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Rev Invest Clin. 2024;76(4):185-98 ORIGINAL ARTICLE

LINC01614 Activated by SP1 Promoted Malignant Behavior of Triple-Negative Breast Cancer Cells via the WNT/β-Catenin Signaling Pathway

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ABSTRACT

Background: Triple-negative breast cancer (TNBC) represents the most aggressive subtype of breast cancer (BC), characterized by a dismal prognosis. Dysregulated long non-coding RNA *LINC01614* might be a potential biomarker for BC as previously reported. Nevertheless, its functions and mechanism in TNBC cells are unclear. **Objectives:** The study aimed to study the effects of *LINC01614* on TNBC cell migration, invasion, and epithelial-mesenchymal transition (EMT) process as well as the related mechanism. **Methods**: Reverse transcription quantitative polymerase chain reaction was performed to detect the expression of *LINC01614* and SP1 in TNBC cells and tissues. The cellular localization of *LINC01614* was determined by subcellular fraction assays. Cell counting kit-8 and Transwell invasion assays were conducted for measurement of TNBC cell viability and invasive ability. Cell migration was performed using wound healing assays and Transwell migration assays. Chromatin immunoprecipitation assays and luciferase reporter assays were used to explore the interaction between SP1 and *LINC01614*. Western blotting was used to assess protein levels of factors involved in EMT process and Wnt/β-catenin signaling in TNBC cells. **Results:** *LINC01614* expression was elevated in TNBC tissues and cells. *LINC01614* knockdown inhibited cell viability as well as migratory and invasive abilities of TNBC cells. *LINC01614* knockdown also obstructed EMT process, as shown by E-cadherin upregulation and vimentin downregulation in TNBC cells. SP1 directly bound to the promoter of *LINC01614* and activated *LINC01614* expression. SP1 overexpression reversed the suppressive effect of *LINC01614* knockdown on TNBC cell migration, invasion, and EMT process. Protein levels of Wnt and β-catenin were diminished by *LINC01614* knockdown, and the trend was partially rescued by SP1 overexpression. **Conclusion:** SP1-induced *LINC01614* promoted malignant behavior of TNBC cells by activating the Wnt/β-catenin signaling pathway. (REV INVEST CLIN. 2024;76(4):185-98)

Keywords: *LINC01614*. SP1. Transcription factor. Triple-negative breast cancer. Wnt/β-catenin pathway.

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INTRODUCTION

Breast cancer (BC) is a prevalent malignancy among women worldwide, with its incidence continuing to rise[1]. According to recent data published by American Cancer Society, BC alone accounts for 32% of all new cancer diagnoses in women and it is a leading cause of cancer death for women younger than age 50 in the United States[2]. BC can be categorized into various types according to the expression of molecular markers such as human epidermal growth factor receptor 2 (HER2), progesterone, and estrogen[3,4]. Triple-negative BC (TNBC) is characterized by the absence of HER2, progesterone receptor, and estrogen receptor[5]. TNBC accounts for approximately 10-20% of total BC cases and is more prevalent in women < 50 years[6]. Treatments including surgery, chemotherapy, and radiotherapy have been widely applied and have made advances to some extent[7]. Nevertheless, many patients have missed the surgical window at the time of diagnosis[7]. Tumor recurrence poses another obstacle in the treatment of TNBC. For patients with TNBC, the median time to relapse ranges from approximately 19-40 months, and there is a 75% mortality rate within the first 3 months following relapse[5,8]. Moreover, therapeutic resistance of TNBC also deserve mentioning, including enhanced antioxidant ability, increased drug efflux and metabolism, and enhanced DNA repair during treatment[6]. Thus, it is imperative to find more effective therapeutical strategies.

Increasingly studies have identified long non-coding RNAs (lncRNA) as key players in various types of cancer, playing either tumor-suppressive or oncogenic roles, and holding promise for the development of effective targeted therapies in TNBC[9]. For example, lncRNA SEM13B-AS1 knockdown promoted the proliferation, migration, and invasion of TNBC cells (BT-549 and MDA-MB-231) by acting as a competing endogenous RNA against miR-3940 to regulate the downstream gene KLLN[10]. The transcription factor SP1-induced high expression of lncRNA AFAP1-AS1 facilitates tumorigenesis in TNBC by activating the mTOR signaling[11]. *LINC01614* is a novel oncogene and was first demonstrated as a lung cancer-associated lncRNA in 2018[12]. After that, Wang et al. further confirmed that *LINC01614* is an epithelial-mesenchymal transition (EMT)-associated oncogene[13]. Based on these two studies, researchers have increasingly investigated its role in various types of cancer. For example, *LINC01614* acts as an oncogene in gastric cancer and reduces the chemosensitivity of gastric cancer cells to 5-fluorouracil[14]. Cai et al. reported that *LINC01614* promoted the proliferative and invasive ability of osteosarcoma cells[15]. Especially, Vishnubalaji et al. analyzed that *LINC01614* was upregulated in patients with BC and was correlated with worse disease-free survival, having the potential to be an unfavorable prognostic marker[16]. Zhang et al. reported that the interaction of *LINC01614* with COL3A1 can predict drug sensitivity in TNBC patients, although the accuracy still needs improvement[17]. Nevertheless, its specific role and underlying mechanisms in TNBC have not been discussed yet.

Transcriptional activation is critical for the upregulation of lncRNAs[18]. Transcription factor SP1 was reported to upregulate *LINC01614* in glioma and thereby promotes cancer progression[19]. In addition, SP1 was reported to affect TNBC progression by activating the expression of other lncRNAs such as AFAP1-AS1[11] and LINC01234[20]. Hence, it was hypothesized that SP1 may also contribute to the transcriptional upregulation of *LINC01614* in TNBC cells.

The current study aimed to explore the influence of *LINC01614* on the viability, migration, invasion, and EMT process of TNBC cell lines (BT-549 and MDA-MB-231). Experiments were designed to confirm its oncogenic role and interaction with the transcription factor SP1 in TNBC cells. This study may provide a novel theoretical basis for the targeted treatment for TNBC.

METHODS

Patient samples

TNBC tissues (n = 78) and matching non-cancerous tissues (n = 7 8) were obtained from the patients diagnosed with TNBC (Stage I–IIA) from Yichang Central People's Hospital. All the patients gave written informed consent, and the protocols for the use of human tissues were approved by the Ethics Committee of Yichang Central People's Hospital (2022-164-01).

Before sampling, none of the patients underwent radiotherapy, chemotherapy, or hormone therapy. The tissue samples were confirmed by histopathological examination. All the samples were then immediately frozen in liquid nitrogen and stored at −80°C for the subsequent experiments.

Cell culture and transfection

TNBC cell lines (CAL-51, BT-549, MDA-MB-231, and MDA-MB-468) and normal human breast epithelial cells (MCF-10A) were purchased from ATCC and cultured in DMEM medium containing fetal bovine serum (10%), penicillin, and streptomycin were used for cell culture. The culture conditions were 5% CO_2 at 37°C in a humidified incubator.

BT-549 and MDA-MB-231 cells were seeded in 6-well plates for 24 h. Short hairpin RNA targeting *LINC01614* (sh-*LINC01614*#1 and sh-*LINC01614*#2) was used for the knockdown of *LINC01614*. The full sequence of SP1 was transfected into pcDNA3.1 vectors to amplify SP1 expression. These plasmids together with their negative controls (sh-NC and empty pcDNA3.1 vectors) were all obtained from GenePharma (Shanghai, China) and were transfected into TNBC cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, USA). After 48 h of transfection, subsequent *in vitro* experiments were conducted.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted and then reverse-transcribed into complementary DNA. Quantitative PCR experiments were performed on the ABI 7500 Fast Real-Time PCR System with SYBR Fast qPCR Mix (Invitrogen). Relative expression of *LINC01614* and SP1 was quantified using the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. Sequences of primers used in the study were listed as follows:

LINC01614: (forward) 5'- CAACCAAGAGCGAAGCCAAG-3' and (reverse) 5'- CGCCCCAAAACAACTGAGTC-3'.

