAFG-AS1 *in vivo*.

CONCLUSIONS: MAFG-AS1 appears to play an oncogene role in breast carcinoma by regulating the miR-339-5p/MMP15.

Key Words:

Breast cancer, Metastasis, MAFG-AS1, MMP15, MiR-339-5p.

Introduction

Breast cancer has been recognized as one of the most common types of malignant cancers

and is the leading cause of cancer-related deaths worldwide¹. Recently, treatment strategies, such as chemotherapy, radiotherapy and molecular targeting treatment significantly improve the therapeutic outcome of patients². However, the clinical outcome of patients with breast cancer needs to improve. The metastasis of cancer cells is one major difficulty of overcoming the poor prognosis of breast cancer patients. The epithelial-mesenchymal transition (EMT) process of cancer cells is a crucial step during metastasis^{3,4}. Therefore, inhibition of EMT may be helpful for inhibiting breast cancer cell metastasis and for improving the poor clinical outcome of patients.

Earlier investigations have proved that lncRNAs epigenetically regulate their target genes and play crucial roles in the development of cancers⁵⁻⁷. For example, lncRNA maternally expressed gene 3 (MEG3) suppresses the growth and metastasis of gastric cancer cell *via* regulating the p53 signaling pathway⁸. In colorectal cancer, lncRNA MAFG-AS1 promotes the development of tumor through regulating miR-147b and activating NADH dehydrogenase (Ubiquinone) 1 Alpha Subcomplex, 4 (NDUFA4)⁹. MicroRNAs (miRNAs), which are small endogenous nucleotides, regulate the expression of target genes. Currently, more than 2000 miRNAs have been identified and have been proved to be related to diseases, including cancer^{10,11}.

Here, we demonstrated that MAFG-AS1 was over-regulated in breast cancer tissue compared to that in adjacent normal tissue. Moreover, we identified that miR-339-5p was modulated by MAFG-AS1 in breast cancer. Additionally, we verified that the aggressiveness of breast cancer cell was enhanced by MAFG-AS1. Functional experiments demonstrated that lncRNA MAFG-

AS1 facilitated the aggressiveness of breast cancer through the modulation of miR-339-5p-MMP15.

Materials and Methods

Breast Carcinoma Tissues and Cells

Breast carcinoma cells (MDA-MB-231, HCC1937, MCF-7, and MDA-MB-468) and the normal epithelial breast cell line, MCF-10A, were obtained from Nanjing Cobioer Biotechnology Co., Ltd (Nanjing, Jiangsu, China). The cells were cultured using Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% of FBS (Thermo Fisher Scientific, Waltham, MA, USA). 42 cases of breast cancer and normal tissues were obtained from the Tai'an Central Hospital (Tai'an, Shandong, China). The experiment protocol was approved by the Ethics Committee of the Tai'an Central Hospital.

Transfections

MiRNA-control inhibitor (miR-NC inhibitor), miR-339-5p inhibitor, miRNA control (miR-NC) and miR-339-5p were obtained from GeneCopoeia (Guangzhou, Guangdong, China). Scramble or MAFG-AS1, which was provided by GeneCopoeia and was cloned into the pcDNA3.1 (GeneCopoeia). MDA-MB-231 cell was transfected with miRNAs using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA).

Migration Assay

5×10^5 MDA-MB-231 or MCF-7 cell was seeded into 6 well plates. After 24 h, a wound was made by a 100 μ l pipette tip. The picture of the wound width was taken at 0 h and 48 h, respectively.

Invasion Assay

200 μ l MCF-7 or MDA-MB-231 cells (1×10^5) were seeded in the upper chamber of the transwell (Corning, Shanghai, China). 600 μ l medium contained 20% FBS was used as a chemo-attractant and plated into the lower chamber. After 24 h, 4% of paraformaldehyde and 1% of crystal violet were used to fix and stain the invaded cells, respectively.

Quantitative Real-time (qRT-PCR) Assay

RNAs were extracted with TRIzol (Thermo Fisher Scientific, Waltham, MA, USA). For the analysis the level of MMP15, RNA was tran-

scribed by PrimeScript RT Reagent Kit and oligo dT primer (TaKaRa, Dalian, Liaoning, China). The SYBR PrimeScript miRNA RT PCR kit (TaKaRa, Dalian, Liaoning, China) was used to detect the level of miRNA-363. The $2^{-\Delta\Delta Ct}$ method was adapted to the analysis of MMP15, lncRNA MAFG-AS1, and miR-339-5p. The primer sequences are the as following: MMP15 forward, 5'-ATGACGACCCCAATAAAGGA-3', and reverse, 5'-CACCGACATGGTTACCAGC-3'; GAPDH forward, 5'-AGGTCGGTGTGAACG-GATTTG-3', and reverse: 5'-TGTAGACCAT-GTAGTTGAGGTCA-3'; miR-339-5p, forward 5'-GGGTCCCTGTCTCCA-3' and reverse: 5'-TGCGTGTCTGTGGAGTC-3'; U6 forward, 5'-CTCGCTTCGGCAGCACATATACT-3', and reverse, 5'-ACGCTTCACGAATTTGCGT-GTC-3'; MAFG-AS1 forward, 5'-ATGACGAC-CCCAATAAAGGA-3', and reverse, 5'-CACCGACATGGTTACCAGC-3'.

