European Review for Medical and Pharmacological Sciences 2020; 24: 1899-1912

Up-regulation of SNHG15 facilitates cell proliferation, migration, invasion and suppresses cell apoptosis in breast cancer by regulating miR-411-5p/VASP axis

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Abstract. – OBJECTIVE: Breast cancer (BC) is an intractable cancer with a rising incidence. Small nucleolar RNA host gene 15 (SNHG15) is a novel biomarker of multiple cancers. However, the molecular mechanism of SNHG15 during oncogenesis of BC is still poorly understood.

MATERIALS AND METHODS: Expression of SNHG15, microRNA (miR)-411-5p and vasodilator stimulated phosphoprotein (VASP) was measured by quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation was evaluated by colony formation and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. Cell apoptosis was determined by flow cytometry and caspase-3 activity assay. Cell migration and invasion were examined by transwell assay. The interaction between miR-411-5p and SNHG15 or VASP was validated by dual-luciferase reporter assay. Protein expression of VASP, B cell lymphoma (Bcl-2), Bcl-2 associated X (Bax), vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP-9, MMP-14) was measured by Western blot. Xenograft mice were established by subcutaneously injecting SKBR-3 cells transfected with sh-SNHG15 and sh-NC.

RESULTS: SNHG15 and VASP were over-expressed whereas miR-411-5p was low-expressed in BC tumors and cells compared with the normal counterparts. Next, SNHG15 knockdown attenuated cell proliferation, migration, invasion and stimulated cell apoptosis in BC. In addition, SNHG15 acted as a sponge while VASP acted as a target of miR-411-5p. Rescue experiment revealed that miR-411-5p inhibitor could alleviate SNHG15 silencing-induced inhibitive effects on cell proliferation, migration, invasion and promotive effects on cell apoptosis. Similarly, VASP attenuated the regulatory effects of SNHG15 silencing on BC cell progression. Furthermore, SNHG15 elimination hindered tumor growth *in vivo*.

CONCLUSIONS: SNHG15 contributes to BC cell progression by sponging miR-411-5p and enhancing VASP expression, providing essential biomarkers for BC therapy.

Key Words:
SNHG15, MiR-411-5p, VASP, Progression, BC.

Introduction

Breast cancer (BC) is a malignant tumor which threatens the health of millions of people, in particular women[1]. The pathogenesis of BC is complicated, including hereditary, smoking, alcohol consumption, late age of pregnancy, no breastfeeding and delayed menopause[2-4]. Despite earlier diagnosis and advanced treatment methods, the 5-year survival rate is still unpleasant[5,6]. Therefore, it is of great importance to disclose the molecular mechanism implicated in BC cell progression.

Long non-coding RNAs (lncRNAs) are fundamental modulators of many pathological processes, such as cell survival, metabolism, differentiation, migration, inflammation and apoptosis[7-9]. Small nucleolar RNA host gene 15 (SNHG15), a long transcript mapped on human chromosome 7p13, was involved in the oncogenesis of various cancers[10]. However, its role was controversial in different cancers. SNHG15 acted as competing

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endogenous RNA (ceRNA) to expedite osteosarcoma cell progression by interacting with miR-141[11]. In addition, SNHG15 improved the aggressiveness of prostate cancer by accelerating cell proliferation and epithelial to mesenchymal transition (EMT) via sponging miR-338-3p and increasing FKBP1A expression[12]. On the contrary, SNHG15 exerted its suppressive function in thyroid cancer to restrain cell growth, migration and invasion[13]. Therefore, the exact role of SNHG15 during the initiation and progression of BC requires in-depth exploration.

MicroRNAs (miRNAs) are signal-stranded transcripts with 17-26 nucleotides in length[14]. MiRNAs could negatively regulate gene expression by interacting with the messenger RNA (mRNA) and leading to mRNA degradation and protein translation repression[15,16]. They were closely linked with carcinogenesis by affecting cell cycle, EMT, migration, autophagy, apoptosis and death[17-19]. For instance, miR-889-3p served as tumor promoter to accelerate cell growth in osteosarcoma by interacting with MNDA[20]. By contrast, miR-202-3p acted as tumor suppressor to repress colorectal carcinoma cell growth by targeting ARL5A[21]. In addition, miR-411 could alleviate the malignancy of gastric cancer by restricting cell viability and migration via regulating SETD6[22]. However, the regulatory effects of miR-411-5p on BC cell development remain unclear.

Owing to the controversial role of SNHG15 in cancer cells, we aimed to disclose the exact regulatory role and molecular mechanism of SNHG15 in BC progression. The expression of SNHG15, miR-411-5p and vasodilator stimulated phosphoprotein (VASP) was measured to reveal their potential role. Rescue experiment was performed to clarify the regulatory effects of SNHG15, miR-411-5p and VASP on NC cell progression. To the best of our knowledge, this is the first study to explore the influence of SNHG15/miR-411-5p/VASP feedback loop on BC cell development.

Materials and Methods

Patient Samples

BC patients (n=35) were recruited from the Affiliated Nanhua Hospital, University of South China. Those patients have not received pre-operative therapies, including chemotherapy, radiotherapy and immunotherapy. Fresh BC tumors and normal tissues were collected from the participants by surgery. All the patients have signed informed consents and the protocols have been approved by Ethics Committee of Affiliated Nanhua Hospital, University of South China.

Cell Transfection

BT-474, MDA-MB-468, SKBR-3, MCF-7 cells and human breast epithelial cells MCF-10A were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in complete Dulbecco's Modified Eagle's Medium (DMEM) medium (Gibco, Rockville, MD, USA). Small harboring RNA targeting SNHG15 (sh-SNHG15), negative control (sh-NC), pcDNA, SNHG15 and VASP overexpression vectors were synthesized by Genepharma (Shanghai, China). MiR-411-5p, miR-411-5p inhibitor (anti-miR-411-5p), negative control (miR-NC) and negative control inhibitor (anti-miR-NC) were purchased from RIBOBIO (Guangzhou, China). The vectors were transfected into SKBR-3 and MCF-7 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The tissues and the cells were re-suspended in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) to extract total RNA. The cDNA for SNHG15, miR-411-5p and VASP was synthesized by All-in-One™ Kit (FulenGen, Guangzhou, China). Next, SYBR green (Applied Biosystems, Foster City, CA, USA) was exploited for qRT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were employed as internal references. The primers for SNHG15, miR-411-5p, VASP, GAPDH and U6 were listed: SNHG15, (Forward, 5'-TTGTGAAGCCCAGTGAAAGTACTGC-3'; Reverse, 5'-TTCACTGTGGAGACTGTCGTTGGT-3'); miR-411-5p, (Forward, 5'-CCGGAACCCCCTCCTTACTC-3'; Reverse, 5'-AATGGGATGTGTCCGAAGGA-3'); VASP (Forward, 5'-CTGGGAGAAGAACAGCACAACC-3'; Reverse, 5'-CTGGGAGAAGAACAGCACAACC-3'); GAPDH, (Forward, 5'-AGGTCGGTGTGAACGGATTTG-3'; Reverse, 5'-GGGGTCGTTGATGGCAACA-3'); U6, (Forward, 5'-ACCCTGAGAAATACCCTCACAT-3'; Reverse, 5'-GACGACTGAGCCCCTGATG-3').