SP: (Forward) 5'- GGGTACTTCAGGAATCCAGG-3' and (reverse) 5'-TGAGCTCCATGATCACCTG-3'.

GAPDH: (Forward) 5'-GATCATCAGCAATGCCTCC -3' and (reverse) 5'-TCCACGATACCAAAGTTGTC -3'.

Subcellular fraction assay

To determine the location of *LINC01614* in TNBC cells, the PARIS Kit (Life Technologies, Carlsbad, CA, USA) was used. Cells were harvested, lysed on ice for 10 min, and subjected to centrifugation at 12000 g for 3 min. The precipitation part was mainly the nucleus, and the supernatant was the cytoplasm. The distribution of *LINC01614* in the cytoplasmic or nuclear part was measured by RT-qPCR. U6 and GAPDH were regarded as the nuclear and cytoplasmic controls, respectively.

Cell counting kit-8 (CCK-8) assay

After the indicated transfection, TNBC cells were plated into 96-well plates (2000 cells/well) and incubated overnight. Then, cell viability was evaluated by adding 15 μL of CCK-8 solution (Glpbio, Montclair, USA) to each well at the time points of 24 h, 48 h, and 72 h, and the absorbance at 450 nm was examined by a microplate reader (BioTek, Vermont, USA).

Wound healing assay

The assay was performed to assess cell migration by measuring the widths of scratches at 0 h and 24 h. TNBC cells (3×10^5 cells) were seeded into 6-well plates and subjected to cell transfection. After that, cells were incubated with a serum-free medium for 24 h. Scratches were made on cell layers using a 200 μL pipette tip. Next, cells were cultured in a complete medium for 24 h. Cell fragments were removed by washing with phosphate-buffered saline, and the images of wound closures at 0 and 24 h after scratch were taken using an inverted microscope (Olympus, Tokyo, Japan). The experiment was repeated in triplicate.

Transwell assay

Transwell assays were conducted to evaluate cell migration and invasion using 8 μm pore Transwell chambers (Corning Incorporated, Corning, USA).

For cell invasion assays, TNBC cells with indicated transfection (2×10^4 cells) were suspended in 300 µL of serum-free medium and added to the upper chamber pre-coated with Matrigel (Sigma Aldrich, St. Louis, USA) 2 h before cell inoculation. The lower chamber was added with 500 µL complete medium containing 10% fetal bovine serum. After 24 h, cells invading the lower chamber were fixed with 4% paraformaldehyde (Zeye Biotechnology, Shanghai, China), stained with crystal violet (0.2%, Beyotime, Shanghai, China), and then imaged using a light microscopy (Olympus). For cell migration experiments, similar procedures were performed except for the precoat of Matrigel. Each assay was performed in triplicate.

Western blot analysis

Proteins were isolated from the TNBC cells using cell lysis buffer (Abcam, UK), and the protein concentrations were confirmed by Bicinchoninic acid assay kits (Thermo Fisher Scientific, Waltham, USA). Then, equal quantities of proteins were separated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (10%) and transferred onto polyvinylidene difluoride membranes. Primary antibodies against E-cadherin (ab40772; 1:1000), vimentin (ab92547: 1:1000), Wnt (ab219412; 1:1000), β-catenin (ab32572; 1:5000) and GAPDH (ab8245; 1:500) were purchased from Abcam and prepared as recommended. After blocking with 5% non-fat milk for 1 h, the membranes were incubated with these primary antibodies overnight at 4°C. The next day, secondary antibodies were incubated with the membranes for another 1 h. Finally, the blots were illuminated using enhanced chemiluminescence kit (Absin, Shanghai, China), and the band intensity was calculated with the ImageJ software (National Institutes of Health, Bethesda, USA).

Chromatin immunoprecipitation (ChIP) assay

The assay was performed using ChIP assay kit (Beyotime). TNBC cells were first cross-linked with 1% formaldehyde for 10 min and then fragmented through ultrasonication to 200-500 base pairs. Cell lysates were incubated with protein A/G beads coated with immunoglobulin G antibody (anti-IgG; ab6757, Abcam) and anti-SP1 (ab231778, Abcam). The IgG group acted as the negative control. Finally, DNA fragments were purified from the protein-DNA complex using a DNA extraction kit (Beckman Coulter, Miami, USA), and the precipitated DNA was analyzed by qPCR.

Luciferase reporter assay

The wild type (Wt) or mutant (Mut) promoter region of *LINC01614* containing SP1 binding area was cloned into the pGL3-basic vector (Promega, Madison, USA) to construct Wt-pGL3-*LINC01614* and Mut-pGL3-*LINC01614*. TNBC cells were cotransfected with the above Wt/Mut vectors and pcDNA3.1-SP1/empty pcDNA3.1 vectors for 48 h. After that, the luciferase activity was analyzed using the Dual-Luciferase Reporter Assay System (Promega). Firefly luciferase activity/renilla activity was calculated in the Wt or Mut group.

Statistical analysis

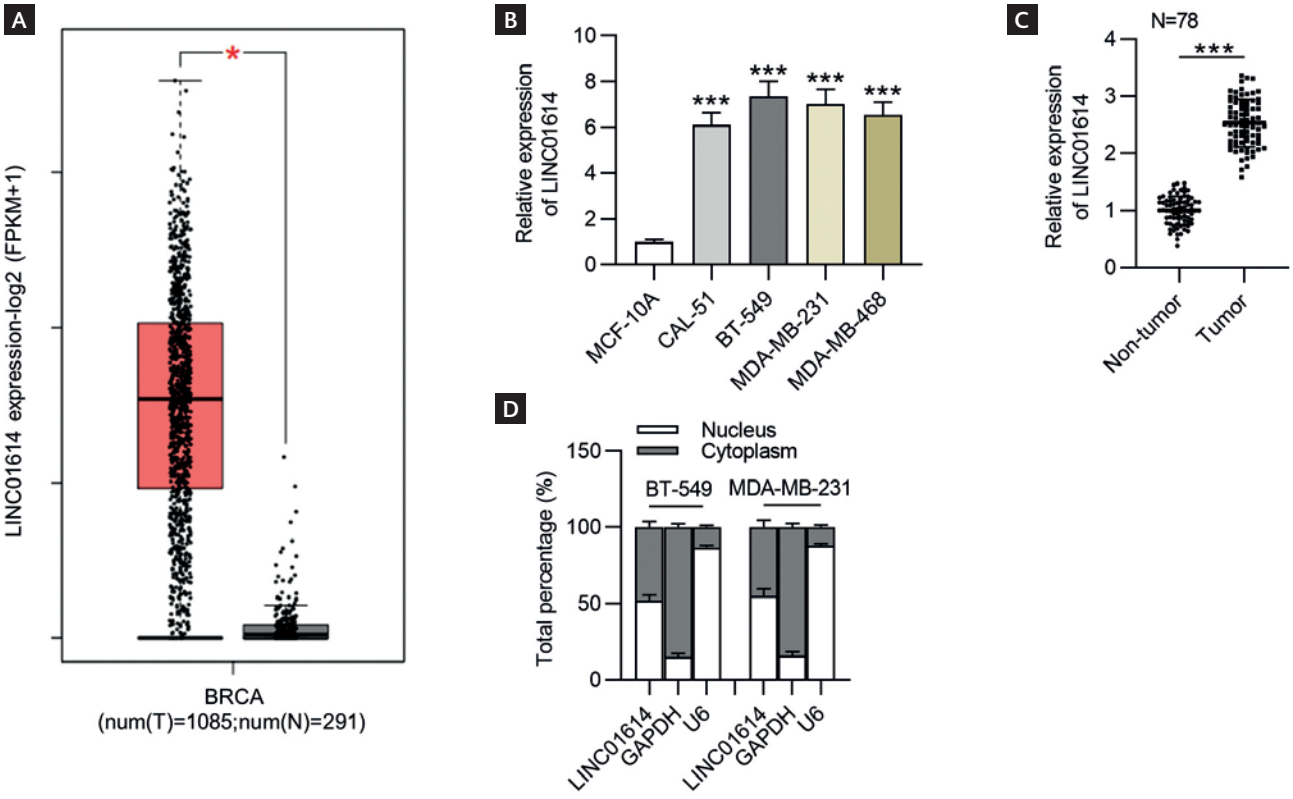
All the experiments were repeated in triplicate and data were processed by GraphPad Prism (La Jolla, USA). All data are presented as mean ± SD. Student's *t*-test was performed to compare differences between two groups. One-way analysis of variance followed by Tukey's *post hoc* analysis was conducted to evaluate the significance among multiple groups. A value of $p < 0.05$ was regarded as statistically significant.