Luciferase Reporter Gene Assay

The pmirGLO Dual-Luciferase miRNA Target Expression Vector was obtained from Promega (Madison, WI, USA). The wild type 3'-UTR of MMP15 or MAFG-AS1 (wt-MMP15 or wt-MAFG-AS1) was inserted into the vector. The mutated type 3'-UTR of MAFG-AS1 or MMP15 (mut-MAFG-AS1 or mut-MMP15) was produced using the quikChange XL site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA, USA). MAFG-AS1 and miR-339-5p or miR-339-5p and MMP15 were co-transfected into MDA-MB-231 cell using the Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA). The luciferase reporter assay kit (Promega, Madison, WI, USA) was applied to the analysis of the luciferase activity.

Transplanted Tumor Model

100 μ l MDA-MB-231 cells (2×10^7) were subcutaneous injected subcutaneously into nude mice. The tumor size was weekly recorded and the tumor volumes were calculated. Volume = $0.5 \times \text{length} \times \text{width}^2$. After 35 days, all mice were sacrificed and the tumor was extracted for the subsequent immunohistochemical staining analysis.

Experimental Metastasis Model

MAFG-AS1 transfected MDA-MB-231 cell (5×10^5) was injected into BALB/c mice through the tail vein. After two weeks, the mice were sacrificed and lung tissues were applied for H&E staining. All animal experiments were approved

by the Ethics Committee of Tai'an Central Hospital (Shandong, China).

Statistical Analysis

Results were expressed as the Mean $\pm$ SD. The difference was analyzed using either two-tailed Student's *t*-test or one-way ANOVA followed by post-hoc Dunnett's test. $p < 0.05$ was statistically significant.

Results

MAFG-AS1 Is Up-regulated in Breast Carcinoma

The statistical analysis of dysregulation of lncRNAs was conducted using the dataset (GSE113851) which contained both cancer and normal tissues¹². We found that MAFG-AS1 was overexpressed in breast carcinoma tissue in contrast to that in the normal tissue (Figure 1A). Meanwhile, MAFG-AS1 in breast cancer, as well as the adjacent normal tissue, was analyzed. We also observed that its level was significantly

higher in breast carcinoma in contrast to normal (Figure 1B). Meanwhile, MAFG-AS1 was distinctly over-expressed in breast carcinoma cells, including MDA-MB-231, MCF-7, MDA-MB-468, and HCC1937) than that in the normal epithelial breast cell, MCF-10A (Figure 1C). These data suggested that MAFG-AS1 was up-regulated in breast carcinoma.

The Aggressive Phenotypes of Breast Carcinoma was Enhanced by MAFG-AS1

The pcDNA3.1 containing MAFG-AS1 was transfected into the MDA-MB-231 cell to raise MAFG-AS1 level (Figure 2A). Transwell invasion and wound closure analysis were applied to analyze the function of MAFG-AS1 on the MDA-MB-231 cell invasion and migration. We observed that the migration and invasion of MDA-MB-231 cell were enhanced by the transfection of MAFG-AS1 (Figure 2B-2C). At the same time, the aggressiveness of the MCF-7 cell was also promoted by MAFG-AS1 (Figure 2D-2E). In addition, the level of the mesenchymal-related marker, N-cadherin was raised by MAFG-AS1, while MAFG-AS1 in-

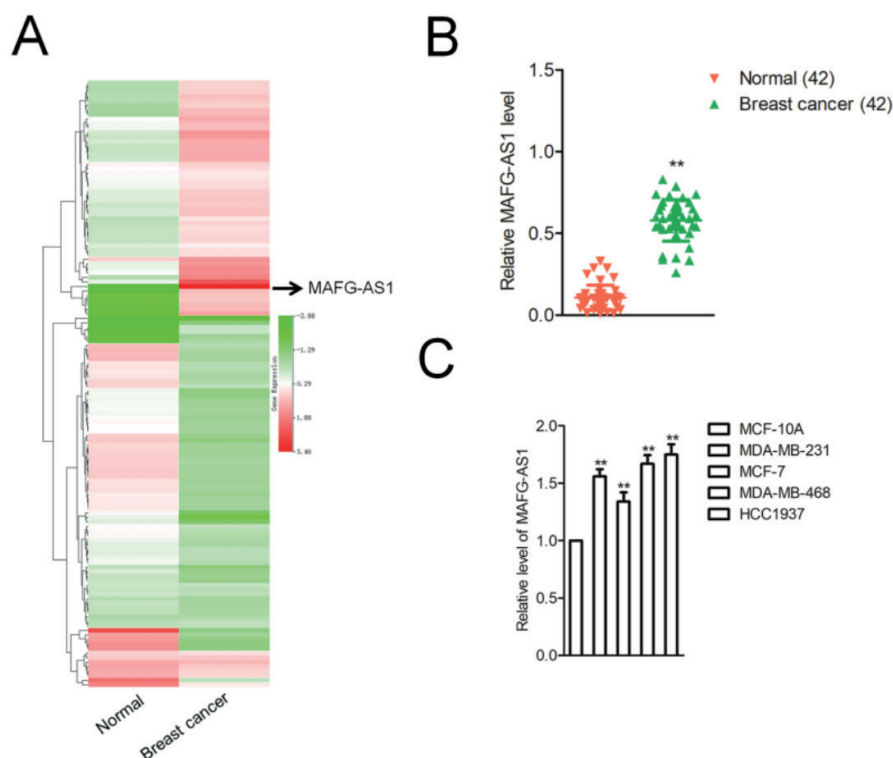


Figure 1. MAFG-AS1 is up-regulated in breast cancer. **A**, Representative down-expressed lncRNAs in breast cancer and non-cancer tissues was shown in the heatmap. **B**, Levels of MAFG-AS1 in breast cancer and normal tissues were detected using qRT-PCR. $**p < 0.01$, compared to normal. **C**, Levels of MAFG-AS1 in breast cancer cells and MCF-10A were analyzed using qRT-PCR assay. $**p < 0.01$, compared to MCF-10A.

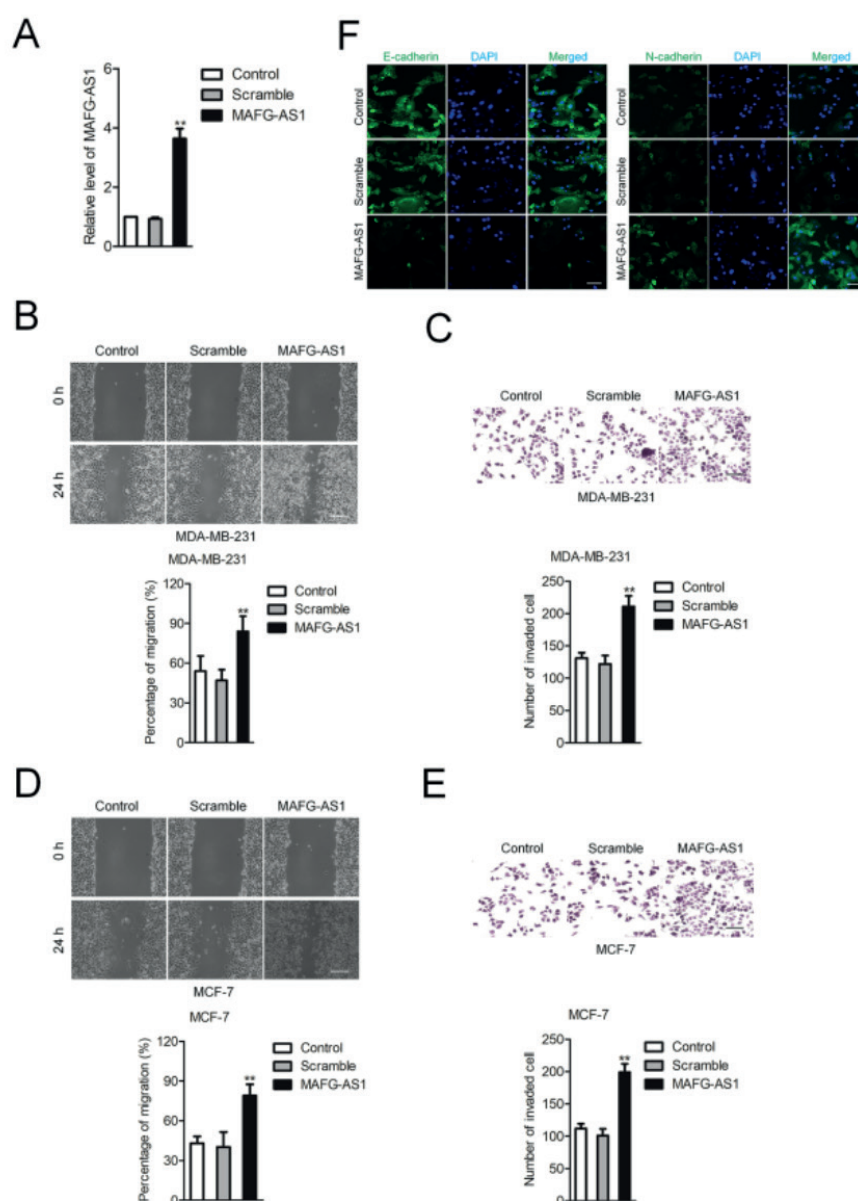


Figure 2. MAFG-AS1 increases the migration, invasion, and EMT of breast cancer cells *in vitro*. **A.** MDA-MB-231 cell was transfected with pcDNA3.1-MAFG-AS1 or Scramble and level of MAFG-AS1 was analyzed using qRT-PCR. **B.** Migration of MDA-MB-231 cell was analyzed using the wound healing test. **C.** The invasion of MDA-MB-231 cell was analyzed using transwell invasion test. **D.** MCF-7 cell was transfected with pcDNA3.1-MAFG-AS1 or Scramble. The migration of MCF-7 cell was analyzed using wound healing test. **E.** Invasion of the MCF-7 cell was detected using transwell invasion test. **F.** Levels of E-cadherin and N-cadherin were detected by Western blotting assay. ** $p < 0.01$, compared to control.

hibited the epithelial-related marker, E-cadherin level in MDA-MB-231 cell (Figure 2F). These data demonstrated the oncogene function of MAFG-AS1 in the MDA-MB-231 cell *in vitro*.