Colony Formation

Transfected BC cells were mixed with top agar and seeded on the base agar in 6-well plates. After 2 weeks' incubation, the cells were stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis,

MO, USA). Finally, the colonies ≥ 0.5 mm were analyzed by a microscope.

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay

Transfected BC cells were inoculated in 96-well plates. Then, the cells were incubated for 24 h, 48 h and 72 h and reacted with 10 μL MTT (Beyotime, Shanghai, China) for 4 h. Next, 100 μL dimethyl sulfoxide (DMSO) (Sigma-Aldrich) was added in the cells for 2 h. Finally, the optical density (OD) value at 490 nm was detected by a spectrophotometer.

Flow Cytometry

Transfected BC cells were inoculated in 24-well plates. After incubating for 48 h, the cells were collected and stained by fluorescein isothiocyanate tagged Annexin V (Annexin V-FITC)/ propidium iodide (PI) detection kit (Invitrogen). Then, the cells were washed for 3 times and the apoptotic rate was analyzed by a flow cytometer.

Western Blot

Proteins VASP, B cell lymphoma (Bcl-2), Bcl-2 associated X (Bax), vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMP-9, MMP-14) and GAPDH were extracted from BC cells. The proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking by 5% nonfat milk, the membranes were incubated with primary antibodies against VASP, Bcl-2, Bax, VEGF, MMP-9, MMP-14 and GAPDH (Abcam, Cambridge, MA, USA) and horseradish peroxidase (HRP)-conjugated secondary antibody (Sangon, Shanghai, China).

Detection of Caspase-3 Activity

Caspase-3 activity of transfected BC cells was detected by caspase-3 activity kit (Beyotime). In brief, transfected BC cells were plated on 24-well plates and incubated for 48 h. Later, the cells were collected, and caspase-3 activity was determined by caspase-3 activity kit following the manufacturer's instructions.

Transwell Assay

For cell migration, transfected BC cells were inoculated on the upper chamber of transwell (Corning, Corning, NY, USA). For invasion assay, transfected BC cells were inoculated on the upper chamber pre-treated with Matrigel (Sigma-Aldrich). After migrating or invading for 48 h, the cells at the lower chamber of transwell were stained with 0.1% crystal violet (Sigma-Aldrich). Lastly, the migration and invasion rate were analyzed by a microscope.

Dual-Luciferase Reporter Assay

The interaction between miR-411-5p and SNHG15 or VASP was certified by dual-luciferase reporter assay. In brief, wild type SNHG15 (SNHG15-WT) and mutant type SNHG15 (SNHG15-MUT) luciferase vectors were constructed. Meanwhile, wild type VASP (VASP 3'UTR-WT) and mutant type VASP (VASP 3'UTR-MUT) luciferase vectors were constructed. Next, they were co-transfected in BC cells with miR-411-5p or miR-NC to construct dual-luciferase system. Luciferase activities were determined using a luminometer.

Establishment of Xenograft Mice

Female nude mice of 5-week old were purchased from Jinan Pengyue Animal Breeding Center (Jinan, China). Animal experiment was divided into two groups: sh-SNHG15 and sh-NC group (n=6). The xenograft mice were established by subcutaneously injecting SKBR-3 cells stably transfected with sh-SNHG15 or sh-NC. To detect the expression of SNHG15, miR-411-5p and VASP in tumor sites, the mice were sacrificed, and the tumor tissues were collected after 27 days measurement of tumor volume. All the animal experiment protocols were approved by the National Animal Care and Ethics Institution and Ethics Committee of Affiliated Nanhua Hospital, University of South China.

Statistical Analysis

All the data were presented as means ± standard deviation (SD). Statistical analysis was performed by GraphPad Prism 7 (San Diego, CA, USA). The correlation between miR-411-5p and SNHG15 or VASP was analyzed by Pearson's correlation coefficient. p-value less than 0.05 ($p<0.05$) was considered statistically significant.

Results

Up-Regulation of SNHG15 in BC

The expression of SNHG15 was evaluated by qRT-PCR to explore the role of SNHG15 during BC tumorigenesis and development. As illustrat-

ed in Figure 1A, SNHG15 expression was up-regulated in BC tumors compared with the adjacent normal tissues. Meanwhile, SNHG15 expression was evidently higher in BC cells (BT-474, MDA-MB-468, SKBR-3, MCF-7) than that of human breast epithelial cells MCF-10A, especially SKBR-3 and MCF-7 cells (Figure 1B). Hence, SKBR-3 and MCF-7 cells were exploited for the following experiments. In short, overexpression of SNHG15 in BC indicated the oncogenic role of SNHG15 in BC.

Elimination of SNHG15 Inhibited Cell Proliferation and Stimulated Cell Apoptosis in BC

Loss-of-function experiment was conducted to elucidate the function of SNHG15 in BC. Firstly, SKBR-3 and MCF-7 cells were stably transfected with sh-NC and sh-SNHG15. Next, qRT-PCR was performed to detect the transfection efficiency. Apparently, SNHG15 expression was reduced in SKBR-3 and MCF-7 cells transfected with sh-SNHG15 compared with sh-NC, revealing the transfection efficiency was high (Figure 2A). The colony formation result exhibited that the number of colonies was decreased by SNHG15 silencing (Figure 2B). What's more, inhibition of SNHG15 weakened cell viability while strengthened cell apoptosis in BC (Figure 2C-D). The influences of SNHG15 on cell apoptosis were further assessed by Western blot and caspase-3 activity detection assay. As expected, pro-apoptotic protein Bax was boosted whereas anti-apoptotic protein Bcl-2 was blocked by SNHG15 silencing (Figure 2F-G). Enhanced caspase-3 activity in SKBR-3 and MCF-7 cells transfected with sh-SNHG15 indicated SNHG15 knockdown boosted BC cell apoptosis (Figure 2H). Taken together, SNHG15 depletion restricted BC cell growth.

SNHG15 Knockdown Weakened Migration and Invasion of BC Cells

Transwell and Western blot were further conducted to investigate the importance of SNHG15 in BC cell migration and invasion. Transwell result revealed that depletion of SNHG15 displayed negative effects on cell migration and invasion (Figure 3A-B). In addition, the expression of migration and invasion associated proteins VEGF, MMP-9 and MMP-14 was declined by SNHG15 silencing (Figure 3C-D). All the data clarified that the lack of SNHG15 attenuated migration and invasion of BC cells.

