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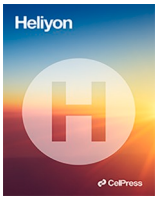

Research article

LINC01503 promotes the cell proliferation, migration and invasion of triple-negative breast cancer as a ceRNA to elevate SPNS2 expression by sponging miR-335–5p

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ABSTRACT

Objective: Triple-negative breast cancer (TNBC) is a common cancer with high aggressiveness and high mortality in women. Recently, a plenty of studies have indicated that long non-coding RNAs (lncRNAs) exert the crucial function in human cancers, TNBC is included. The carcinogenicity of lncRNA long intergenic non-protein coding RNA 1503 (LINC01503) has been confirmed in several cancers, nevertheless, its function in TNBC still unclear. Therefore, our study aimed to reveal the underlying mechanism of LINC01503 in TNBC.

Methods: In our study, RT-qPCR was performed to detect the expression of LINC01503 in TNBC cells. The proliferative, invasive, migratory and apoptotic abilities of TNBC cells were detected by functional assay such as CCK-8, clone formation, EdU staining, transwell, and flow cytometry. RIP, RNA pull down, and luciferase assay revealed interactions between LINC01503, miR-335–5p, and sphingolipid transporter protein 2 (SPNS2). Finally, rescue experiments were performed to validate the previous results.

Results: LINC01503 expression was singularly high in TNBC cells. LINC01503 knockdown could restrain cell proliferation, invasion and migration, but accelerated cell apoptosis in TNBC. What's more, miR-335–5p could be sponged by LINC01503 in TNBC. We also found that overexpressed miR-335–5p could inhibit cell proliferation, migration and invasion and facilitates cell apoptosis. Moreover, SPNS2 was the target gene of miR-335–5p and it functioned as an oncogene in TNBC cells. Finally, we found that overexpressed SPNS2 or inhibited miR-335–5p could reverse the suppressive function of silencing LINC01503 on TNBC progression.

Conclusion: LINC01503 could facilitate cell proliferation, migration and invasion of TNBC by sponging miR-335–5p to elevate SPNS2 expression.

1. Introduction

Breast cancer (BC) is a common cancer in women and one of the major factors of cancer-related deaths in all around the world [1]. In the past decade, the incidence and fatality rate of BC have continued to rise [2]. As we know, BC is a heterogeneous disease with different subtypes, and triple-negative breast cancer (TNBC) is the most aggressive subtype. It is characterized by the deficiency of estrogen, progesterone receptors and human epidermal growth factor receptor 2 (HER2) [3]. At the same time, its high aggressiveness is also closely related to the high metastasis rate. At present, the more common treatment methods include surgery, chemotherapy and

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radiotherapy [4]. But for advanced patients, the treatment effect is minimal and the survival rate is extremely low [5]. Therefore, it is urgent to deeply understand the molecular mechanism of TNBC progression.

A growing number of studies have shown that non-coding RNAs, including circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs), play an important role in TNBC [6–8]. LncRNAs are defined as the group of non-coding RNAs with more than 200 nucleotides in length [9]. Meanwhile, lncRNAs are incapable to encode proteins [9]. An increasing number of researched has unclosed that lncRNAs possess the notable expression in assorted human disease, including different kind of cancers, and exert the vital and immeasurable functions in tumorigenesis and development [10–12]. LncRNAs frequently function as oncogenes or anti-oncogenes in dissimilar cancers depended on their expression [13]. For example, SNHG1 was verified to possess a high expression cervical cancer cells and enhanced cell proliferation and migration of cervical cancer [14]. MEG3 was reported to lowly express in gastric cancer cells and repress metastasis of gastric cancer [15]. Also, lncRNA long intergenic non-protein coding RNA 1503 (LINC01503) has been confirmed to exert the carcinogenic effect in several types of cancer. For example, upregulated LINC01503 was proven to accelerate cell growth of esophageal squamous cell carcinoma [16]. Also, it was reported that LINC01503 expedited the occurrence and development of colorectal cancer through modulating miR-4492/FOXK1 signaling [17]. Moreover, LINC01503 was confirmed to facilitate cell growth and epithelial-mesenchymal transition (EMT) progress in cholangiocarcinoma [18]. Nevertheless, the specific function and mechanism of LINC01503 in TNBC remains to be probed.

Recently, the competitive endogenous RNA (ceRNA) network has been searched and identified in various cancers [19]. The ceRNA mechanism refers to that lncRNA can bind with miRNAs to relieve the inhibition of microRNAs (miRNAs) on messenger RNAs (mRNAs) [19,20]. For instance, HCP5 accelerated TNBC development via serving as a ceRNA to sponge miR-219a-5p [21]. GAS5 suppressed TNBC via inhibition of proliferation via serving as a ceRNA to sponge miR-196a-5p [22].

In our research, we aimed to investigate the specific function and mechanism of LINC01503 in TNBC. At the same time, we also detected whether LINC01503 could function as a ceRNA to exert the regulatory role on TNBC.

2. Materials and methods

2.1. Cell lines

The normal mammary epithelial cell line (HMEC) and TNBC cell lines (HCC70, MDA-MB-231, MDA-MB-468 and BT549) were obtained from ATCC cell bank (Rockville, Maryland). HMEC cells were cultivated in Mammary Epithelial Cell Basal Medium (Invitrogen, Carlsbad, CA). HCC70 and BT549 cells were cultivated in RPMI-1640 Medium with 10 % fetal bovine serum (FBS; Invitrogen). MDA-MB-231 and MDA-MB-468 cells were cultivated in Leibovitz's L-15 Medium with 10 % FBS (Invitrogen). These cells were deposited in 5 % CO_2 at 37 °C.

2.2. Real-time quantitative PCR (RT-qPCR)

Based on the protocols of supplier, the total RNA was extracted by TRIzol method (Invitrogen). Then the cDNA was acquired via reverse transcription. For the sake of measuring genes expressions, RT-qPCR was conducted utilizing SYBR green Supermix (Takara, Shiga, Japan) and $2^{-\Delta\Delta Ct}$ method was applied for calculating the expression level. GAPDH or U6 served as control. The experiment was repeated at least three times.

2.3. