LncRNA SNHG15 facilitates development of breast cancer (BC) by upregulating c-Myc through sponging miR-451

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Research article

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Abstract

Background: Emerging evidences suggested that LncRNA SNHG15 functioned as an oncogene to promote breast cancer (BC) progression, but the detailed mechanisms are still not fully delineated.

Methods: The expression levels of the associated genes were examined by using the Real-Time qPCR and Western Blot. Dual-luciferase reporter gene system was performed to validated the potential targeting sites. Cell counting kit-8 and colony formation assay were used to measure cell proliferation, and trypan blue staining and Annexin V-FITC/PI double staining assay were performed to determine cell viability and apoptosis. Cell invasion and migration were examined by transwell and wound scratch assay, respectively. The tumor-bearing mice models were established, and immunohistochemistry (IHC) was conducted to examine expression and localization of Ki67 protein in tumor tissues.

Results: Here we identified that LncRNA SNHG15 upregulated c-Myc to facilitate BC progression by sponging miR-451 in a competing endogenous RNA (ceRNA)-dependent manner *in vitro* and *in vivo*. Mechanistically, LncRNA SNHG15 and c-Myc were upregulated, while miR-451 was downregulated in BC cells and clinical tissues, compared to their normal counterparts. As expected, the Pearson correlation analysis results indicated that miR-451 negatively correlated with LncRNA SNHG15 and c-Myc, and LncRNA SNHG15 was positively relevant to c-Myc in BC tissues. Next, we validated that LncRNA SNHG15 sponged miR-451 to upregulate c-Myc in BC cells. Further gain- and loss-function experiments evidenced that LncRNA SNHG15 promoted, while miR-451 inhibited malignant phenotypes, including cell proliferation, viability, migration, invasion and epithelial-mesenchymal transition (EMT) in BC cells. Interestingly, the inhibiting effects of LncRNA SNHG15 ablation on BC progression were abrogated by both silencing miR-451 and overexpressing c-Myc.

Conclusions: Collectively, the present study evidenced that targeting LncRNA SNHG15/miR-451/c-Myc signaling cascade was novel to hamper BC progression, and the potential underlying mechanisms were also uncovered, which broadened our knowledge in this field, and provided potential biomarkers for BC diagnosis and treatment.

Background

Breast cancer (BC) is a huge health burden for women worldwide [1, 2], although great advances had been reached in early diagnosis and treatment therapies, BC in some patients still developed into metastatic stage and caused high mortality and worse prognosis [3–5]. Based on the previous publications [1, 2], researchers agreed that uncovering the underlying mechanisms of BC pathogenesis and developing new BC associated biomarkers became urgent and necessary for BC treatment in clinic. Recently, researchers identified that long-non coding RNAs (LncRNAs) functioned as oncogenes and tumor suppressors to modulate BC progression [6–8], and targeting LncRNAs was effective to inhibit malignant phenotypes in BC [9, 10]. Among all the LncRNAs, LncRNA SNHG15 acted as an oncogene to promote cancer development in multiple cancer types, such as lung cancer [11], prostate cancer [12], colorectal cancer [13],

BC [14], etc.. Specifically, Qingli Kong et al. noticed that LncRNA SNHG15 promoted BC proliferation, migration and invasion [14]. However, the detailed mechanisms of LncRNA SNHG15 in regulating BC progression are still largely unknown, hence investigations on this issue were meaningful and necessary.

MicroRNAs (miRNAs) are a group of small non-coding RNAs with about 22 nucleotides, which involved in regulating cancer progression [15, 16]. Specifically, multiple miRNAs had been identified to regulate BC pathogenesis, such as miR-200c-3p [17], miR-203a-3p [18], miR-451 [19, 20], etc.. Interestingly, by performing online starBase software (http://starbase.sysu.edu.cn/), our team noticed that there existed potential targeting sites between miR-451 and LncRNA SNHG15, and previous work also validated that LncRNA SNHG15 sponged miR-451 to exert its biological functions in lung adenocarcinoma (LUAD) in a competing endogenous RNA (ceRNA) mechanism dependent manner [21], which rendered the possibility that LncRNA SNHG15 might regulate BC progression through miR-451. In addition, miR-451 served as a tumor suppressor to hamper cancer progression in multiple cancers, such as lung cancer [22], colon cancer [23], glioma [24], etc.. Although previous data had suggested that miR-451 was closely related with BC pathogenesis [19, 20], the detailed mechanisms are not fully delineated.

According to the previous literatures, miRNAs always regulated cell functions through targeting the 3' untranslated regions (3'UTRs) of their downstream targets, resulting in the degradation of these genes [25, 26]. Given the fact that miRNAs targeted 3'UTRs of cancer associated genes, by screening the existed publications in the online Pubmed database (https://pubmed.ncbi.nlm.nih.gov/), we noticed that c-Myc was proved to be the downstream target of miR-451 [27, 28], and researchers found that miR-451 targeted c-Myc to promote the development of bladder cancer [27] and lung adenocarcinoma [28], but the role of miR-451/c-Myc axis in regulating BC progression is still unknown. In addition, c-Myc acted as a proto-oncogene in BC, and Ho Yeon Lee et al. found that c-Myc driven BC metastasis to brain [29], suggesting that c-Myc was crucial for regulating BC metastasis.

Based on the existed information, by conducting *in vitro* and *in vivo* experiments, this study proposed to investigate the role of LncRNA SNHG15/miR-451/c-Myc signaling cascade in regulating BC progression, and uncover the possible underlying mechanisms, which will broaden our knowledge in this filed, and provide potential diagnostic and prognostic biomarkers for BC in clinic.

Methods

Collection and analysis of clinical samples

The BC clinical tissues and their corresponding normal adjacent tissues (N = 30) were collected in the 3rd Affiliated Teaching Hospital of Xinjiang Medical University (Affiliated Cancer Hospital) from 2014 to 2018, and the above tissues were stored at -70 ℃ conditions immediately. The participates did not accept any treatments before surgical resection. All the clinical experiments were approved by the Ethics Committee

of the 3rd Affiliated Teaching Hospital of Xinjiang Medical University (Affiliated Cancer Hospital), and the informed consent had been obtained from all the participants.

Cell culture and vectors transfection

The BC cell lines (MDA-MB-231, MCF7, SK-BR3 and T-47D) and normal human breast epithelial cell line MCF10A were purchased from American Type Culture Collection (ATCC). The above cells were maintained in the Dulbecco's Modified Eagle's medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA), and the cells were cultivated in an incubator with humidified atmosphere containing 5 % CO_2 at 37 ℃. The overexpression and downregulation vectors for LncRNA SNHG15, miR-451 mimic and inhibitor, and c-Myc overexpression vectors were designed and synthesized by Sangon Biotech (Shanghai, China), and the Lipofectamine™ 2000 transfection reagent (Invitrogen, USA) in keeping with the manufacturer's protocol.

Real-Time qPCR

The total RNA was extracted from BC tissues and cells by using the commercial TRIzol reagent (Invitrogen, USA) according to the producer's protocol. Next, LncRNA SNHG15, miR-451 and c-Myc mRNA were reversely transcribed by using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, USA) and TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA), respectively. Finally, the relative expression levels of the associated genes were determined by using the commercial SYBR Green PCR MasterMix (Applied Biosystems, USA) based on the instructions provided by the producer. LncRNA SNHG15 and c-Myc were normalized by β-actin, and miR-451 was normalized by U6. The primer sequences were listed as follows: LncRNA SNHG15 (Forward: 5'-GGT GAC GGT CTC AAA GTG GA-3', Reverse:5'- GCC TCC CAG TTT CAT GGA CA-3'), miR-451 (Forward: 5'-ACA CTC CAG CTG GGA AAC CGT TAC CAT TA-3', Reverse: 5'-TGG TGT CGT GGA GTC G-3'), c-Myc (Forward: 5'- GGA GGC TAT TCT GCC CAT TT-3', Reverse: 5'-AGG TCA TAG TTC CTG TTG GT-3'), β-actin (Forward: 5'-CTC CAT CCT GGC CTC GCT GT-3', Reverse: 5'-GCT GCT ACC TTC ACC GTT CC-3') and U6 (Forward: 5'-GACTATCATATGCTTACCGT-3', Reverse: 5'-GGG CAG GAA GAG GGC CCT AT-3').

Western Blot analysis

The RIPA lysis buffer (Beyotime, Shanghai, China) was purchased to extract and purify the total protein from BC cells and tissues, and Western Blot analysis was conducted to examine the expression levels of c-Myc and N-cadherin based on the experimental procedures provided by the previous studies [6-8], which were normalized by β-actin. The primary antibodies against c-Myc (1:1500, Abcam, UK), N-cadherin (1:1000, Abcam, UK) and β-actin (1:2000, Abcam, UK).

Dual-luciferase reporter gene system

According to the experimental procedures provided by the previous publications [6-8], we used the online starBase software (http://starbase.sysu.edu.cn/) to predict the targeting sites among LncRNA SNHG15,

miR-451 and 3' UTRs of c-Myc mRNA, which were validated by the following dual-luciferase reporter gene system assay. Briefly, the targeting sites in LncRNA SNHG15 and c-Myc mRNA were mutated and were named as Mut-SNHG15 and Mut-Myc, respectively, and the corresponding wild-type genes were named as Wt-SNHG15 and Wt-Myc. Next, the above sequences were cloned into the pmirGLO Expression vectors (Sangon Biotech, Shanghai, China), and co-transfecting with miR-451 mimic and inhibitor into the BC cells by using the Lipofectamine^{TM} 2000 transfection reagent. Finally, the dual-luciferase reporter gene system (Promega, USA) was employed to examine the relative luciferase activities.

Cell counting kit-8 (CCK-8) assay

The BC cells were subjected to differential vectors transfection, and cell proliferation was measured by using the commercial CCK-8 kit purchased from YEASEN (Shanghai, China) based on the experimental procedures provided by the producer. Briefly, the cells were cultured in the 96-well plates under standard culturing conditions for 0 h, 24 h, 72 h and 96 h, and were incubated with CCK-8 reaction reagent for 2 h in the incubator. After that, the plates were shattered and the optical density (OD) values were measured at the wavelength of 450 nm to reflect relative cell proliferation abilities.

Colony formation assay

The BC cells were pre-transfected with different vectors, and were seeded into the 24-well plates at the density of 1000 cells per well. Next, the cells were cultured in the incubator with standard culturing conditions for 14 days to form colonies. After that, the plates were stained with crystal violet (Beyotime Technology, Shanghai, China) for 15 min at room temperature. The light microscope was used to observe and photograph the colonies, and the colonies containing at least 10 cells were counted to reflect BC cell growth.

Annexin V-FITC/PI double staining assay

The commercial apoptosis detection kit (HaiGene Corporation, China) was purchased to examine cell apoptosis in keeping with the manufacturer's protocol. Briefly, the BC cells were sequentially stained with Annexin V-FITC and PI for 25 min at room temperature without light exposure. After that, a flow cytometer (ThermoFisher Scientific, USA) was employed to measure cell apoptosis ratio.

Transwell assay

The vectors were delivered into BC cells, and the transwell assay was performed to determine cell invasion abilities. Briefly, the BC cells were cultured in the upper chamber of transwell plates (Corning Co-Star, USA) in the serum-free DMEM medium (Gibco, USA), at the density of 2×10^4 cells/well. The lower chamber in the transwell system was added with DMEM medium containing 10 % fetal bovine serum (FBS, Gibco, USA). At 24 hours post-culture, the filters were fixed by using the 4 % paraformaldehyde and stained by 0.1 % crystal violet for visualization. Finally, the cells were photographed by the light microscope, and cell number was counted to reflect cell invasion abilities.

Wound scratch assay

The BC cells were cultivated in the 6-well plates at the density of 6×10^5 cells per plate, and the cells were cultured under standard conditions until the cell confluency reached 100 %. After that, the 200 pipette tips were used to equally generate the wound scratches, and the plates were placed back to the incubator and the cells were observed every day by using the light microscope. The scratch distance was calculated to reflect relative cell migration abilities.

Establishment of xenograft tumor-bearing mice models

The BC cells were subcutaneously injected into the back flank of nude mice (6-8 weeks) at the concentration of 5×10^6 cells per mouse, each group had 5 mice, and the mice were fed under the standard conditions. At 25 days post-injection, the mice were anesthetized by Barbiturate (100 mg/kg) and sacrificed, the mice tumors were isolated and weighed to reflect tumorigenesis. Each group included at least 3 mice. All the animal experiments were approved by the Ethics Committee of the 3rd Affiliated Teaching Hospital of Xinjiang Medical University (Affiliated Cancer Hospital).

Immunohistochemistry (IHC)

The mice tumor tissues were prepared and sliced into sections, the IHC was conducted to determine the expression patterns (expression levels and localization) of Ki67 protein in the tissues. The Ki67 protein antibody was purchased from Abcam (1:400, #ab245113, UK), and the detailed experimental procedures for IHC were well recorded in the previous publications [6-8].

Data collection and analysis

The data was presented as Means ± Standard Deviation (SD), and SPSS 18.0 software was used to analyze the statistical significance. Specifically, the Student's t-test was used for the comparisons between two groups, and the one-way ANOVA analysis was conducted to compare the means from multiple groups. In addition, the Pearson correlation analysis was used to analyze the correlations of genes expressions in the clinical samples. $*P < 0.05$ was regarded as statistical significance. Each experiment was triplicated.

Results

Upregulated LncRNA SNHG15 and c-Myc, and downregulated miR-451 were observed in BC cells and tissues

The cancerous and their paired normal adjacent tissues were collected from BC patients (N = 30), and the expression status of LncRNA SNHG15, miR-451 and c-Myc were determined by Real-Time qPCR and Western Blot. As shown in Figure 1A-C, the data indicated that the expression levels of LncRNA SNHG15 (Figure 1A) and c-Myc mRNA (Figure 1C) were increased, while miR-451 (Figure 1B) was decreased in cancer tissues, instead of their paired normal tissues. Consistently, the 4 paired clinical samples were

randomly selected from the patients, and the results in Figure 1D validated that c-Myc was also upregulated in BC tissues at translated levels. Next, by conducting the Pearson correlation analysis, we evidenced that miR-451 negatively related to both LncRNA SNHG15 (Figure 1E) and c-Myc mRNA (Figure 1F), while the expression levels of LncRNA SNHG15 and c-Myc mRNA showed positive correlations in BC tissues (Figure 1G). Furthermore, the BC cell lines (MDA-MB-231, MCF7, SK-BR3 and T-47D) and normal human breast epithelial cell line MCF10A were obtained and analyzed (Figure 1H-K). As expected, LncRNA SNHG15 (Figure 1H) and c-Myc (Figure 1J, K) were prone to be high-expressed, but miR-451 tended to be low-expressed in BC cells (Figure 1I), in contrast with the normal MCF10A cells. Since LncRNA SNHG15 was especially upregulated in MDA-MB-231 and MCF7 cells, instead of the SK-BR3 and T-47D cells (Figure 1H), we selected the two cell lines for further investigations.

LncRNA SNHG15 sponged miR-451 to upregulate c-Myc in BC cells

Based on the information from the online starBase software (http://starbase.sysu.edu.cn/) and the data from the previous publications [27, 28], we assured that miR-451 potentially bound to LncRNA SNHG15 (Figure 2A) and 3' untranslated region (3'UTR) of c-Myc mRNA (Figure 2D). Next, the wild-type targeting sites in LncRNA SNHG15 (Wt-SNHG15) and 3' UTR of c-Myc (Wt-Myc) were mutated, and named as Mut-SNHG15 and Mut-Myc, respectively, the dual-luciferase reporter gene system assay results indicated that the relative luciferase activity was decreased by miR-451 mimic, and was increased by miR-451 inhibitor in BC cells co-transfecting with Wt-SNHG15 (Figure 2B, C) and Wt-Myc (Figure 2E, F), instead of their corresponding mutant counterparts, suggesting that we had validated the binding sites among LncRNA SNHG15, miR-451 and c-Myc. Furthermore, the overexpression and downregulation vectors for LncRNA SNHG15 and miR-451 were delivered into BC cells, and we evidenced that LncRNA SNHG15 positively regulated (Figure 2G-I), while miR-451 negatively modulated c-Myc in BC cells (Figure 2J-L). Also, the data in Figure 2M-O suggested that the inhibiting effects of LncRNA SNHG15 ablation on c-Myc expressions were reversed by downregulating miR-451, suggesting that LncRNA SNHG15 upregulated c-Myc through sponging miR-451 in BC cells.

The role of LncRNA SNHG15 in regulating malignant phenotypes in BC cells

Previous data suggested that LncRNA SNHG15 functioned as an oncogene to accelerate BC progression [14], which were validated by our work. As shown in Figure 3A-B, upregulation of LncRNA SNHG15 promoted cell proliferation in BC cells, which were inhibited by downregulating LncRNA SNHG15. Consistently, the colony formation assay results validated that LncRNA SNHG15 also positively regulated colony formation abilities in BC cells (Figure 3C, D). Next, the Annexin V-FITC/PI double staining assay was used to examine cell apoptosis, and the results in Figure 3E-F showed that knock-down of LncRNA SNHG15 triggered apoptotic cell death in BC cells. In addition, we noticed that upregulated LncRNA SNHG15 increased the expression levels of N-cadherin to promote epithelial-mesenchymal transition (EMT), which were inhibited by silencing LncRNA SNHG15 (Figure 3G). Furthermore, LncRNA SNHG15 overexpression promoted cell invasion (Figure 3H) and migration (Figure 3I) in BC cells, while LncRNA SNHG15 ablation had opposite effects on cell mobility.

MiR-451 acted as a tumor suppressor to impede BC progression

Next, we performed further experiments to investigate the role of miR-451 in regulating BC progression. To achieve this, the miR-451 mimic and inhibitor were transfected into BC cells to overexpress and downregulate miR-451, and CCK-8 assay was performed to examine cell proliferation (Figure 4A, B). As shown in Figure 4A-B, the results showed that miR-451 negatively regulated cell proliferation in BC cells. Also, by performing the colony formation assay, we also verified that overexpression of miR-451 inhibited colony formation abilities in BC cells, but miR-451 ablation had opposite effects (Figure 4C, D). Similarly, the data in Figure 4E indicated that upregulation of miR-451 also induced cell apoptosis in BC cells. Furthermore, we proved that upregulated miR-451 inhibited N-cadherin to reverse EMT in BC cells, which could be promoted by downregulating miR-451 (Figure 4G). Finally, our data evidenced that miR-451 overexpression inhibited cell invasion (Figure 4H) and migration (Figure 4I) in BC cells, while knock-down of miR-451 promoted cell mobility.

Silencing of LncRNA SNHG15 inhibited BC development by targeting miR-451 and c-Myc

Given the fact that there existed regulatory mechanisms among LncRNA SNHG15, miR-451 and c-Myc, we speculated that LncRNA SNHG15 might regulate malignant phenotypes in BC cells by targeting miR-451/c-Myc axis. To validate this hypothesis, the LncRNA SNHG15 downregulation vectors, miR-451 inhibitor and c-Myc overexpression vectors were successfully transfected into BC cells, and were divided into 4 groups, including control, LncRNA SNHG15 knock-down (KD-SNHG15), KD-SNHG15 + KD-miR-451 and KD-SNHG15 + OE-Myc. The above cells were cultured under the standard conditions for 0 h, 24 h, 48 h and 72 h, respectively. The CCK-8 assay results showed that the inhibiting effects of silencing LncRNA SNHG15 on BC cell proliferation were reversed by both downregulating miR-451 and upregulating c-Myc (Figure 5A, B), and the above results were validated by the following colony formation assay (Figure 5C, D). Consistently, data in Figure 5 suggested that knock-down of LncRNA SNHG15 triggered apoptotic cell death in BC cells by regulating miR-451/c-Myc axis in a similar manner (Figure 5E, F). Next, the Western Blot analysis results evidenced that miR-451 ablation and c-Myc overexpression also reversed the inhibiting effects of LncRNA SNHG15 silence on EMT in BC cells (Figure 5G). Finally, the BC cells were used to establish xenograft tumor bearing mice models, and the results showed that knock-down of LncRNA SNHG15 inhibited tumor weight (Figure 5H, I) and Ki67 expressions (Figure 5J) in mice tumor tissues to hinder tumorigenesis of BC cells *in vivo*, which were all reversed by both silencing miR-451 and upregulating c-Myc.

Discussion

Although recent data had assured that LncRNA SNHG15 acted as an oncogene to promote cancer development in multiple cancers [11–14], the detailed underlying mechanisms of LncRNA SNHG15 in regulating BC pathogenesis are still not fully delineated. To investigate this issue, the present study collected clinical tissues from 30 BC patients, and validated that LncRNA SNHG15 tended to be enriched in cancer tissues, instead of their corresponding normal adjacent tissues. Consistently, high-expressed

LncRNA SNHG15 was also observed in BC cells, compared to the normal MCF10A cells, indicating that LncRNA SNHG15 was aberrantly upregulated in BC cells and tissues. Next, by performing the gain- and loss-function experiments, we found that knock-down of LncRNA SNHG15 inhibited cell proliferation, colonies formation, epithelia-mesenchymal transition (EMT), invasion, and migration, and induced apoptotic cell death in BC cells, while LncRNA SNHG15 had opposite effects on the above malignant phenotypes. Consistently, the *in vivo* experiments evidenced that silencing of LncRNA SNHG15 slowed down tumor growth and inhibited Ki67 protein levels to hinder tumorigenesis of BC cells in xenograft mice models. The above results suggested that LncRNA SNHG15 promoted BC progression, which were supported by the previous work [14].

Previous publications indicated that miR-451 was closely associated with BC progression and prognosis [19, 20], but the detailed mechanisms are still largely unknown. The present study investigated this issue and found that miR-451 functioned as a tumor suppressor to hinder BC development *in vitro*. Mechanistically, miR-451 was downregulated in both BC tissues and cells, in contrast with their counterparts. Next, the miR-451 mimic and inhibitor were transfected into BC cells to overexpress and silence miR-451, respectively. As expected, the results showed that overexpression of miR-451 inhibited BC cell growth and mobility, and promoted cell apoptosis, but miR-451 downregulation facilitated BC progression, indicating that miR-451 acted as a tumor suppressor to hamper BC development, which were supported by the data in lung cancer [22], colon cancer [23], glioma [24], etc.. According to the previous work [21], we validated that LncRNA SNHG15 sponged miR-451 in BC cells in a ceRNA-dependent manner, and further experiments showed that the inhibiting effects of LncRNA SNHG15 ablation on the malignant phenotypes in BC cells were all reversed by silencing miR-451, implying that LncRNA SNHG15 promoted BC progression by sponging miR-451.

Ho Yeon Lee et al. proved that the proto-oncogene c-Myc was a crucial “driver” to promote BC metastasis to brain [29], which also played an oncogenic role in multiple cancers [30–32]. Expectedly, we noticed that c-Myc tended to be overexpressed in BC tissues and cells, instead of their normal counterparts, which indirectly reflected that high-expressed c-Myc indicated a worse outcome in BC. In addition, we validated that miR-451 targeted 3’UTR of c-Myc mRNA for degradation, and LncRNA SNHG15 positively regulated c-Myc in BC cells by targeting miR-451, implying that LncRNA SNHG15 sponged miR-451 to upregulate c-Myc in BC cells. Next, we confirmed that LncRNA SNHG15 regulated BC development by upregulating c-Myc. Specifically, the inhibiting effects of LncRNA SNHG15 ablation on the malignant phenotypes in BC cells were all reversed by overexpressing c-Myc.

Conclusions

Taken together, this study validated that targeting LncRNA SNHG15 impeded BC progression *in vivo* and *in vitro* by modulating miR-451/c-Myc pathway. The present study uncovered the possible underlying mechanisms of BC development, and provided LncRNA SNHG15, miR-451 and c-Myc as novel biomarkers for BC diagnosis and prognosis.

Declarations

Ethics approval and consent to participates

All the clinical and animal experiments were approved by the Ethics Committee of the 3rd Affiliated Teaching Hospital of Xinjiang Medical University (Affiliated Cancer Hospital). The informed consent forms had been signed by all the participants.

Consent for publication

All the co-authors agreed to publish the final version of this manuscript.

Availability of data and materials

All the data involved in this study had been included in the manuscript, and the corresponding raw data could be acquired from the corresponding author upon reasonable request.

Competing interests

None.

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Authors' contributions

Dr. JD was responsible for the conception and design of this study, and also conducted most of the experiments and drafted the manuscript. Dr. HZ provided a lot of assistance for this work, and finished the rest of the experiments, and was also responsible for data collection, analysis and visualization. Dr. BM provided guidance for this work, and proofread the manuscript. In addition, Dr. BM acquired the funding and submitted the manuscript for potential publication. All authors have read and approved the manuscript.

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Figures

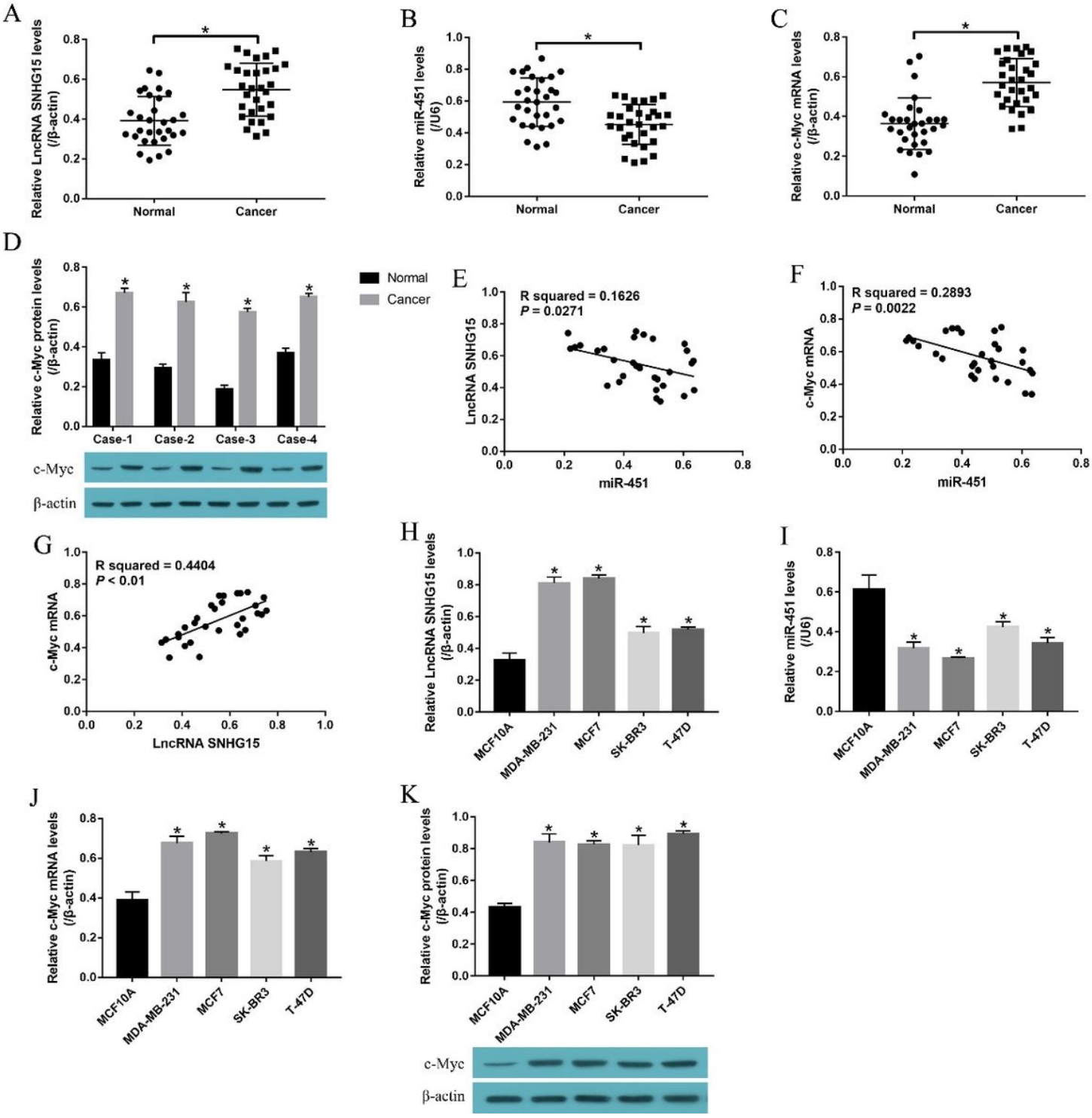

Figure 1

LncRNA SNHG15, miR-451 and c-Myc were aberrantly expressed in BC clinical specimens and cells. The BC tissues and their paired adjacent normal tissues were collected, and Real-Time qPCR was conducted to determine the expression levels of (A) LncRNA SNHG15, (B) miR-451 and (C) c-Myc mRNA. (D) Western Blot analysis was employed to examine c-Myc protein levels in the clinical tissues. (E-G) Pearson correlation analysis was used to analyze the correlations among LncRNA SNHG15, miR-451 and c-Myc in BC tissues. The BC cell lines (MDA-MB-231, MCF7, SK-BR3 and T-47D) and normal human breast

epithelial cell line MCF10A were cultured in vitro, and the expression levels of (H) LncRNA SNHG15, (I) miR-451 and (J) c-Myc mRNA were determined by Real-Time qPCR. (K) The c-Myc protein expressions in the cells were measured by using the Western Blot analysis. Each experiment repeated at least 3 times, and * P < 0.05 indicated statistical significance.