SNHG15 Acted as a Sponge of miR-411-5p

According to prediction by starBase, there were potential binding sites between SNHG15 and miR-411-5p (Figure 4A). To validate the prediction, dual-luciferase reporter system was established by co-transfecting with SNHG15-WT or SNHG15-MUT and miR-411-5p or miR-NC in SKBR-3 and MCF-7 cells. As displayed in Figure 4B-C, luciferase activity of SKBR-3 and MCF-7 cells transfected with miR-411-5p was inhibited by SNHG15-WT compared with SNHG15-MUT. Furthermore, the expression of miR-411-5p was

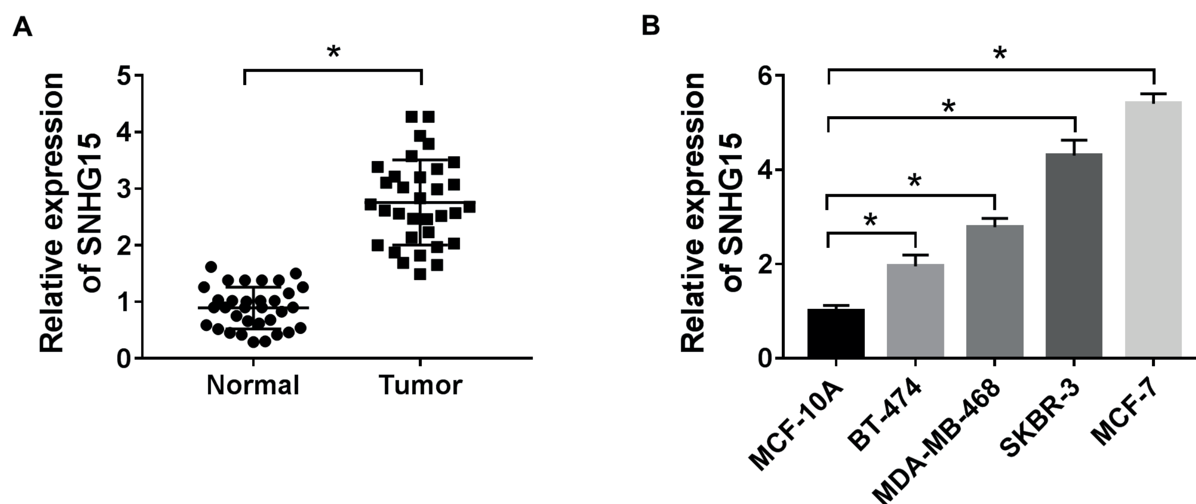

Figure 1. SNHG15 was up-regulated in BC. **(A)** SNHG15 expression in BC tumors and normal tissues was measured by qRT-PCR. **(B)** SNHG15 expression in BC cells (BT-474, MDA-MB-468, SKBR-3, MCF-7) and human breast epithelial cells MCF-10A was detected by qRT-PCR. *$p<0.05$.

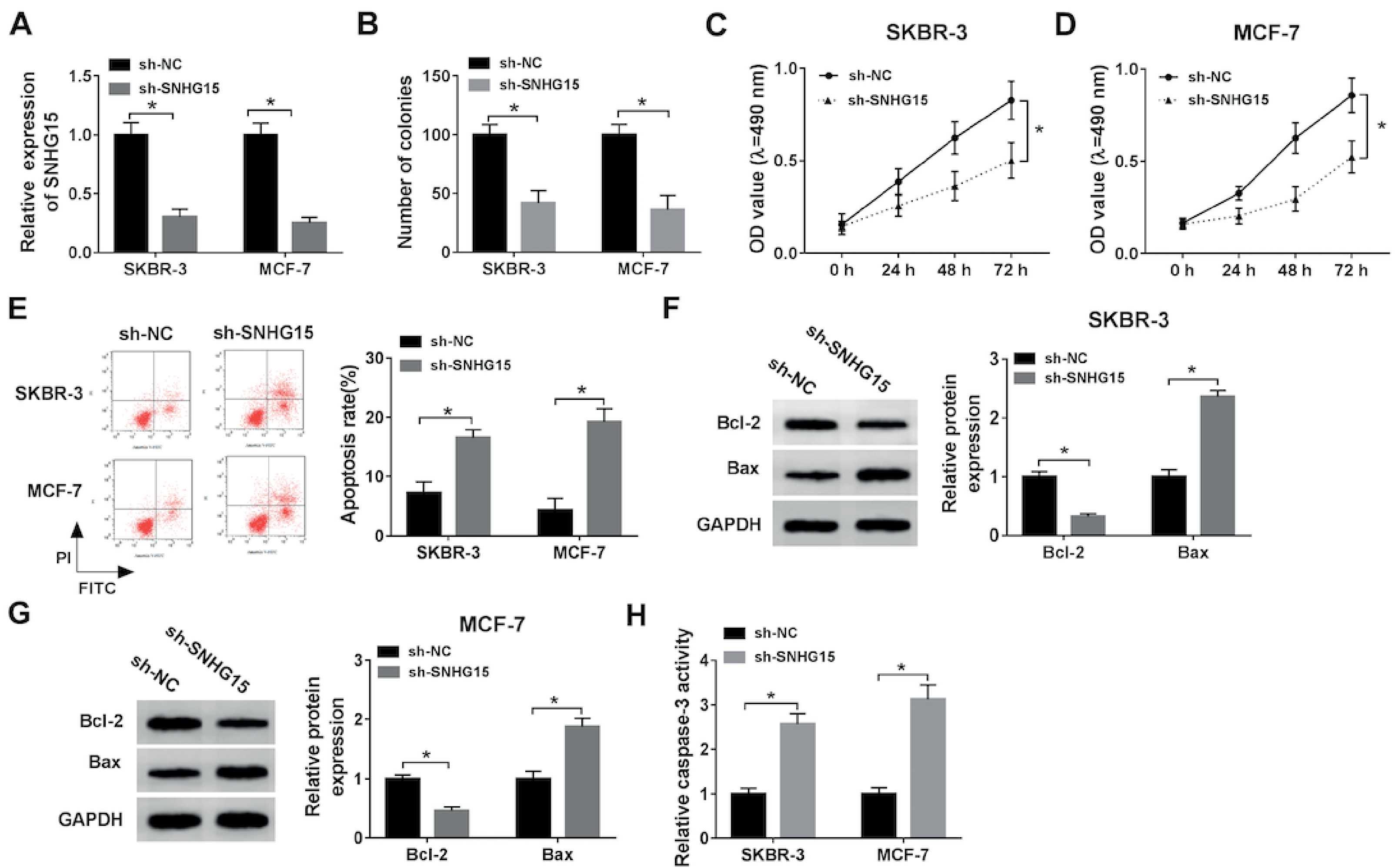

Figure 2. SNHG15 knockdown repressed cell proliferation and induced cell apoptosis in BC. SKBR-3 and MCF-7 cells were stably transfected with sh-NC and sh-SNHG15. (A) SNHG15 expression in transfected SKBR-3 and MCF-7 cells was evaluated by qRT-PCR. (B) The number of colonies was examined by colony formation assay. (C-D) Cell viability was measured by MTT assay. (E) Cell apoptosis was analyzed by flow cytometry. (F-G) Protein expression of Bcl-2 and Bax was evaluated by Western blot. (H) Caspase-3 activity was measured by caspase-3 activity kit. *$p<0.05$.