MAFG-AS1 Binds to MiR-339-5p

For the purpose of revealing the potential mechanisms of MAFG-AS1 in the modulation of the development of breast carcinoma, the starBase

analysis tool was adopted to identify the target of MAFG-AS1¹³. Herein, we found that lncRNA MAFG-AS1 was bound with miR-339-5p (Figure 3A). Then, the result of qRT-PCR indicated that miR-339-5p level was significantly lower in breast cancer (Figure 3B-3C). Moreover, MAFG-AS1 negatively regulated the expression of miR-339-5p (Figure 3D). Next, the luciferase reporter gene assay was adopted to verify MAFG-AS1

binding with miR-339-5p. As shown in Figure 3E, miR-339-5p significantly inhibited the luciferase activity in wt 3'-UTR MAFG-AS1 transfected MDA-MB-231 cell, whereas the luciferase activity in mut 3'-UTR MAFG-AS1 transfected MDA-MB-231 was not significantly inhibited. All data implied that MAFG-AS1 inversely modulated the level of miR-339-5p.

MMP15 Is The Target of MiR-339-5p

The TargetScan analysis tool was selected and we disclosed that MMP15 was a possible target gene of miR-339-5p (Figure 4A)¹⁴. Then, the luciferase reporter gene test was confirmed that MMP15 was definitely the precise target of miR-339-5p. MiR-339-5p suppressed luciferase activity in MDA-MB-231 cell transfected with wt 3'-UTR MMP15 (Figure 4B). Meanwhile, we found that the MAFG-AS1 level was inversely correlated to miR-339-5p level in breast cancer tissues

(Figure 4C). Next, we revealed that the MMP15 level was increased by MAFG-AS1 transfection while MMP15 was suppressed in cell co-transfected with lncRNA MAFG-AS1 and miR-339-5p (Figure 4D). Furthermore, the transfection of miR-339-5p reduced the aggressiveness of MDA-MB-231 cell when compared to miR-NC group. Nevertheless, the miR-339-5p combination with MAFG-AS1 cotransfection rescued the aggressiveness of MDA-MB-231 cell compared to the sole only miR-339-5p transfection (Figure 4E and 4F). As expected, the same consequences were found from the assays using the MCF-7 cell (Figure 4G-4H). All results suggested that miR-339-5p modulated MMP15 level in breast carcinoma.

Progression of Breast Carcinoma Cell in Vivo Is Facilitated by MAFG-AS1

We investigated the influence of lncRNA MAFG-AS1 on MDA-MB-231 cell growth in

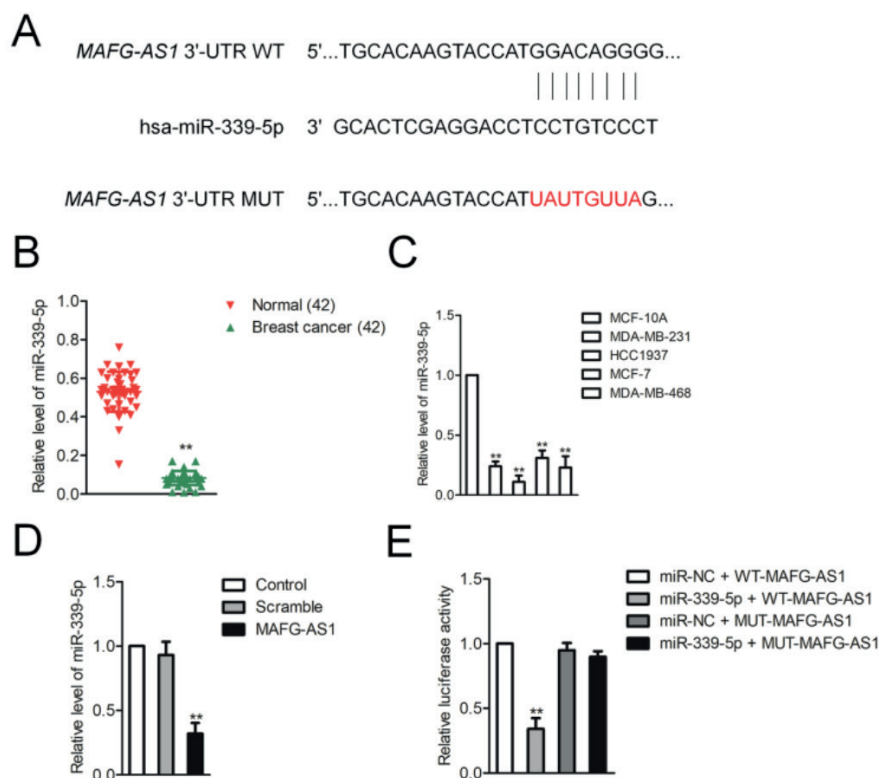


Figure 3. MiR-339-5p is the target of MAFG-AS1. **A.** Bind sites of miR-339-5p between MAFG-AS1 were predicted using the online bioinformatics analysis tool. **B.** Levels of miR-339-5p in breast cancer and normal tissues were detected using qRT-PCR. ** $p<0.01$ compared with normal. **C.** Levels of miR-339-5p in breast cancer cells were measured using qRT-PCR. ** $p<0.01$ compared with MCF-10A. **D.** Level of miR-339-5p in MDA-MB-231 cell which was transfected with MAFG-AS1 alone or cotransfected with MAFG-AS1 in combination with miR-339-5p was detected using qRT-PCR. ** $p<0.01$ compared with control. **E.** Interaction between MAFG-AS1 and miR-339-5p was confirmed by luciferase reporter gene assay. ** $p<0.01$, compared to miR-NC + WT-MAFG-AS1.