RESULTS

LINC01614 was highly expressed in TNBC tissues and cell lines

The bioinformatics tool GEPIA (http://gepia2.cancer-pku.cn/#analysis) was used to analyze the expression of *LINC01614* (ENSG00000230838) in 1085 breast invasive carcinoma (BRCA) samples and 291 corresponding normal samples. The box plot showed that *LINC01614* was highly expressed in BRCA tissues in contrast to its expression in normal tissues (Fig. 1A, $p < 0.05$). Since TNBC accounts

Figure 1. *LINC01614* was highly expressed in TNBC tissues and cell lines. **A**: *LINC01614* (ENSG00000230838) expression in breast invasive carcinoma samples (n = 1085) and corresponding normal samples (n = 291) was analyzed using bioinformatics tool GEPIA. **B**: RT-qPCR was performed to measure *LINC01614* expression in four TNBC cell lines with normalization to its expression in normal human breast epithelial cells (MCF-10A). **C**: *LINC01614* expression levels in the collected TNBC tissues (n = 78) and matched noncancerous tissues (n = 78) were measured by RT-qPCR. **D**: subcellular fractionation assays were conducted to determine the localization of *LINC01614* in cytoplasmic and nuclear parts of TNBC cells. *p < 0.05, ***p < 0.001. TNBC: triple-negative breast cancer.



for 15-20% of all BRCA[21], this study aimed to explore whether *LINC01614* is also abnormally expressed in TNBC cell lines. RT-qPCR revealed that the *LINC01614* expression was elevated significantly in TNBC cell lines compared with the normal cells (more than 6 folds) (Fig. 1B). Consistently, *LINC01614* expression was increased significantly in the TNBC tissues (n = 78) relative to that in the non-cancerous tissues (n = 78) (Fig. 1C). Therefore, two TNBC cell lines (BT-549 and MDA-MB-231) with the highest *LINC01614* expression were selected for subsequent experiments. The localization of *LINC01614* in TNBC cells was determined using subcellular fraction assay. As displayed in Fig. 1D, *LINC01614* was distributed in both nucleus and cytoplasm of TNBC cells.

Knockdown of *LINC01614* suppressed the migration, invasion, and EMT process of TNBC cells

In this part, loss-of-function experiments were performed to explore the effect of *LINC01614* depletion in the TNBC cell process. After shRNAs were transfected into TNBC cells, *LINC01614* expression was markedly diminished in the sh-*LINC01614* groups compared to that in sh-NC group (p < 0.001, Fig. 2A). In response to *LINC01614* deficiency, there was a significant decrease in the viability of both BT-549 and MDA-MB-231 cells (Fig. 2B, p < 0.001). Wound healing and transwell migration assays revealed that wound closure rate and the number of migrated cells were noticeably reduced in the context of *LINC01614*

Figure 2. Knockdown of *LINC01614* suppressed TNBC cell migration, invasion, and EMT process. **A**: RT-qPCR was performed to assess *LINC01614* expression in TNBC cells transfected with sh-*LINC01614*#1 and sh-*LINC01614*#2. **B**: CCK-8 assays were conducted for detection of cell viability in response to *LINC01614* knockdown. **C** and **D:** wound healing assays and Transwell migration assays were carried out to evaluate the effect of sh- *LINC01614* on the migratory ability of TNBC cells. **E**: Transwell invasion assays were performed to examine cell invasive ability in the context of *LINC01614* depletion. ***p < 0.001. TNBC: triple-negative breast cancer; CCK-8: cell counting kit-8; EMT: epithelial-mesenchymal transition; RT-qPCR: reverse transcription quantitative polymerase chain reaction *(continues)*.

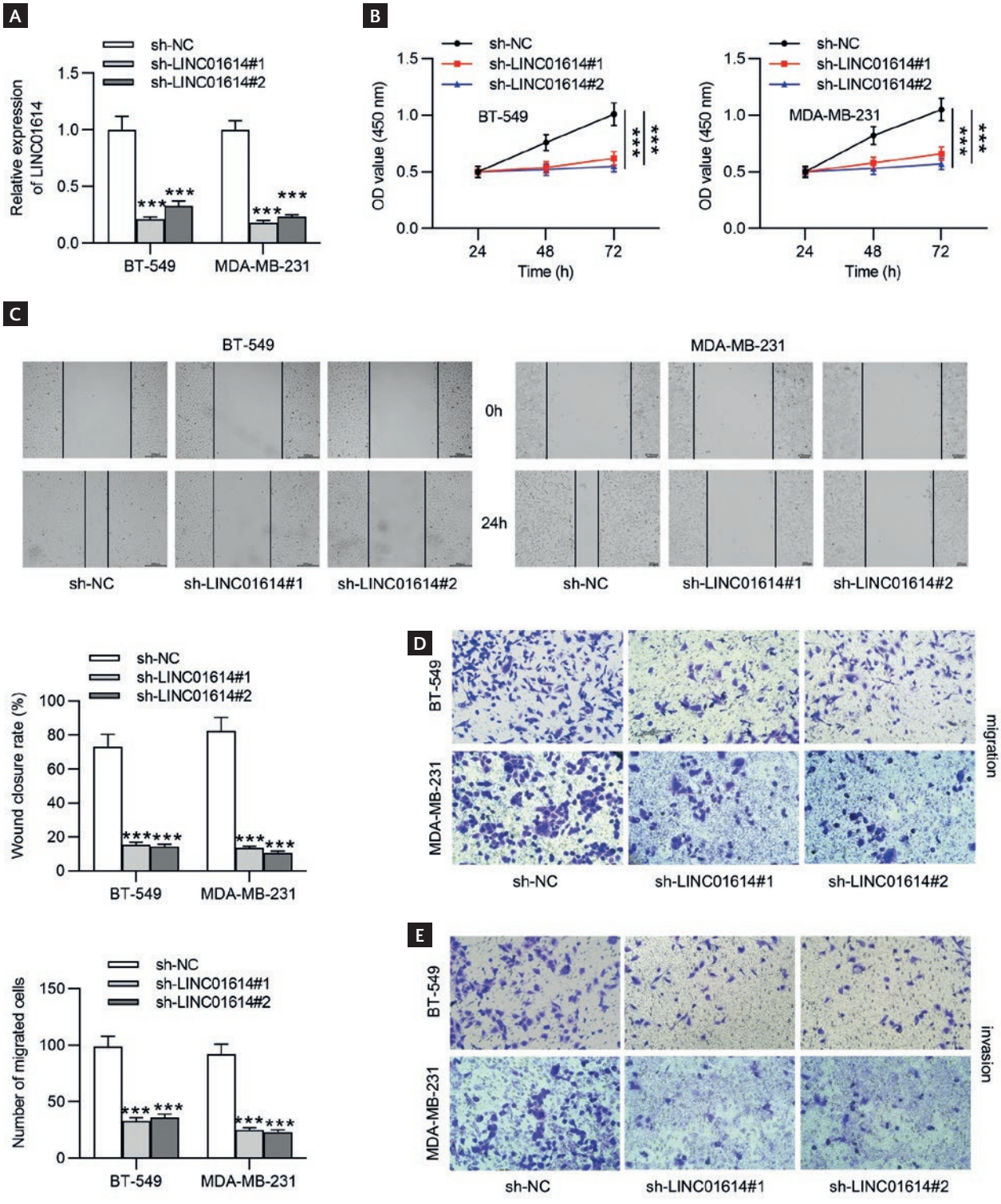
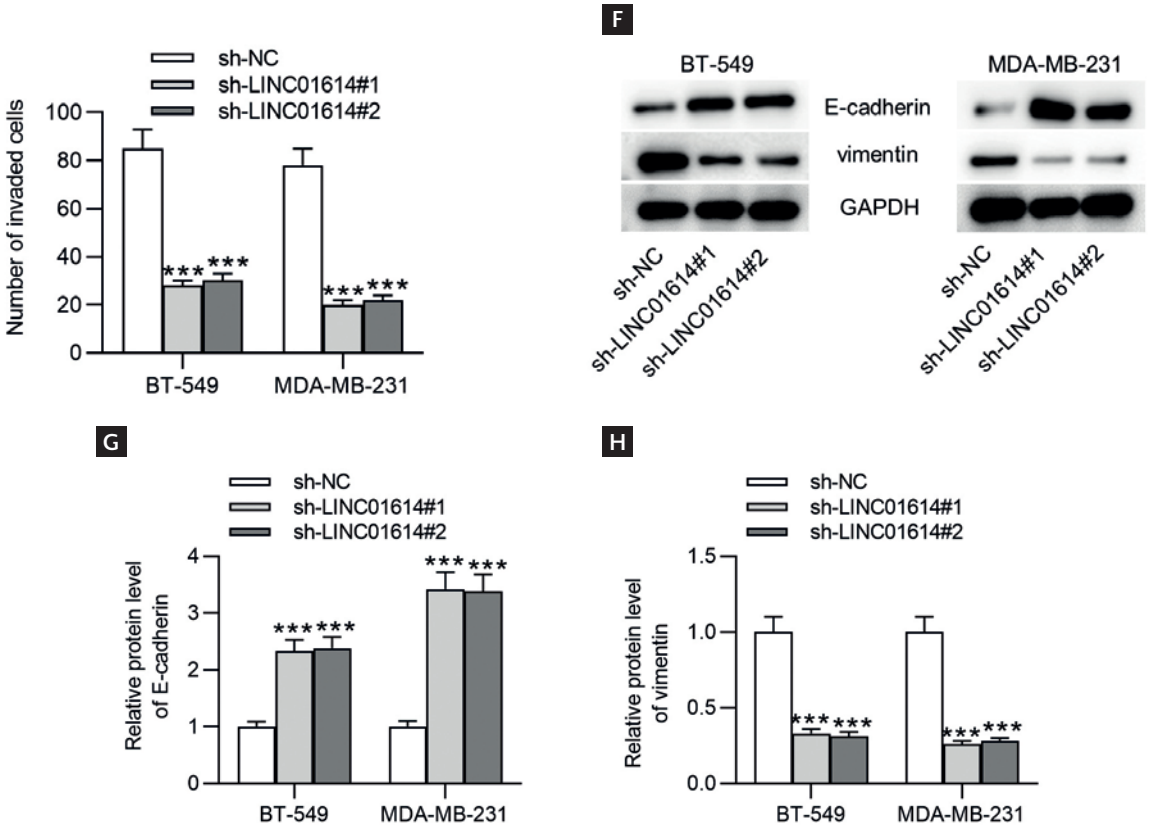