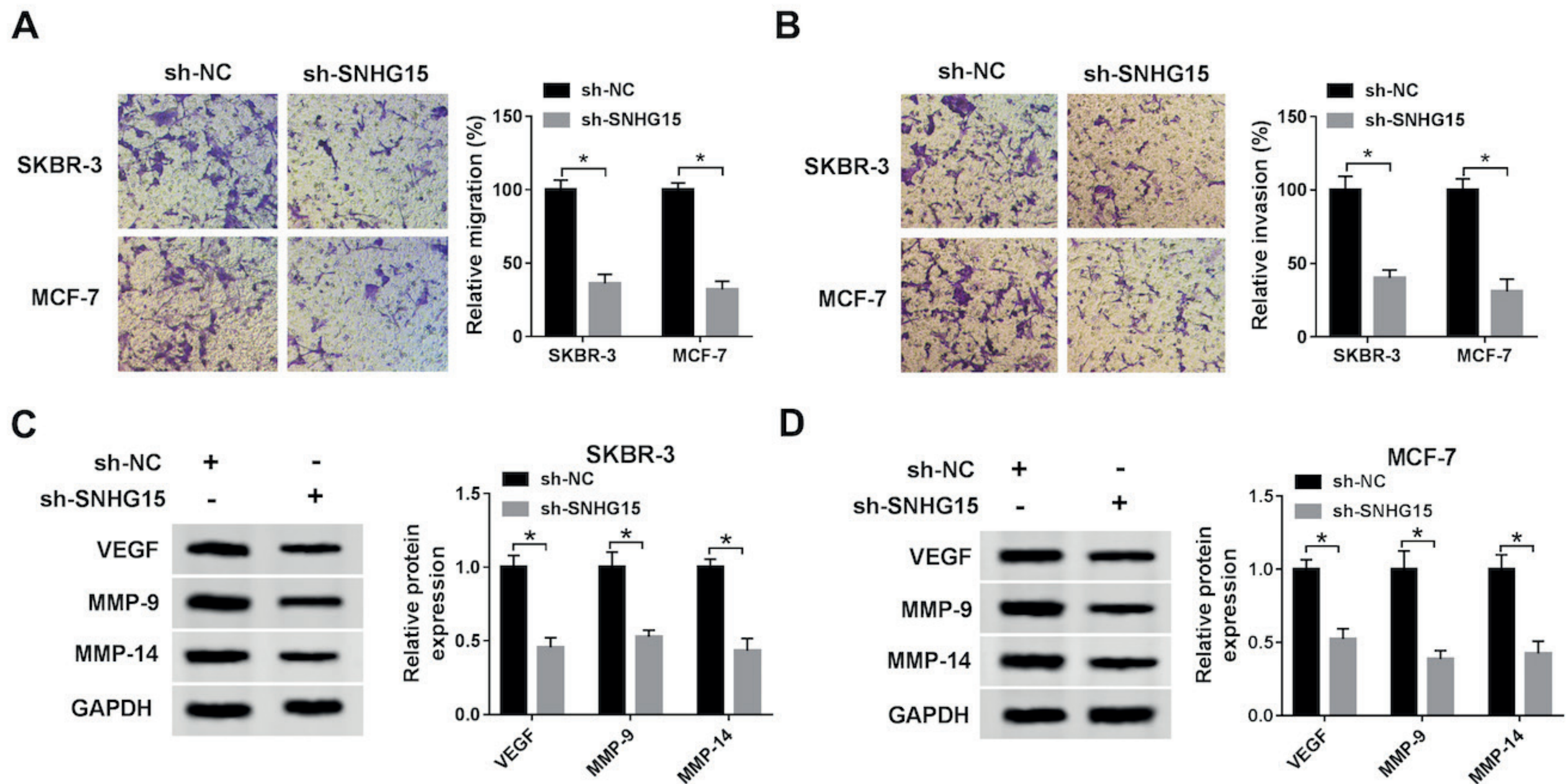

Figure 3. SNHG15 depletion restrained cell migration and invasion in BC**.** SKBR-3 and MCF-7 cells were stably transfected with sh-NC or sh-SNHG15. **(A-B)** Cell migration and invasion were examined by transwell assay (100×). **(C-D)** Protein expression of VEGF, MMP-9 and MMP-14 was analyzed by Western blot. *p<0.05.