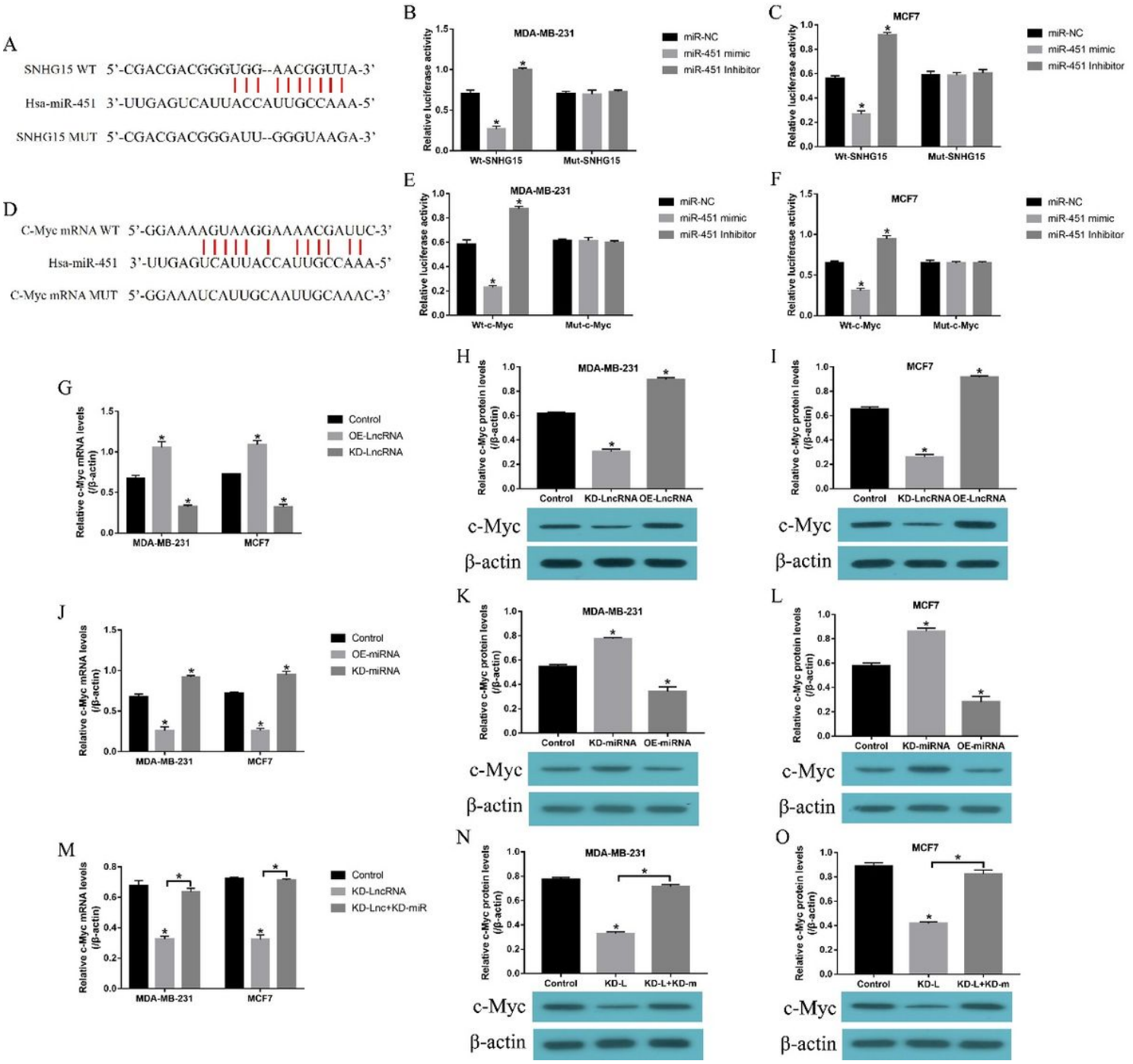

Figure 2

The regulatory mechanisms among LncRNA SNHG15, miR-451 and c-Myc were investigated. The online starBase software (http://starbase.sysu.edu.cn/) was employed to predict the binding sites of miR-451 with (A) LncRNA SNHG15 and (D) 3'UTR of c-Myc mRNA. Dual luciferase reporter gene system was performed to validate the targeting sites of miR-451 with (B, C) LncRNA SNHG15 and (E, F) 3'UTR of c-

Myc mRNA in BC cells. By performing Real-Time qPCR and Western Blot, we evidenced that (G-I) LncRNA SNHG15 positively regulated c-Myc, and (J, K) miR-451 negatively regulated c-Myc in BC cells. In addition, (M-O) silencing of LncRNA SNHG15 inhibited c-Myc expressions through upregulating miR-451. Each experiment repeated at least 3 times, and * P < 0.05 indicated statistical significance.