Figure 2. *(continues)* Knockdown of *LINC01614* suppressed TNBC cell migration, invasion, and EMT process. **F-H**: Western blotting was used to measure the effect of *LINC01614* knockdown on protein levels of EMT markers in TNBC cells. GAPDH acts as a loading control. ***p < 0.001. TNBC: triple-negative breast cancer; CCK-8: cell counting kit-8; EMT: epithelial-mesenchymal transition; RT-qPCR: reverse transcription quantitative polymerase chain reaction.

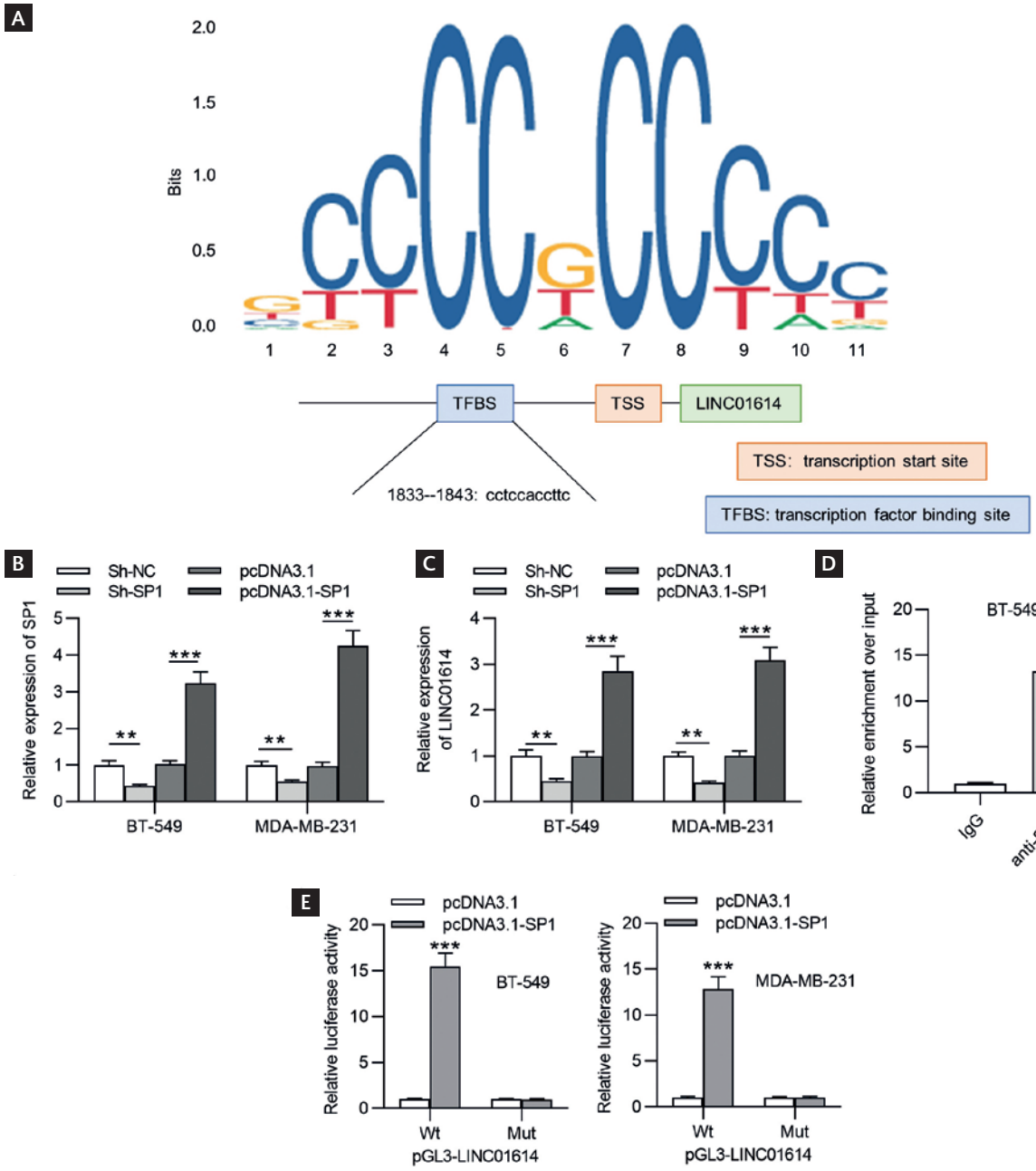


deficiency compared with those in the sh-NC group (Fig. 2C and D). The findings indicate the repressive effect of sh-*LINC01614* on cell migration. In addition, cell invasive ability was also inhibited by the silencing of *LINC01614*, as evidenced by less invaded cells in the sh-*LINC01614* groups in comparison to cell number in the sh-NC group (Fig. 2E). Meanwhile, *LINC01614* knockdown led to the upregulation of E-cadherin protein levels and the downregulation of vimentin levels compared with their levels in the sh-NC group (Fig. 2F-H). The EMT process is characterized by the loss of epithelial surface markers (notably E-cadherin) and the acquisition of mesenchymal markers such as N-cadherin and vimentin[22]. Hence, the data suggest the inhibitory effect of *LINC01614* knockdown on the EMT process of TNBC cells.