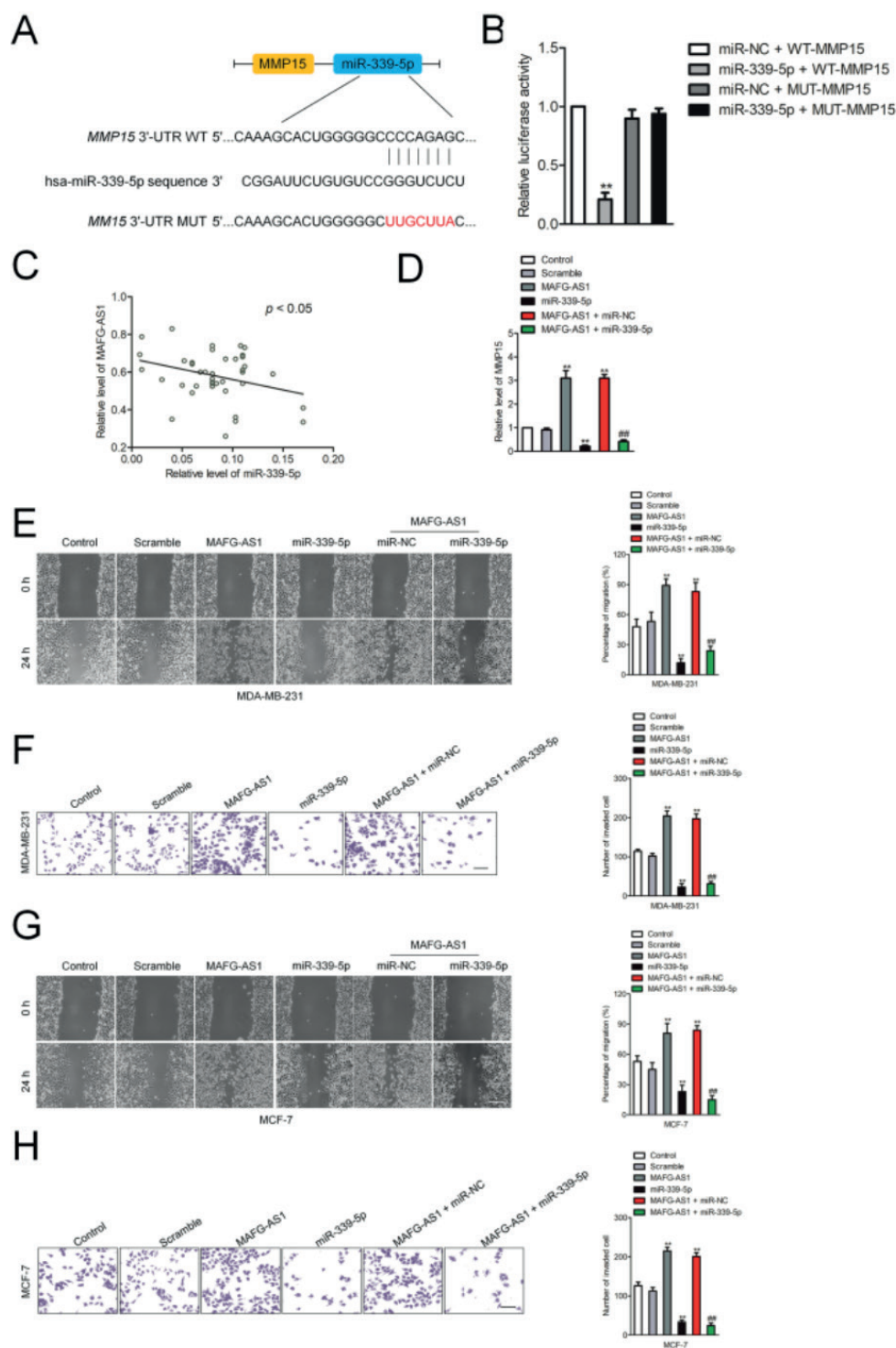


Figure 4. MMP15 is regulated by MAFG-AS1 and miR-339-5p. **A.** Binding sites between miR-339-5p with the 3'-UTR of MMP15 were predicted using the online bioinformatics analysis tool. **B.** Interaction between miR-339-5p and MMP15 was confirmed by the luciferase reporter assay. $^{**}p < 0.01$, compared to miR-NC + WT-MMP15. **C.** Correlation between MAFG-AS1 and miR-339-5p was analyzed using qRT-PCR. **D.** Level of MMP15 in MDA-MB-231 cell was measured by Western blotting. **E.** Migration of MDA-MB-231 cell was analyzed using the wound healing test. **F.** Invasion of MDA-MB-231 cell was analyzed using transwell invasion test. **G.** Migration of MCF-7 cell was measured using the wound healing test. **H.** Invasion of MCF-7 cell was analyzed using the transwell invasion assay. $^{**}p < 0.01$, compared to control, $^{##}p < 0.01$, compared to MAFG-AS1 + miR-NC.

nude mice. As demonstrated in Figure 5A, the growth of MDA-MB-231 *in vivo* was promoted by MAFG-AS1 transfection. Meanwhile, the E-cadherin level was reduced and the level of N-cadherin was raised by the MAFG-AS1 *in vivo*, respectively (Figure 5B). Besides, in contrast to the control, the level of MMP15 was increased, and the miR-339-5p level was reduced in tumor tis-

sue formed by the MAFG-AS1 transfected MDA-MB-231 cell, respectively (Figure 5C-5D). In the experimental lung metastasis model, we observed that lung metastasis of MAFG-AS1 transfected MDA-MB-231 was increased *in vivo* in comparison with control group (Figure 5E-5F). Based on the results, we concluded that MAFG-AS1 facilitated the development of breast carcinoma *in vivo*.