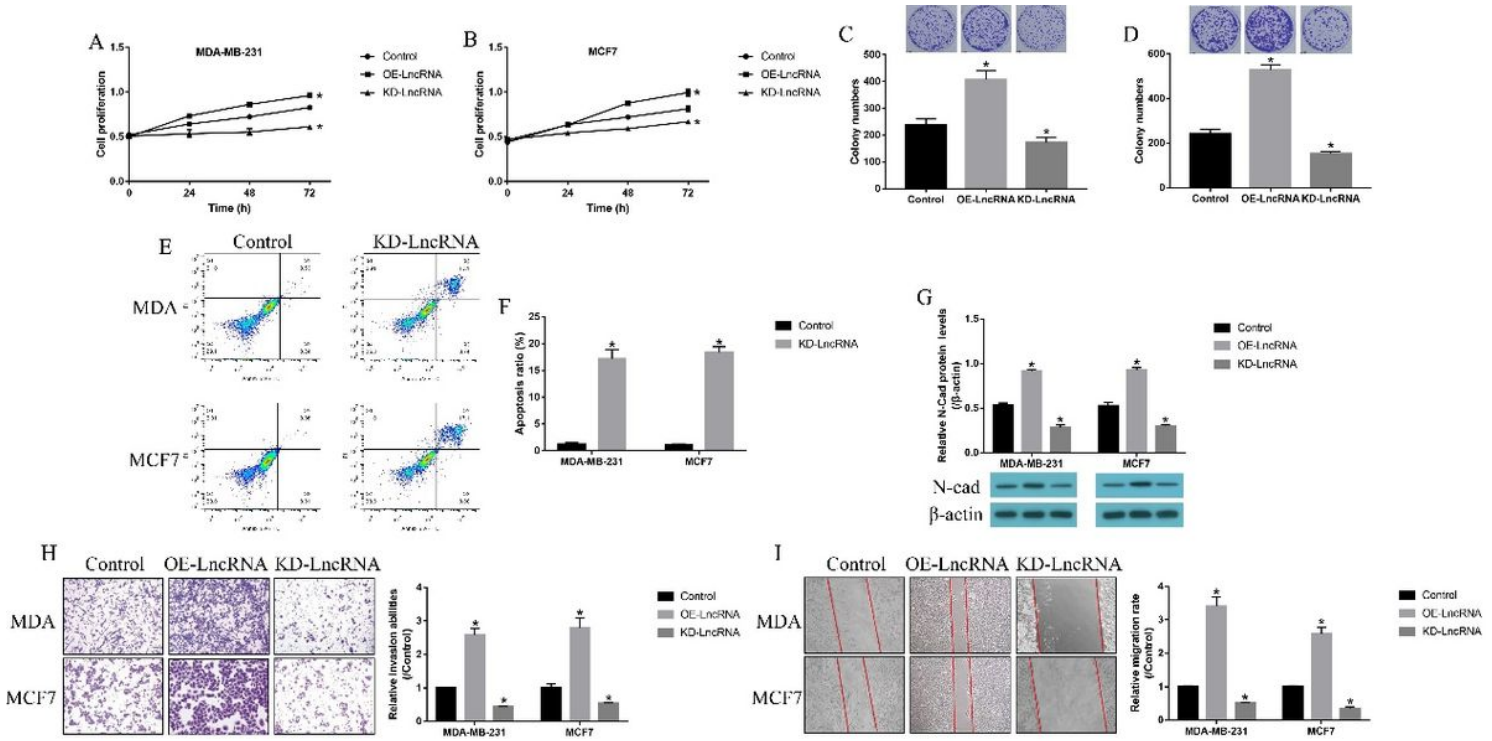

Figure 3

LncRNA SNHG15 promoted malignant phenotypes in BC cells. (A, B) CCK-8 assay was performed to determine cell proliferation abilities. (C, D) Colony formation assay was used to examine colonies formation abilities in BC cells. (E, F) Knock-down of LncRNA SNHG15 promoted cell apoptosis in BC cells, determined by using Annexin V-FITC/PI double staining method. (G) The expression levels of N-cadherin was monitored by using the Western Blot analysis. (H) Cell invasion and (I) migration were detected by transwell and wound scratch assay, respectively. Each experiment repeated at least 3 times, and * P < 0.05 indicated statistical significance.