Transcription factor SP1 directly bound to the promoter of *LINC01614*

The specific SP1 binding sites in the promoter region of *LINC01614* were analyzed using JASPAR tool (Fig. 3A). According to the results of RT-qPCR, SP1 expression was significantly downregulated in TNBC cells transfected with sh-SP1 ($p < 0.01$) and was prominently upregulated in the cells transfected with pcDNA3.1-SP1 vectors (Fig. 3B, $p < 0.001$). In addition, *LINC01614* expression was diminished in TNBC cells silencing SP1 whereas being upregulated in TNBC cells overexpressing SP1 (Fig. 3C). The results indicate the positive expression correlation between SP1 and *LINC01614*. Data from ChIP assays showed that SP1 directly bound to the

Figure 3. Transcription factor SP1 directly bound to the promoter of *LINC01614*. **A**: the specific SP1 binding sites in the promoter region of *LINC01614* were analyzed using JASPAR tool. **B**: RT-qPCR was performed to verify the knockdown or overexpression efficacy of SP1 in TNBC cells. **C**: The effects of SP1 knockdown (or SP1 overexpression) on *LINC01614* expression in TNBC cells were measured by RT-qPCR. **D** and **E**: chromatin immunoprecipitation assays and luciferase reporter assays were conducted to explore the interaction between SP1 and *LINC01614*. **p < 0.01, ***p < 0.001. TNBC: triple-negative breast cancer; RT-qPCR: reverse transcription quantitative polymerase chain reaction.



chromatin fragments of predicted regions of *LINC01614* in BT-549 and MDA-MB-231 cells (Fig. 3D). In addition, luciferase reporter assay was conducted to further validate the activation of SP1 on *LINC01614* transcription. The results showed an increased luciferase activity of Wt-pGL3-*LINC01614* in the TNBC cells overexpressing SP1, while the luciferase activity of Mut-pGL3-*LINC01614* showed no significant difference between pcDNA3.1-SP1 group and corresponding control group (Fig. 3E). The above findings suggest that *LINC01614* acts as a direct transcription target for SP1.

Figure 4. SP1 reversed the inhibitory effect of *LINC01614* knockdown on the migration, invasion, and EMT process of TNBC cells. **A**: RT-qPCR was performed to measure *LINC01614* expression in TNBC cells silencing *LINC01614* and overexpressing SP1. **B**: CCK-8 assays, **C**: wound healing assays, and. **D**: Transwell invasion assays were carried out to evaluate the effects of *LINC01614* knockdown and SP1 overexpression on cell viability, cell migration, and cell invasion, respectively. **p < 0.01, ***p < 0.001. TNBC: triple-negative breast cancer; RT-qPCR: reverse transcription quantitative polymerase chain reaction, EMT: epithelial-mesenchymal transition *(continues)*.

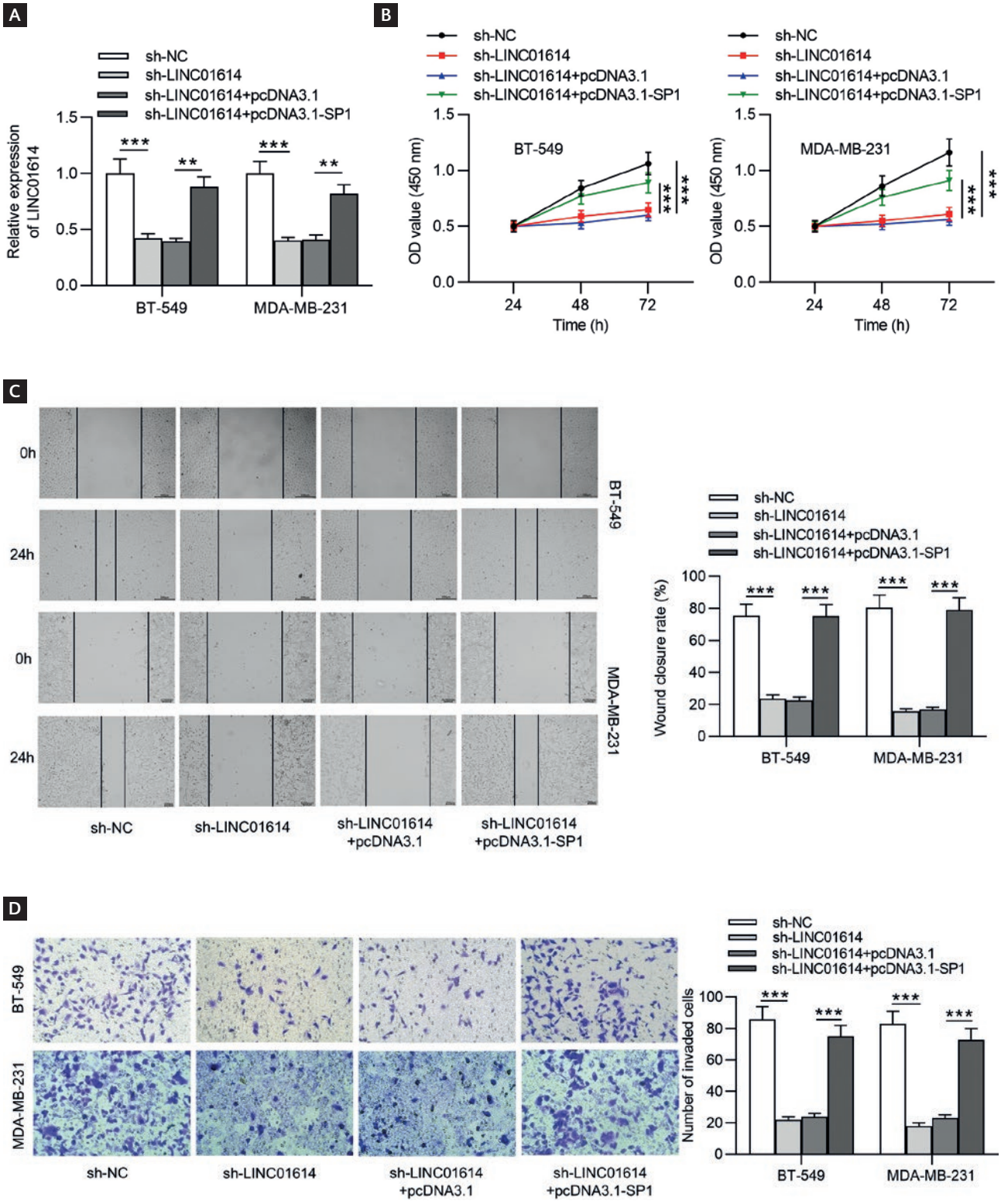
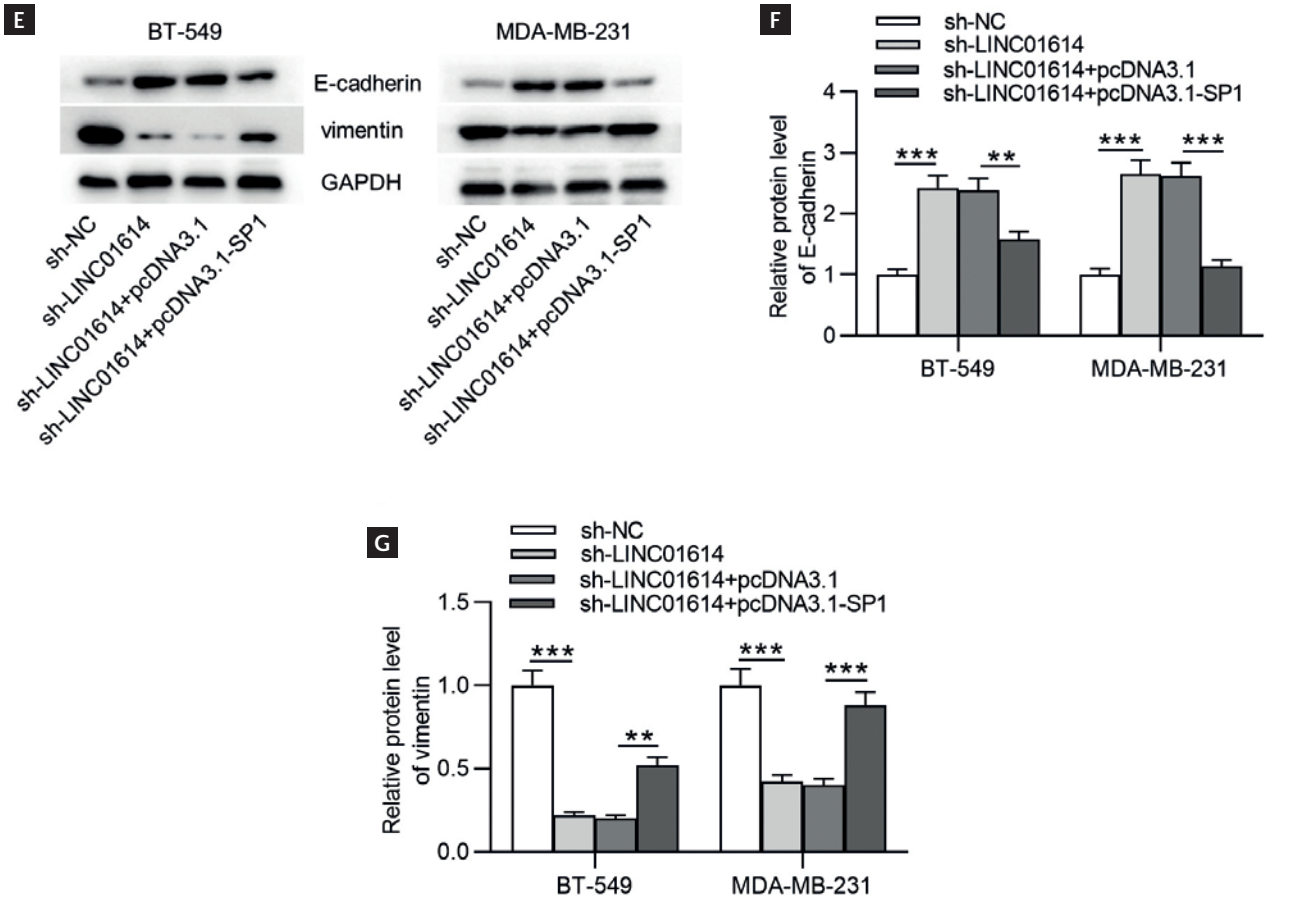