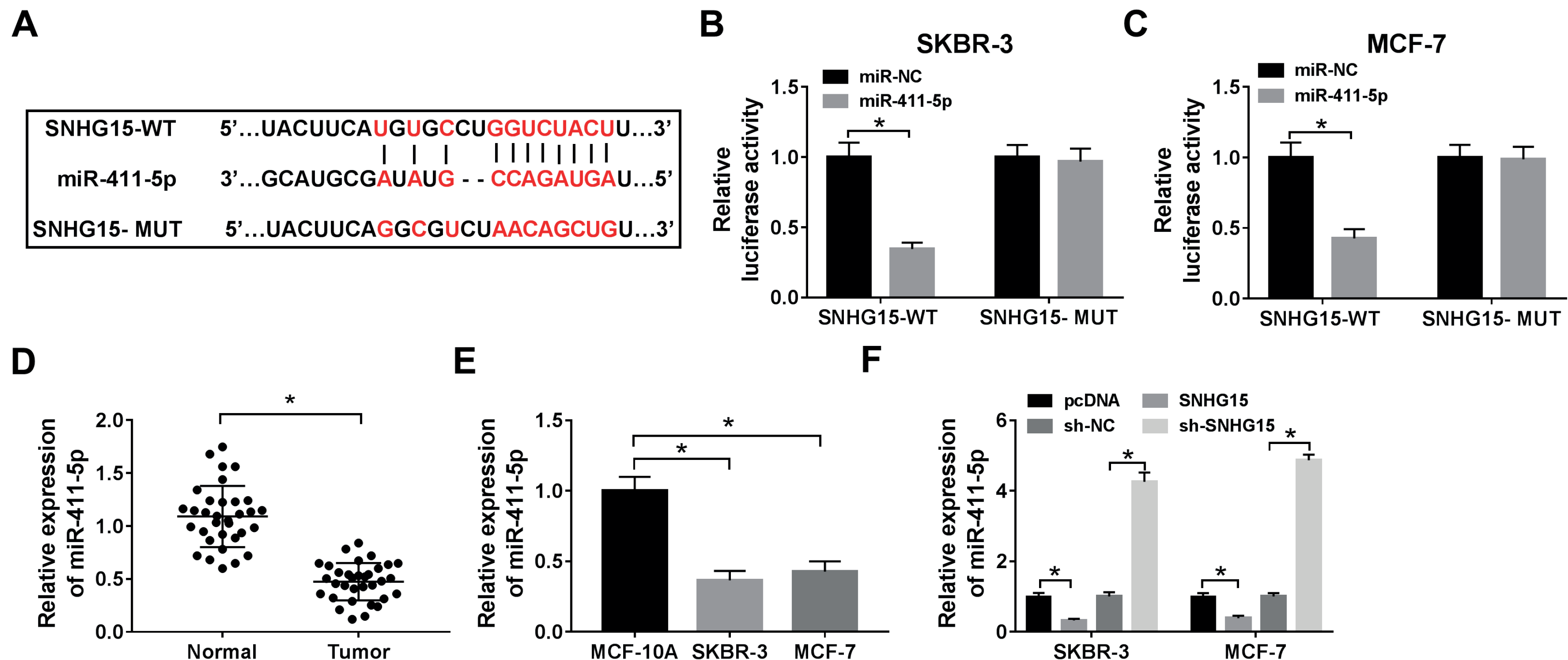

Figure 4. SNHG15 directly interacted with miR-411-5p. (A) The putative binding sites between SNHG15 and miR-411-5p were predicted by starBase. (B-C) Luciferase activity of SKBR-3 and MCF-7 cells co-transfected with SNHG15-WT or SNHG15-MUT and miR-411-5p or miR-NC was determined by dual-luciferase reporter assay. (D-E) The expression of miR-411-5p in BC tumors and cells compared with normal tissues and cells was assessed by qRT-PCR. (F) The expression of miR-411-5p in SKBR-3 and MCF-7 cells transfected with pcDNA, SNHG15, sh-NC and sh-SNHG15. *p<0.05.

down-regulated in BC tumors and cells in comparison with their counterparts (Figure 4D-E). Besides, the expression of miR-411-5p was decreased by SNHG15 overexpression while increased by SNHG15 depletion (Figure 4F). Altogether, SNHG15 could regulate the expression of miR-411-5p in BC.

SNHG15 Modulated Cell Proliferation, Apoptosis, Migration and Invasion by Sponging miR-411-5p in BC

To elucidate the regulatory effects of SNHG15/miR-411-5p axis on BC cell progression, SKBR-3 and MCF-7 cells were transfected with sh-NC, sh-SNHG15, sh-SNHG15+anti-miR-NC and sh-SNHG15+anti-miR-411-5p. The expression of miR-411-5p was elevated by SNHG15 silencing and restrained by miR-411-5p inhibitor (Figure 5A-B). In addition, the abundance of miR-411-5p inhibited whereas the absence of miR-411-5p facilitated the colony formation (Figure 5C). Interestingly, miR-411-5p inhibitor rescued SNHG15 silencing-mediated suppression on cell proliferation (Figure 5D-E) and promotion on cell apoptosis (Figure 5F) in BC. As expected, the expression of Bax was enhanced by SNHG15 silencing and reduced by miR-411-5p inhibitor. However, the expression of Bcl-2 exhibited the opposite trend (Figure 5G-H). Meanwhile, caspase-3 activity was boosted by SNHG15 silencing and blocked by miR-411-5p inhibitor (Figure 5I). Moreover, cell migration and invasion were inhibited by SNHG15 silencing. However, the inhibitive effects were inversed by miR-411-5p inhibitor (Figure 5J-K). Consistently, the expression of protein VEGF, MMP-9 and MMP-14 was blocked by SNHG15 silencing and the blockage was reversed by miR-411-5p inhibitor (Figure 5L-M). Therefore, miR-411-5p inhibitor could abolish SNHG15 silencing-induced regulatory effects on BC cell proliferation, migration, invasion and apoptosis.

Identification of the Interaction between VASP and miR-411-5p

As predicted by bioinformatics analysis tool starBase, miR-411-5p comprised the binding sites of VASP (Figure 6A). Reduction of luciferase activity in SKBR-3 and MCF-7 cells co-transfected with VASP 3'UTR-WT and miR-411-5p certified the interaction between VASP and miR-411-5p (Figure 6B-C). Then, we observed that the expression of VASP mRNA and protein was relatively higher in BC tumors and cells than that of normal tissues (Figure 6D-E) and cells (Figure 6F-G). Moreover, miR-411-5p inhibitor counteracted SNHG15 silencing-induced inhibition in VASP mRNA and protein expression in BC (Figure 6H-K). After analyzing by Pearson's correlation coefficient, we discovered that miR-411-5p was negatively correlated with SNHG15 (R=-0.59, $p<0.0001$) or VASP (R=-0.68, $p<0.0001$) (Figure 6L-M). By contrast, SNHG15 was positively correlated with VASP (R=0.60, $p<0.0001$) (Figure 6N). These findings demonstrated that SNHG15 could enhance VASP expression by interacting with miR-411-5p in BC.

Increased Expression of VASP Abrogated SNHG15 Deficiency-Induced Regulatory Effects on BC Cell Proliferation, Migration, Invasion and Apoptosis

To disclose the molecular mechanism of BC progression, SKBR-3 and MCF-7 cells were transfected with sh-NC, sh-SNHG15, sh-SNHG15+p-cDNA and sh-SNHG15+VASP. The expression of VASP mRNA and protein was inhibited by SNHG15 silencing and promoted by VASP (Figure 7A-C). Interestingly, VASP alleviated SNHG15 silencing-mediated repression on colony formation (Figure 7D). Consistently, restoration of VASP neutralized SNHG15 silencing-induced suppression on cell proliferation (Figure 7E-F), migration, invasion (Figure 7K-L) and promotion on apoptosis (Figure 7G). Western blot result showed that the absence of SNHG15 expedited the expression of Bax and blocked the expression of Bcl-2. However, VASP reversed the regulatory effects on the expression of protein Bcl-2 and Bax (Figure 7H-I). What's more, abundance of VASP accelerated while deficiency of SNHG15 inhibited protein expression of VEGF, MMP-9 and MMP-14 in BC (Figure 7M-N). The results clarified that SNHG15 facilitated proliferation, migration, invasion and weakened apoptosis of BC cells by increasing VASP expression.