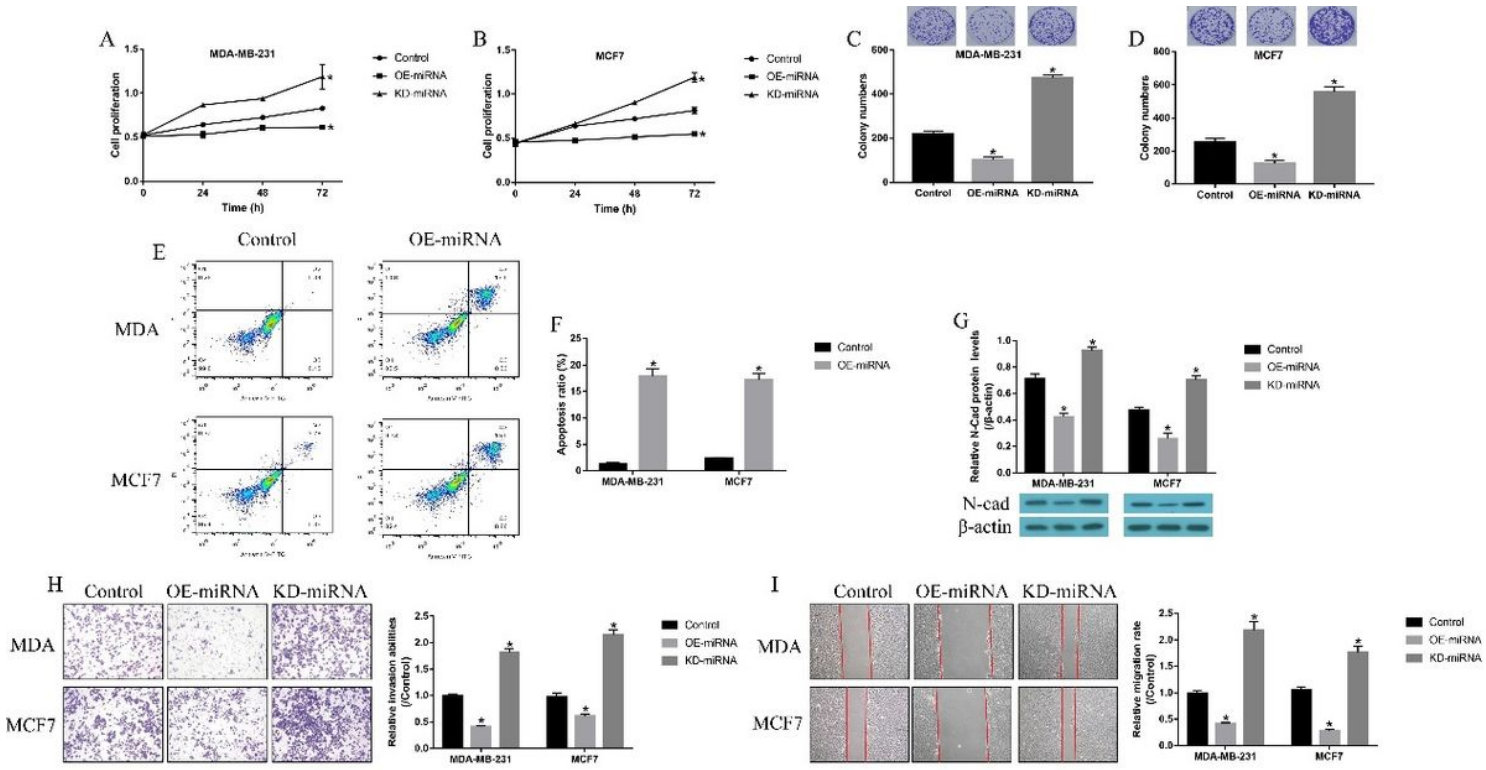

Figure 4

MiR-451 acted as a tumor suppressor to hinder BC progression in vitro. (A, B) The CCK-8 assay results indicated that miR-451 inhibited BC cell proliferation. (C, D) The colonies formation abilities were inhibited by overexpressing miR-451 in BC cells. (E, F) The Annexin V-FITC/PI double staining assay evidenced that upregulation of miR-451 induced cell apoptosis in BC cells. (G) By performing Western Blot, we noticed that miR-451 negatively regulated N-cadherin to inhibit EMT in BC cells. (H, I) Cell mobility was evaluated by using transwell and wound scratch assay, respectively. Each experiment repeated at least 3 times, and * $P < 0.05$ indicated statistical significance.

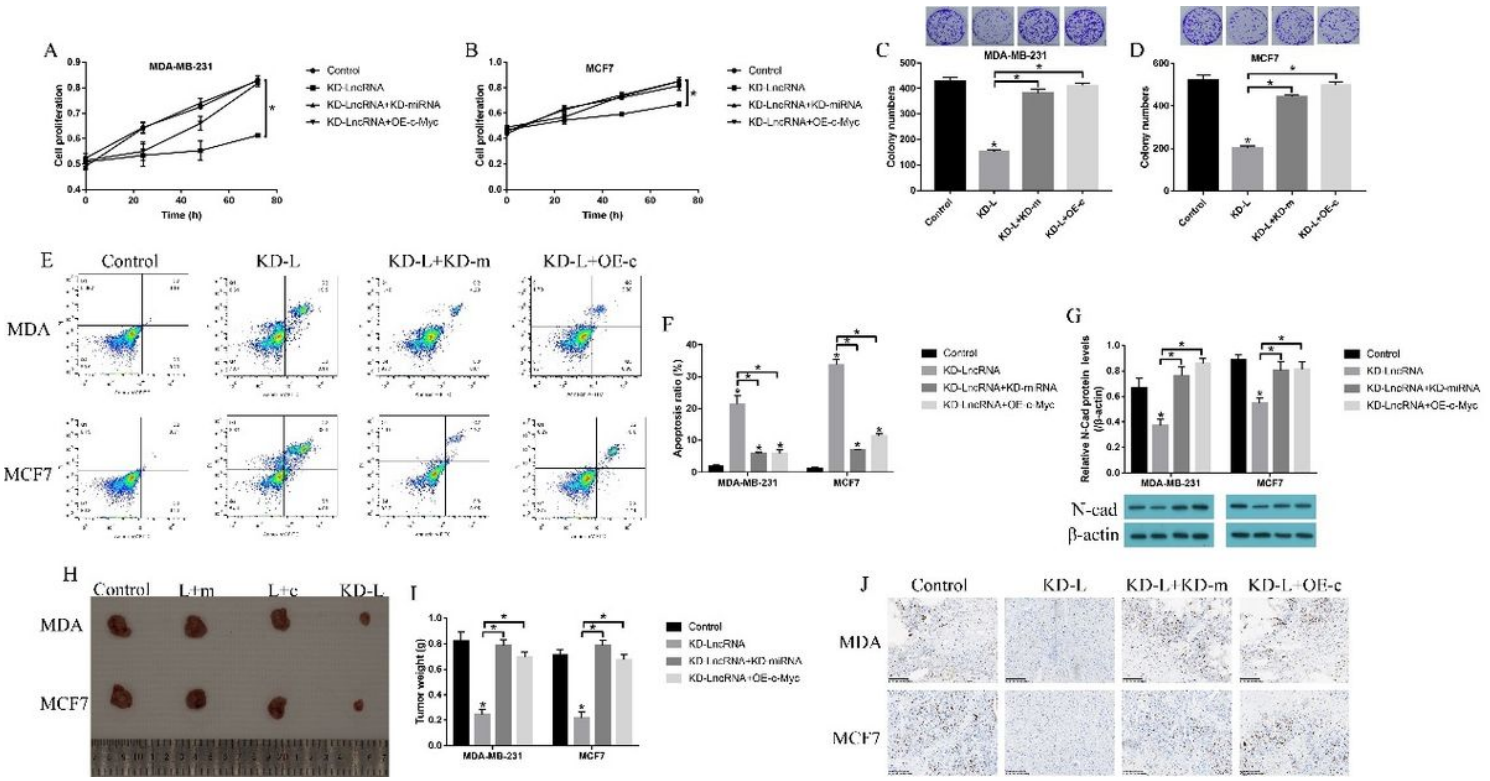

Figure 5

Knock-down of LncRNA SNHG15 inhibited BC progression in vitro and in vivo by targeting miR-451 and c-Myc. (A, B) Cell proliferation was evaluated by using the CCK-8 assay. (C, D) Colony formation abilities were measured by conducting the colony formation assay. (E, F) Annexin V-FITC/PI double staining assay results suggested that silencing of LncRNA SNHG15 promoted cell apoptosis in BC cells by targeting miR-451 and c-Myc. (G) Western Blot analysis was used to detect N-cadherin expressions to determine EMT in BC cells. The BC cells were utilized to establish xenograft tumor-bearing mice models, and (H, I) tumor weight was measured at day 25 post-injection. (J) The immunohistochemistry (IHC) was used to examine the expressions and localization of Ki67 protein in mice tumor tissues. (Note: “KD-L” suggested “Knock-down of LncRNA SNHG15”, “KD-m” indicated “Knock-down of miR-451”, and “OE-c” represented “Overexpression of c-Myc”). Each experiment repeated at least 3 times, and * $P < 0.05$ indicated statistical significance.

Supplementary Files

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