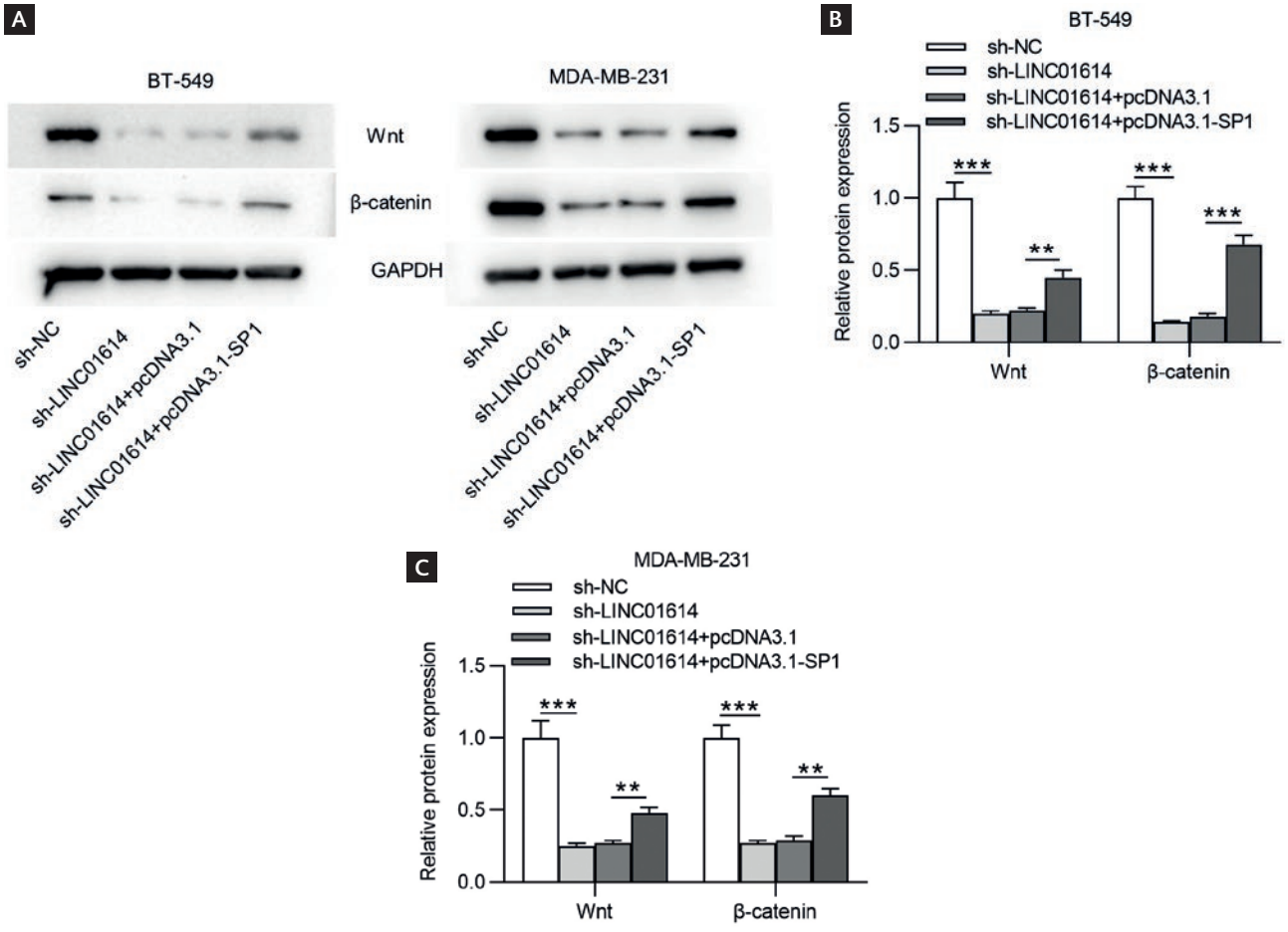
Figure 4. *(continued)* SP1 reversed the inhibitory effect of *LINC01614* knockdown on the migration, invasion, and EMT process of TNBC cells. **E-G**: Western blotting was performed to quantify protein levels of EMT markers in TNBC cells of sh-NC, sh-*LINC01614*, sh-*LINC01614* + pcDNA3.1, and sh-*LINC01614* + pcDNA3.1-SP1 groups. **p < 0.01, ***p < 0.001. TNBC: triple-negative breast cancer; RT-qPCR: reverse transcription quantitative polymerase chain reaction, EMT: epithelial-mesenchymal transition.