Interference of SNHG15 Hindered Tumor Growth in Vivo by Regulating miR-411-5p/VASP Axis

Tumor-bearing mice were established by subcutaneously injecting SKBR-3 cells stably transfected with sh-SNHG15 and sh-NC to evaluate the effects of SNHG15 on tumor growth *in vivo*. As shown in Figure 8A-B, tumor growth was attenuated by SNHG15 deficiency. In addition, the expression of SNHG15 was reduced while miR-411-5p was enhanced in tumors harvested from sh-SNHG15 transfection mice compared with sh-

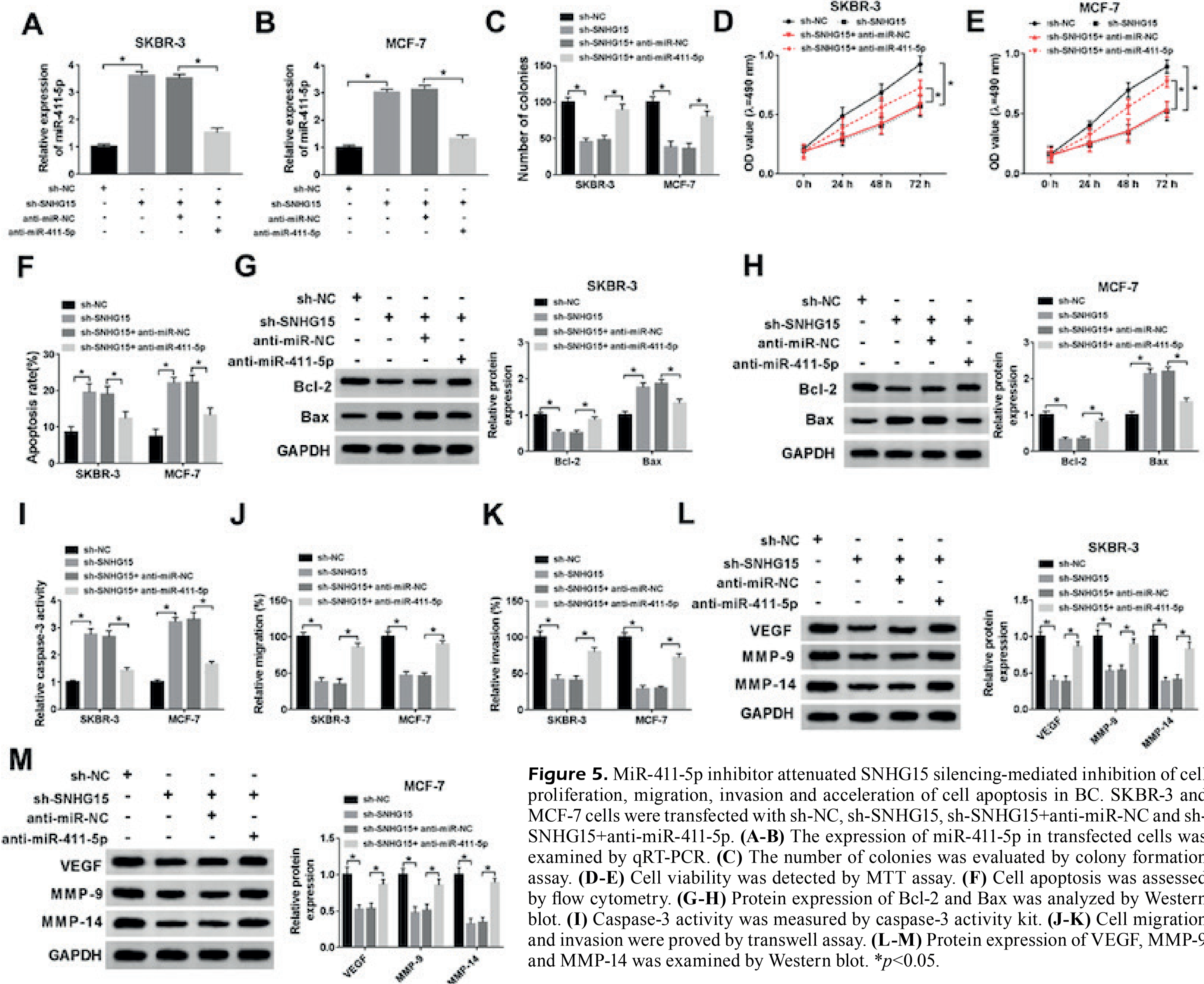

Figure 5. MiR-411-5p inhibitor attenuated SNHG15 silencing-mediated inhibition of cell proliferation, migration, invasion and acceleration of cell apoptosis in BC. SKBR-3 and MCF-7 cells were transfected with sh-NC, sh-SNHG15, sh-SNHG15+anti-miR-NC and sh-SNHG15+anti-miR-411-5p. **(A-B)** The expression of miR-411-5p in transfected cells was examined by qRT-PCR. **(C)** The number of colonies was evaluated by colony formation assay. **(D-E)** Cell viability was detected by MTT assay. **(F)** Cell apoptosis was assessed by flow cytometry. **(G-H)** Protein expression of Bcl-2 and Bax was analyzed by Western blot. **(I)** Caspase-3 activity was measured by caspase-3 activity kit. **(J-K)** Cell migration and invasion were proved by transwell assay. **(L-M)** Protein expression of VEGF, MMP-9 and MMP-14 was examined by Western blot. *p<0.05.