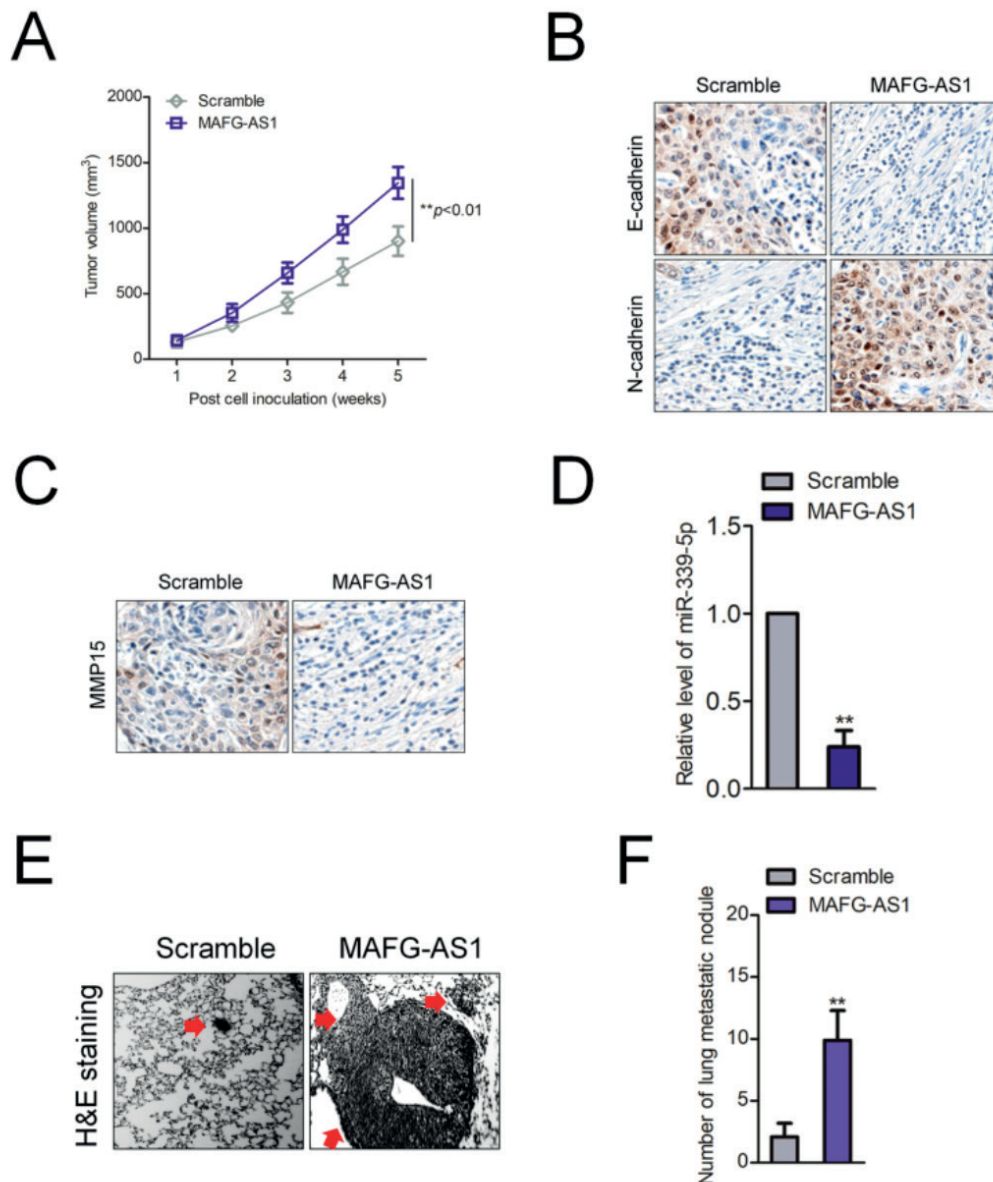


Figure 5. MAFG-AS1 promotes the growth of MDA-MB-231 cell *in vivo*. **A.** MAFG-AS1 transfected MDA-MB-231 cells were inoculated into BALB/c nude mice. The tumor volume was shown. **B.** Levels of N-cadherin and E-cadherin in tumor tissue were detected by immunohistochemical (IHC) staining. **C.** Level of MMP15 in tumor tissue was measured by immunohistochemical (IHC) staining. **D.** Level of miR-300-5p in tumor tissue was analyzed using qRT-PCR. **E.** Representative picture of lung tissue from mice was injected with Scramble or MAFG-AS1 transfected MDA-MB-231 cell. **F.** The number of metastatic nodules was counted and showed. ****** $p < 0.01$, compared to Scramble.