SP1 reversed the inhibitory effect of *LINC01614* knockdown on TNBC cell migration, invasion, and EMT process

Subsequently, rescue experiments were performed to investigate whether SP1 is responsible for the functions of *LINC01614* in TNBC cells. RT-qPCR showed that *LINC01614* expression was noticeably reduced in TNBC cells post-transfection of sh-*LINC01614*, and the alteration was mostly rescued by SP1 overexpression (Fig. 4A). In addition, the reduction of TNBC cell viability induced by *LINC01614* knockdown was partially reversed by SP1 overexpression (Fig. 4B, p < 0.001). According to the results of wound closure assays and transwell invasion assays, SP1 upregulation rescued the decrease in wound closure rate and the number of invaded cells caused by *LINC01614* deficiency (Fig. 4C, p < 0.001). The data suggests that SP1 counteracted the inhibitory impact of *LINC01614* knockdown on cell migration and invasion. Data from Western blotting showed that the gain of E-cadherin and the loss of vimentin induced by sh-*LINC01614* were counteracted in the context of SP1 overexpression (Fig. 4E-G). Overall, SP1 reversed the inhibitory impact of *LINC01614* depletion on TNBC cell viability, migration, invasion, and EMT process. The above data indicate that SP1-induced upregulation of *LINC01614* promotes the malignant behavior of TNBC cells.

Figure 5. SP1-induced *LINC01614* activated the Wnt/β-catenin signaling pathway. **A**: Western blotting was performed to measure protein levels of Wnt and β-catenin in triple-negative breast cancer cells of four groups: sh-NC, sh-*LINC01614*, sh-*LINC01614* + pcDNA3.1, and sh-*LINC01614* + pcDNA3.1-SP1. **B** and **C**: relative protein levels of Wnt and β-catenin in each group were quantified with normalization to GAPDH. **p < 0.01, ***p < 0.001.



SP1-induced *LINC01614* activated the Wnt/β-catenin signaling pathway

The aberrant Wnt/β-catenin signaling can promote cancer cell differentiation, proliferation, and stem cell renewal, and targeting the pathway is a promising strategy for personalized cancer treatment[23]. Previously, *LINC01614* was reported to activate the Wnt signaling in pancreatic cancer[24]. Therefore, protein levels of factors involved in the Wnt/β-catenin pathway were measured by Western blotting. As displayed in Fig. 5A-C, *LINC01614* knockdown prominently reduced protein levels of Wnt and β-catenin in BT-549 and MDA-MB-231 cells, and the trend was partially upregulated by SP1 overexpression. The finding indicates that SP1-induced upregulation of *LINC01614* may promote TNBC cell processes by activating the Wnt/β-catenin signaling pathway.

DISCUSSION

TNBC, a subtype of BC, is related to high morbidity and mortality worldwide[25]. Recently, several lncRNAs, such as DDIT4-AS1[26], MALAT1[27], and SEMA3B-AS1[28], have been reported to modulate TNBC progression by mediating various mechanisms. Nevertheless, the functions and underlying mechanisms of lncRNAs in

TNBC have not been clearly illustrated. The present study validated that nuclear transcription factor SP1 induced upregulation of *LINC01614* to activate Wnt/β-catenin signaling in TNBC cells, thereby promoting cell viability, migration, invasion, and EMT process.

According to previous studies, *LINC01614* can act as an oncogene and exerts regulatory control over diverse cellular processes across various types of cancer. For example, *LINC01614* promotes cell proliferation and represses cell apoptosis in lung adenocarcinoma[12]. *LINC01614* knockdown suppressed pancreatic cancer cell process and inhibit tumor proliferation[24]. Consistent with these articles, the present study also verified the oncogenic role of *LINC01614* in TNBC cells. More specifically, this article showed the upregulation of *LINC01614* in TNBC cells and tissues, and that the silencing of *LINC01614* repressed cell migration, invasion, and EMT process.

As an aggressive type of cancer, TNBC is linked to a high probability of metastasis[29]. EMT process has been reported to associated with tumor metastasis[30], and E-cadherin and Vimentin are regarded as important epithelial and mesenchymal markers, respectively[31]. The process is characterized by the absence of epithelial surface markers and the acquisition of mesenchymal markers[22]. Interestingly, *LINC01614* was analyzed to be an EMT-associated oncogene by a previous study[13]. Consistent with that, the current work revealed that *LINC01614* knockdown led to increased E-cadherin expression and decreased vimentin level, indicating the inhibitory effect of *LINC01614* knockdown on the EMT process of TNBC cells. In another words, *LINC01614* facilitates the EMT process of TNBC cells.

Particularly, subcellular fraction assays confirmed the location of *LINC01614* in both the cytoplasm and nucleus of TNBC cells, indicating that *LINC01614* may exert its role in TNBC cells both transcriptionally and post-transcriptionally. At the post-transcriptional level, the ceRNA role of *LINC01614* has been demonstrated in other types of cancer such as osteosarcoma and lung cancer[12,15]. The study concentrated on the role of *LINC01614* at the transcriptional level.

Recently, an increasing number of transcription and epigenetic regulatory factors have been revealed to influence lncRNA expression levels[32]. SP1 is a transcription factor frequently reported in cancer-related studies[33]. For example, SP1 could interact with the promoter of lncRNA LUCAT1 and induce its expression in cervical cancer cells, thereby facilitating cell growth, migration, and invasion[34]. In addition, overexpression of LINC00511 is significantly motivated by SP1 and contributes to glioma progression[35]. SP1 could bind to the lncRNA TINCR promoter and activate its transcription in colorectal cancer[36]. In line with the regulatory trend mentioned in previous articles, SP1 in this study was shown to directly bind to the promoter of *LINC01614* and significantly activate *LINC01614* expression in TNBC cells. Moreover, the current rescue experiments demonstrated that SP1 overexpression partially reversed the inhibitory effect of *LINC01614* knockdown on the malignant behavior of TNBC cells, suggesting the involvement of SP1 in the oncogenic mechanisms of *LINC01614* in TNBC. The regulatory relationship between SP1 and *LINC01614* is consistent with the article focused on the two players in glioma[19].

Wnt/β-catenin signaling participates in tumor growth and metastasis[37-39]. In a very recent study, Wang et al. revealed that Wnt/β-catenin signaling was a downstream pathway that can be regulated by *LINC01614* in bladder cancer[40]. Consistently, the study discovered that *LINC01614* depletion led to reduced protein expression of Wnt and β-catenin, and the trend was partly rescued by SP1 overexpression. The findings indicate that SP1-induced upregulation of Wnt/β-catenin signaling. It is worth mentioning that SP1 overexpression can partly but not completely recover the effect of *LINC01614* depletion on cell viability and protein levels of EMT markers, Wnt, and β-catenin. That may be due to the involvement of other molecules in the regulatory networks related to SP1 and *LINC01614*. For example, there could be additional potential lncRNAs that are activated by SP1 and positively regulates the Wnt/β-catenin signaling in TNBC cells.

Since TNBC is perceived as a complex clinical entity, the study lacks further exploration of the SP1/*LINC01614*/Wnt/β-catenin axis in TNBC, which is a

limitation of the study. Another limitation is the absence of animal experiments for further verification of *in vitro* findings.

In conclusion, experimental results in this study showed that *LINC01614* upregulation induced by SP1 promoted TNBC cell migration, invasion, and EMT process by activating the Wnt/β-catenin signaling. The study might provide a novel therapeutical strategy for the advancement of targeted therapy in TNBC treatment. In the future, more experiments shall be performed to further explore other molecules involved in the regulatory axis of SP1/*LINC01614*/Wnt/β-catenin, and the post-transcriptional role of *LINC01614* in TNBC can also be investigated.