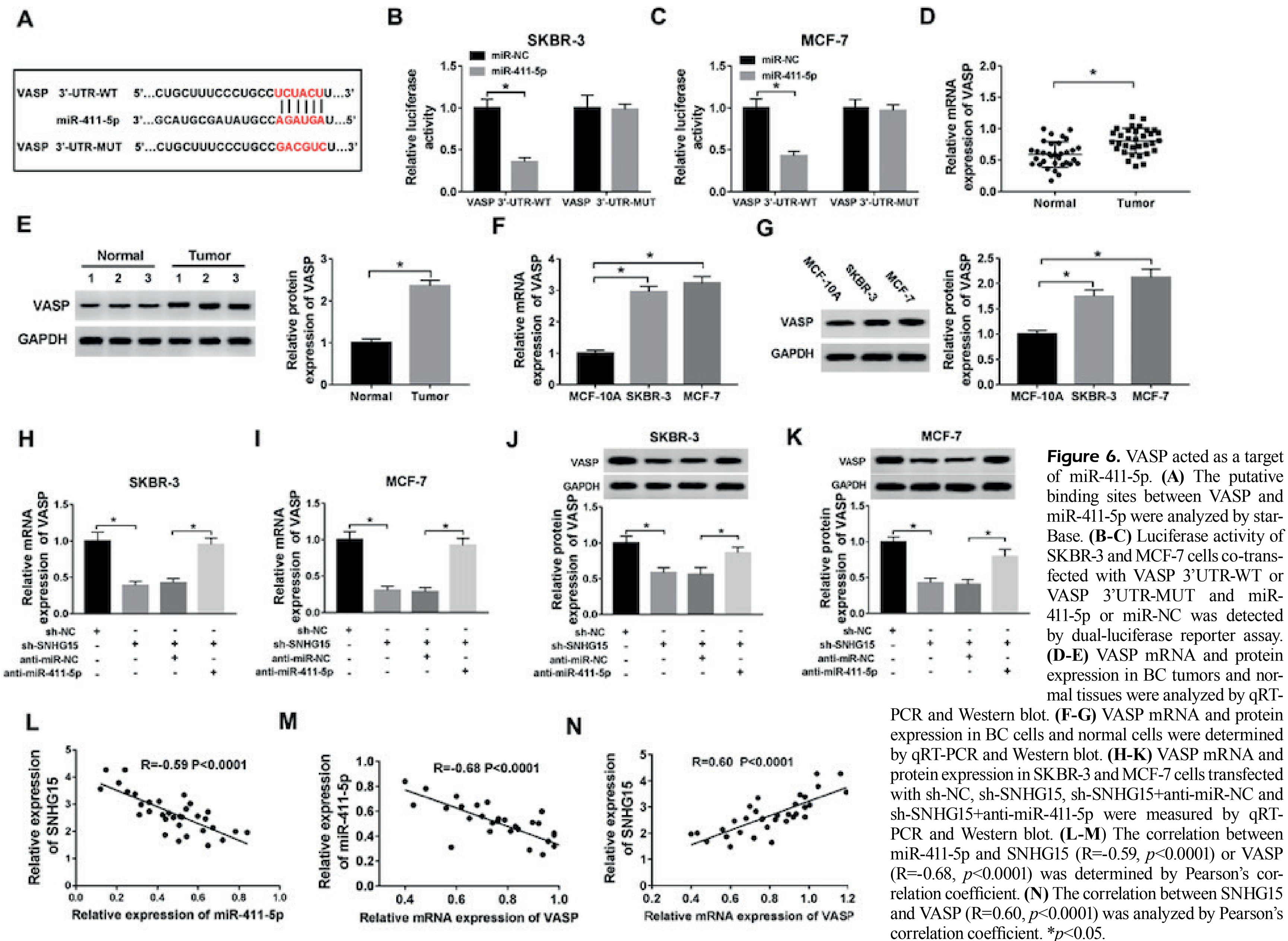

Figure 6. VASP acted as a target of miR-411-5p. **(A)** The putative binding sites between VASP and miR-411-5p were analyzed by starBase. **(B-C)** Luciferase activity of SKBR-3 and MCF-7 cells co-transfected with VASP 3'UTR-WT or VASP 3'UTR-MUT and miR-411-5p or miR-NC was detected by dual-luciferase reporter assay. **(D-E)** VASP mRNA and protein expression in BC tumors and normal tissues were analyzed by qRT-PCR and Western blot. **(F-G)** VASP mRNA and protein expression in BC cells and normal cells were determined by qRT-PCR and Western blot. **(H-K)** VASP mRNA and protein expression in SKBR-3 and MCF-7 cells transfected with sh-NC, sh-SNHG15, sh-SNHG15+anti-miR-NC and sh-SNHG15+anti-miR-411-5p were measured by qRT-PCR and Western blot. **(L-M)** The correlation between miR-411-5p and SNHG15 (R=-0.59, p<0.0001) or VASP (R=-0.68, p<0.0001) was determined by Pearson's correlation coefficient. **(N)** The correlation between SNHG15 and VASP (R=0.60, p<0.0001) was analyzed by Pearson's correlation coefficient. *p<0.05.

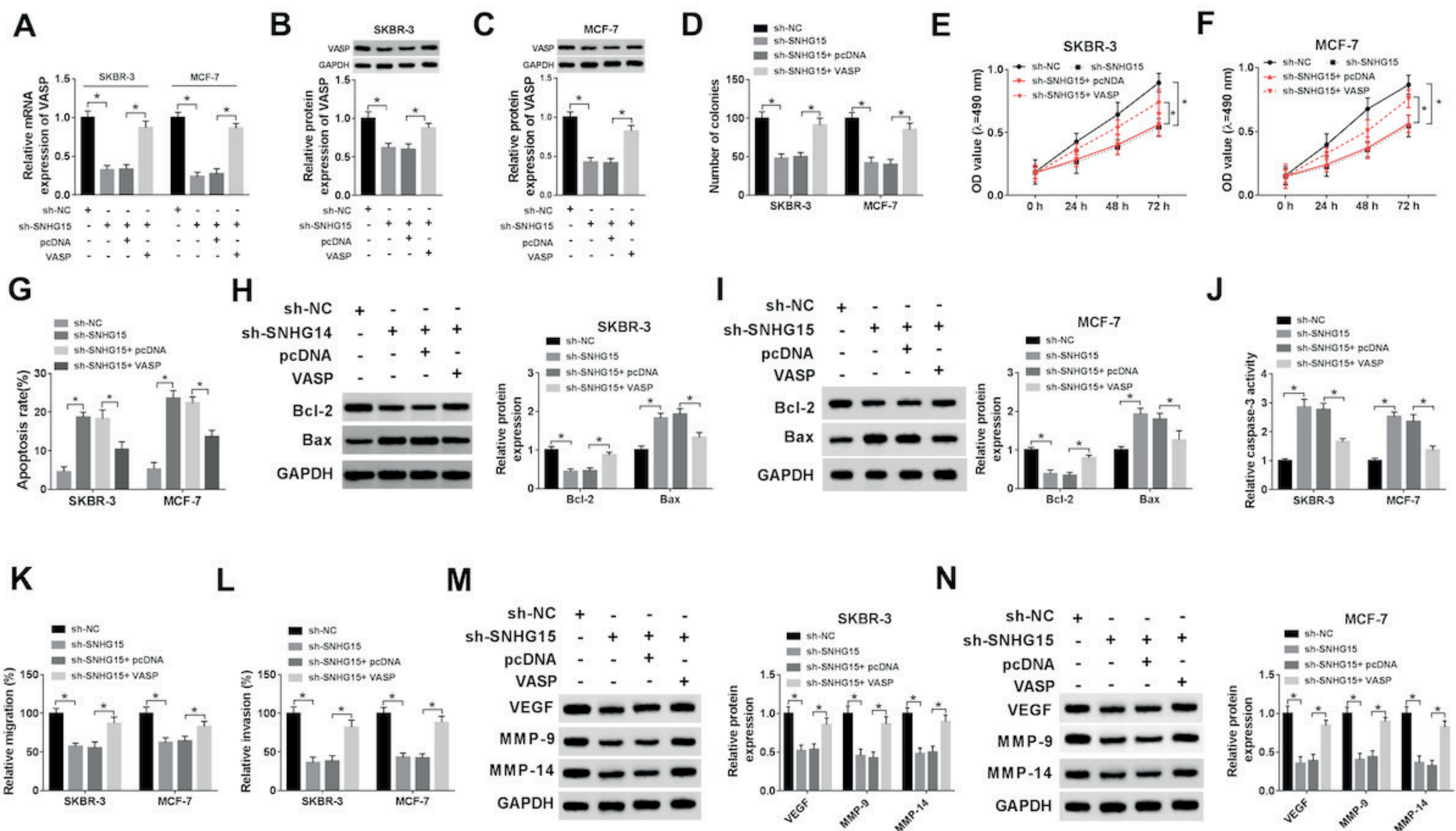

Figure 7. VASP restored SNHG15 silencing-mediated repression on proliferation, migration, invasion and promotion on apoptosis of BC cells. SKBR-3 and MCF-7 cells were transfected with sh-NC, sh-SNHG15, sh-SNHG15+pcDNA and sh-SNHG15+VASP. **(A-C)** The expression VASP mRNA and protein in transfected cells was assessed by qRT-PCR and Western blot. **(D)** The number of colonies was analyzed by colony formation assay. **(E-F)** Cell viability was examined by MTT assay. **(G)** Cell apoptosis was evaluated by flow cytometry. **(H-I)** Protein expression of Bcl-2 and Bax was detected by Western blot. **(J)** Caspase-3 activity was examined by caspase-3 activity kit. **(K-L)** Cell migration and invasion were measured by transwell assay. **(M-N)** Protein expression of VEGF, MMP-9 and MMP-14 was determined by Western blot. *$p<0.05$.