Discussion

The in-depth study of the exact mechanisms of breast cancer cell metastasis can help to improve the prognosis of breast cancer patients^{15,16}. Previous studies¹⁷⁻²⁰ have demonstrated the role of many lncRNAs in cancer cell invasion and migration. We have observed that lncRNA MAFG-AS1 is overexpressed in breast carcinoma cells and tissues. MAFG-AS1 played a vital role in promoting the EMT, invasion and migration of breast carcinoma *via* regulating miR-339-5p-MMP15.

Plenty of lncRNAs have been shown to be overexpressed and serve as carcinogenic factor²¹. Consistent with the previous findings, MAFG-AS1 was significantly up-regulated in breast carcinoma tissue. The similar results were also obtained in breast cancer cell lines. Furthermore, MAFG-AS1 promoted the development of breast cancer. During the metastasis of the cancer cell, EMT is the vital process, in which the epithelial cell transforms into a mesenchymal cell²². Some reports have shown the crucial function of EMT in cancer cell metastasis, and EMT serves as an independent indicator of cancer prognosis²³. The present study demonstrated the facilitating effect of MAFG-AS1 on breast cancer cell invasiveness and migration *in vitro*. Besides, MAFG-AS1 over-expression had an inhibiting and promoting effect on the levels of epithelial and mesenchymal marker, respectively, implying the increasing effect of MAFG-AS1 on breast cancer cell EMT.

MiRNAs play significant roles in regulating pathological processes and other regular physiological activities^{24,25}. In a large number of previous researches, the potential regulating effect of lncRNAs on miRNA level¹⁷ has been detected. In this study, from the bioinformatics, we revealed that the putative target of MAFG-AS1 was miR-339-5p. Besides, in this study, when compared with normal epithelial breast cell and adjacent normal tissues, miR-339-5p was evidently down-regulated in the breast cancer cell and tissue. Furthermore, the miR-339-5p level was largely suppressed by transfection of MAFG-AS1 in the MDA-MB-231 cancer cell. And the binding between lncRNA MAFG-AS1 and miR-339-5p was further verified by the results of the luciferase reporter assay.

Matrix metalloproteinase 15 (MMP15) plays a vital role in breaking down extracellular matrices (ECM) in the process of pathological processes such as cancer cell metastasis and some normal physiological activities, including tissue remodel-

ing, reproduction, embryonic development, etc²⁶. Previous studies have shown that the metastasis and invasion of the cancer cell are inhibited by down-regulation of MMP15²⁷. In this study, both luciferase reporter gene analysis and online bioinformatics analysis indicated that MMP15 gene was a target of miR-339-5p. Besides, based on qRT-PCR and immunoblotting analysis, the level of MMP15 was positively and inversely adjusted by lncRNA MAFG-AS1 and miR-339-5p, respectively. Additionally, miR-339-5p was proved effective in inhibiting the EMT process, invasion, and migration of breast cancer. Consistent with previous findings, we observed that the level of E-cadherin, as well as N-cadherin, was suppressed and promoted by MAFG-AS1, respectively. And MAFG-AS1 also effectively promoted the expression of MMP15 and inhibited miR-339-5p level *in vivo*.

Conclusions

We showed that MAFG-AS1 was up-regulated in breast carcinoma and MAFG-AS1 boosted the aggressive phenotypes of breast cancer *via* modulating miR-339-5p/MMP15.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

- 1) WU ZJ, LI Y, WU YZ, WANG Y, NIAN WO, WANG LL, LI LC, LUO HL, WANG DL. Long non-coding RNA CCAT2 promotes the breast cancer growth and metastasis by regulating TGF-beta signaling pathway. *Eur Rev Med Pharmacol Sci* 2017; 21: 706-714.
- 2) ZHAO J, JIANG GQ. MiR-4282 inhibits proliferation, invasion and metastasis of human breast cancer by targeting Myc. *Eur Rev Med Pharmacol Sci* 2018; 22: 8763-8771.
- 3) LOU KX, LI ZH, WANG P, LIU Z, CHEN Y, WANG XL, CUI HX. Long non-coding RNA BANC1 indicates poor prognosis for breast cancer and promotes cell proliferation and invasion. *Eur Rev Med Pharmacol Sci* 2018; 22: 1358-1365.
- 4) BILL R, CHRISTOFORI G. The relevance of EMT in breast cancer metastasis: correlation or causality? *FEBS Lett* 2015; 589: 1577-1587.
- 5) WAN L, ZHANG L, FAN K, CHENG ZX, SUN QC, WANG JJ. Knockdown of long noncoding RNA PCAT6 inhibits proliferation and invasion in lung cancer cells. *Oncol Res* 2016; 24: 161-170.