SUPPLEMENTARY MATERIAL

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69:7-34.
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74:12-49.
3. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. Annu Rev Med. 2011;62:233-47.
4. Robidoux A, Tang G, Rastogi P, Geyer CE Jr., Azar CA, Atkins JN, et al. Lapatinib as a component of neoadjuvant therapy for HER2-positive operable breast cancer (NSABP protocol B-41): an open-label, randomised phase 3 trial. Lancet Oncol. 2013; 14:1183-92.
5. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res. 2020;22:61.
6. Bai X, Ni J, Beretov J, Graham P, Li Y. Triple-negative breast cancer therapeutic resistance: where is the Achilles' heel? Cancer Lett. 2021;497:100-11.
7. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, et al. Recent advances in therapeutic strategies for triple-negative breast cancer. J Hematol Oncol. 2022;15:121.
8. Lu B, Natarajan E, Balaji Raghavendran HR, Markandan UD. Molecular classification, treatment, and genetic biomarkers in triple-negative breast cancer: a review. Technol Cancer Res Treat. 2023;22:15330338221145246.
9. Xu J, Wu KJ, Jia QJ, Ding XF. Roles of miRNA and lncRNA in triple-negative breast cancer. J Zhejiang Univ Sci B. 2020;21: 673-89.
10. Hu J, Huang H, Xi Z, Ma S, Ming J, Dong F, et al. LncRNA SEMA3B-AS1 inhibits breast cancer progression by targeting miR-3940/KLLN axis. Cell Death Dis. 2022;13:800.
11. Li F, Xian D, Huang J, Nie L, Xie T, Sun Q, et al. SP1-induced upregulation of LncRNA AFAP1-AS1 promotes tumor progression in triple-negative breast cancer by regulating mTOR pathway. Int J Mol Sci. 2023;24:13401.
12. Liu AN, Qu HJ, Yu CY, Sun P. Knockdown of LINC01614 inhibits lung adenocarcinoma cell progression by up-regulating miR-217 and down-regulating FOXP1. J Cell Mol Med. 2018;22:4034-44.
13. Wang D, Zhang H, Fang X, Cao D, Liu HJ. Pan-cancer analysis reveals the role of long non-coding RNA LINC01614 as a highly cancer-dependent oncogene and biomarker. Oncol Lett. 2020;20:1383-99.
14. Sheng W, Zhou W, Cao Y, Zhong Y. Revealing the role of lncRNA CCDC144NL-AS1 and LINC01614 in gastric cancer via integrative bioinformatics analysis and experimental validation. Front Oncol. 2021;11:769563.
15. Cai Q, Zhao X, Wang Y, Li S, Wang J, Xin Z, et al. LINC01614 promotes osteosarcoma progression via miR-520a-3p/SNX3 axis. Cell Signal. 2021;83:109985.
16. Vishnubalaji R, Shaath H, Elkord E, Alajez NM. Long non-coding RNA (lncRNA) transcriptional landscape in breast cancer identifies LINC01614 as non-favorable prognostic biomarker regulated by TGFβ and focal adhesion kinase (FAK) signaling. Cell Death Discov. 2019;5:109.
17. Zhang X, Zeng J, Wang J, Yang Z, Gao S, Liu H, et al. Revealing the potential markers of N(4)-acetylcytidine through acrip-seq in triple-negative breast cancer. Genes (Basel). 2022; 13:2400.
18. Chen Y, Li Z, Chen X, Zhang S. Long non-coding RNAs: from disease code to drug role. Acta Pharm Sin B. 2021;11:340-54.
19. Wang H, Wu J, Guo W. SP1-Mediated upregulation of lncRNA LINC01614 functions a ceRNA for miR-383 to facilitate glioma progression through regulation of ADAM12. Onco Targets Ther. 2020;13:4305-18.
20. Xiao F, Jia H, Wu D, Zhang Z, Li S, Guo J. LINC01234 aggravates cell growth and migration of triple-negative breast cancer by activating the Wnt pathway. Environ Toxicol. 2021;36:1999-2012.
21. Marra A, Curigliano G. Adjuvant and neoadjuvant treatment of triple-negative breast cancer with chemotherapy. Cancer J. 2021;27:41-9.
22. Serrano-Gomez SJ, Maziveyi M, Alahari SK. Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. Mol Cancer. 2016;15:18.
23. Zhang Y, Wang X. Targeting the Wnt/β-catenin signaling pathway in cancer. J Hematol Oncol. 2020;13:165.
24. Chen LJ, Wu L, Wang W, Zhai LL, Xiang F, Li WB, et al. Long non-coding RNA 01614 hyperactivates WNT/β-catenin signaling to promote pancreatic cancer progression by suppressing GSK-3β. Int J Oncol. 2022;61:116.
25. Azari M, Bahreini F, Uversky VN, Rezaei N. Current therapeutic approaches and promising perspectives of using bioengineered peptides in fighting chemoresistance in triple-negative breast cancer. Biochem Pharmacol. 2023;210:115459.
26. Jiang T, Zhu J, Jiang S, Chen Z, Xu P, Gong R, et al. Targeting lncRNA DDIT4-AS1 sensitizes triple negative breast cancer to chemotherapy via suppressing of autophagy. Adv Sci (Weinh). 2023;10:e2207257.
27. Liu J, Shi Y, Wu M, Zhang F, Xu M, He Z, et al. JAG1 enhances angiogenesis in triple-negative breast cancer through promoting the secretion of exosomal lncRNA MALAT1. Genes Dis. 2023; 10:2167-78.
28. Yu H, Wu Y, Huang J, Li S. lncRNA SEMA3B-AS1 Inhibits miR-513c-5p to regulate the progression of triple-negative breast cancer. Anticancer Res. 2023;43:5475-84.
29. Deepak KG, Vempati R, Nagaraju GP, Dasari VR, Nagini S, Rao DN, et al. Tumor microenvironment: challenges and opportunities in targeting metastasis of triple negative breast cancer. Pharmacol Res. 2020;153:104683.
30. Saitoh M. Involvement of partial EMT in cancer progression. J Biochem. 2018;164:257-64.
31. Lei H, Gao Y, Xu X. LncRNA TUG1 influences papillary thyroid cancer cell proliferation, migration and EMT formation through targeting miR-145. Acta Biochim Biophys Sin (Shanghai). 2017; 49:588-97.
32. Jiang Q, Wang J, Wang Y, Ma R, Wu X, Li Y. TF2LncRNA: identifying common transcription factors for a list of lncRNA genes from ChIP-Seq data. Biomed Res Int. 2014;2014:317642.
33. Liu G, Ye Z, Zhao X, Ji Z. SP1-induced up-regulation of lncRNA SNHG14 as a ceRNA promotes migration and invasion of clear cell renal cell carcinoma by regulating N-WASP. Am J Cancer Res. 2017;7:2515-25.
34. Zhang L, Liu S, Song L, Yao HR. SP1-induced up-regulation of lncRNA LUCAT1 promotes proliferation, migration and invasion of cervical cancer by sponging miR-181a. Artif Cells Nanomed Biotechnol. 2019;47:556-64.

35. Li C, Liu H, Yang J, Yang J, Yang L, Wang Y, et al. Long noncoding RNA LINC00511 induced by SP1 accelerates the glioma progression through targeting miR-124-3p/CCND2 axis. J Cell Mol Med. 2019;23:4386-94.
36. Yu S, Wang D, Shao Y, Zhang T, Xie H, Jiang X, et al. SP1-induced lncRNA TINCR overexpression contributes to colorectal cancer progression by sponging miR-7-5p. Aging (Albany NY). 2019; 11:1389-403.
37. Schneider JA, Logan SK. Revisiting the role of Wnt/β-catenin signaling in prostate cancer. Mol Cell Endocrinol. 2018; 462:3-8.
38. Guo YZ, Xie XL, Fu J, Xing GL. SOX9 regulated proliferation and apoptosis of human lung carcinoma cells by the Wnt/β-catenin signaling pathway. Eur Rev Med Pharmacol Sci. 2018;22:4898-907.
39. Shao TR, Zheng ZN, Chen YC, Wu QQ, Huang GZ, Li F, et al. LncRNA AC007271.3 promotes cell proliferation, invasion, migration and inhibits cell apoptosis of OSCC via the Wnt/β-catenin signaling pathway. Life Sci. 2019;239:117087.
40. Wang Z, Yan H, Cheng D, Xu L, Shen T, Chen Y, et al. Novel lncRNA LINC01614 facilitates bladder cancer proliferation, migration and invasion through the miR-217/RUNX2/Wnt/β-catenin axis. Cancer Manag Res. 2021;13:8387-97. Retracted article.