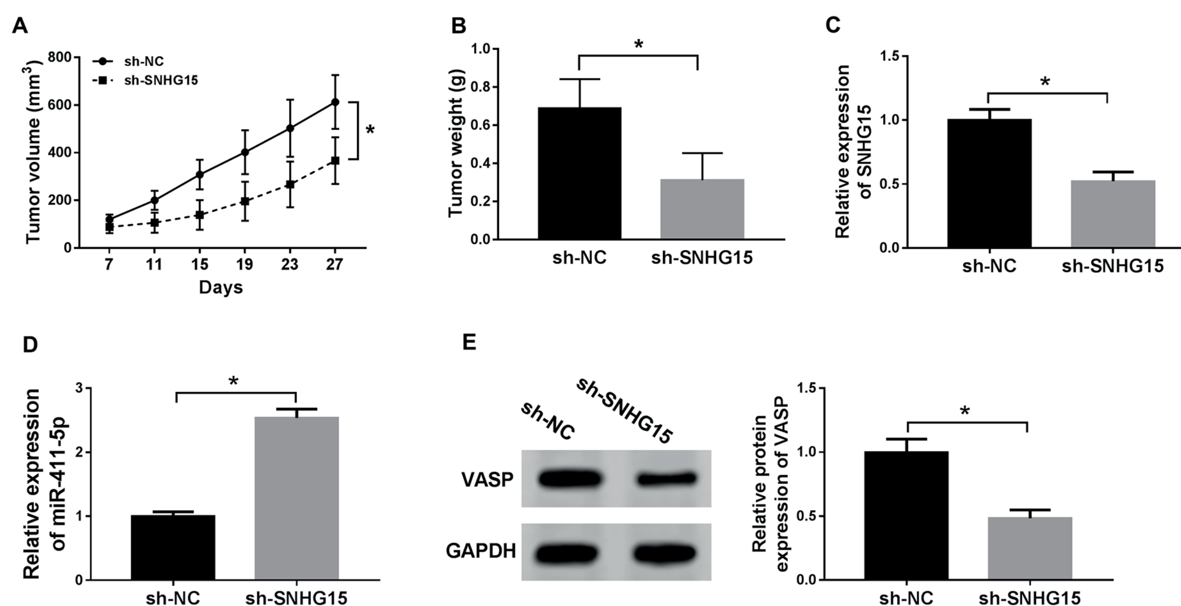

Figure 8. SNHG15 knockdown retarded tumor growth *in vivo*. **(A)** Tumor volume was measured every four days. **(B)** Tumor weight was measured at day 27. **(C-D)** SNHG15 and miR-411-5p expression in tumors collected from the xenograft mice were detected by qRT-PCR. **(E)** VASP protein expression in tumors collected from the xenograft mice was analyzed by Western blot. $^*p<0.05$.

NC group (Figure 8C-D). Meanwhile, decreased expression of VASP protein was observed in tumors collected from sh-SNHG15 transfection mice (Figure 8E). Hence, we considered that SNHG15 knockdown could retard tumor growth *in vivo* by regulating miR-411-5p/VASP axis.

Discussion

Numerous evidences demonstrated that SNHG15 was closely associated with cancer cell survival, invasion, EMT, autophagy and death[23,24]. SNHG15 acted as an oncogene in colorectal carcinoma to facilitate cell proliferation, EMT, invasion and repress cell apoptosis by absorbing miR-141 and enhancing SIRT1 expression via activation of Wnt/β-catenin pathway[25]. In addition, excess of SNHG15 accelerated cell viability, colony formation and blocked cell apoptosis in pancreatic cancer by repressing P15 and KLF2 expression[26]. Enhanced expression of SNHG15 contributed to cell metastasis by sponging miR-486 to improve CDK14 expression in non-small cell lung cancer[27]. Consistently, SNHG15 was reported to contribute to cell viability and invasion by interacting with miR-141-3p or miR-338-3p in hepatocellular carcinoma or colorectal cancer[28,29]. Therefore, we assumed that SNHG15 played an essential role in BC.

Based on the prediction by bioinformatics analysis tool starBase, SNHG15 could specifically bind to miR-411-5p. Dual-luciferase reporter assay also validated the interaction between SNHG15 and miR-411-5p in BC cells. Hence, we considered that SNHG15 might participate in BC cell development by interacting with miR-411-5p. According to previous researches, the dysregulation of miR-411-5p was the pathogenesis of a variety of diseases[30,31]. MiR-411-5p functioned as tumor suppressor in BC to attenuate cell growth and metastasis by regulating GRB2 through AKT/ERK pathway[32]. Wu et al[33] reported that miR-411-5p was down-regulated in non-small cell lung cancer and miR-411-5p was capable of suppressing cell proliferation and stimulating cell apoptosis by binding to PUM1. Therefore, we assumed that SNHG15 could regulate cell progression by acting as a sponge of miR-411-5p in BC.

To prove the assumption, we measured the expression of SNHG15, miR-411-5p and VASP in BC tumors and cells by qRT-PCR. Increased expression of SNHG15 and VASP in BC tumors and cells implied that they might serve as oncogenes. However, a decreased expression of miR-411-5p implicated the inhibitive role of miR-411-5p in BC. In

addition, inhibition of SNHG15 repressed BC cell proliferation, migration, invasion and induced cell apoptosis. What's more, elimination of SNHG15 hindered tumor growth *in vivo* by regulating miR-411-5p/VASP axis. Afterwords, we discovered that SNHG15 could boost VASP expression by sponging miR-411-5p in BC using dual-luciferase reporter, qRT-PCR and Western blot assay. Besides, reduced miR-411-5p expression reversed SNHG15 silencing-mediated suppression on proliferation, migration, invasion and promotion on apoptosis of BC cells. Likewise, restoration of VASP counteracted SNHG15 deficiency-induced regulatory effects on cell progression in BC.

Conclusions

We discovered the molecular mechanism of SNHG15 in BC progression. The results demonstrated that SNHG15 acted as a competing endogenous RNA (ceRNA) to facilitate proliferation, migration, invasion and block apoptosis of BC cells by competitively binding to miR-411-5p and enhancing VASP expression. Our study represented potential biomarkers for the diagnosis and prospective targeted therapies of BC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

Funding

This work was supported by China Hunan Provincial Science and Technology Department (No.2017SK50216); Hengyang Science and Technology Board (No.2017KJ335).

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