- 6) HUANG Y, ZHANG J, HOU L, WANG G, LIU H, ZHANG R, CHEN X, ZHU J. LncRNA AK023391 promotes tumorigenesis and invasion of gastric cancer through activation of the PI3K/Akt signaling pathway. *J Exp Clin Cancer Res* 2017; 36: 194.
- 7) YANG W, NING N, JIN X. The lncRNA H19 promotes cell proliferation by competitively binding to miR-200a and derepressing β -catenin expression in colorectal cancer. *Biomed Res Int* 2017; 2017: 2767484.
- 8) WEI GH, WANG X. LncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. *Eur Rev Med Pharmacol Sci* 2017; 21: 3850-3856.
- 9) CUI S, YANG X, ZHANG L, ZHAO Y, YAN W. LncRNA MAFG-AS1 promotes the progression of colorectal cancer by sponging miR-147b and activation of NDUFA4. *Biochem Biophys Res Commun* 2018; 506: 251-258.
- 10) ZARE M, BASTAMI M, SOLALI S, ALIVAND MR. Aberrant miRNA promoter methylation and EMT-involving miRNAs in breast cancer metastasis: diagnosis and therapeutic implications. *J Cell Physiol* 2018; 233: 3729-3744.
- 11) VANDENBOOM I, TG, LI Y, PHILIP PA, SARKAR FH. MicroRNA and cancer: tiny molecules with major implications. *Curr Genomics* 2008; 9: 97-109.
- 12) POLYCARPOU-SCHWARZ M, GROSS M, MESTDAGH P, SCHOTT J, GRUND SE, HILDENBRAND C, ROM J, AULMANN S, SINN HP, VANDESOMPELE J, DIEDERICH S. The cancer-associated microprotein CASIMO1 controls cell proliferation and interacts with squalene epoxidase modulating lipid droplet formation. *Oncogene* 2018; 37: 4750-4768.
- 13) LI JH, LIU S, ZHOU H, QU LH, YANG JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res* 2014; 42: D92-97.
- 14) LEWIS BP, SHIH IH, JONES-RHOADES MW, BARTEL DP, BURGE CB. Prediction of mammalian microRNA targets. *Cell* 2003; 115: 787-798.
- 15) ZHANG K, ZHANG Y, LIU C, XIONG Y, ZHANG J. MicroRNAs in the diagnosis and prognosis of breast cancer and their therapeutic potential (review). *Int J Oncol* 2014; 45: 950-958.
- 16) BERTOLI G, CAVA C, CASTIGLIONI I. MicroRNAs: New biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. *Theranostics* 2015; 5: 1122-1143.
- 17) XIAO B, ZHANG W, CHEN L, HANG J, WANG L, ZHANG R, LIAO Y, CHEN J, MA Q, SUN Z, LI L. Analysis of the miRNA-mRNA-lncRNA network in human estrogen receptor-positive and estrogen receptor-negative breast cancer based on TCGA data. *Gene* 2018; 658: 28-35.
- 18) JIANG Y, LIN L, ZHONG S, CAI Y, ZHANG F, WANG X, MIAO R, ZHANG B, GAO S, HU X. Overexpression of novel lncRNA NLIPMT inhibits metastasis by reducing phosphorylated glycogen synthase kinase 3 β in breast cancer. *J Cell Physiol* 2019; 234: 10698-10708.
- 19) WANG S, KE H, ZHANG H, MA Y, AO L, ZOU L, YANG Q, ZHU H, NIE J, WU C, JIAO B. LncRNA MIR100HG promotes cell proliferation in triple-negative breast cancer through triplex formation with p27 loci. *Cell Death Dis* 2018; 9: 805.
- 20) LI Z, XU L, LIU Y, FU S, TU J, HU Y, XIONG Q. LncRNA MALAT1 promotes relapse of breast cancer patients with postoperative fever. *Am J Transl Res* 2018; 10: 3186-3197.
- 21) ZHOU CX, WANG X, YANG N, XUE SK, LI WC, XIE PP. LncRNA LET function as a tumor suppressor in breast cancer development. *Eur Rev Med Pharmacol Sci* 2018; 22: 6002-6007.
- 22) XUE Y, ZHANG L, ZHU Y, KE X, WANG Q, MIN H. Regulation of proliferation and epithelial-to-mesenchymal transition (EMT) of gastric cancer by ZEB1 via modulating Wnt5a and related mechanisms. *Med Sci Monit* 2019; 25: 1663-1670.
- 23) CHO ES, KANG HE, KIM NH, YOOK JI. Therapeutic implications of cancer epithelial-mesenchymal transition (EMT). *Arch Pharm Res* 2019; 42: 14-24.
- 24) JIN W, CHEN F, WANG K, SONG Y, FEI X, WU B. MiR-15a/miR-16 cluster inhibits invasion of prostate cancer cells by suppressing TGF- β signaling pathway. *Biomed Pharmacother* 2018; 104: 637-644.
- 25) BANDI N, ZBINDEN S, GUGGER M, ARNOLD M, KOCHER V, HASAN L, KAPPELER A, BRUNNER T, VASSELLA E. MiR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer. *Cancer Res* 2009; 69: 5553-5559.
- 26) PITTAYAPRUEK P, MEEPHANSAN J, PRAPAPAN O, KOMINE M, OHTSUKI M. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *Int J Mol Sci* 2016; 17: pii: E868.
- 27) BODNAR M, SZYLBERG L, KAZMIERCZAK W, MARSZALEK A. Differentiated expression of membrane type metalloproteinases (MMP-14, MMP-15) and pro-MMP2 in laryngeal squamous cell carcinoma. A novel mechanism. *J Oral Pathol Med* 2013; 42: 267-274.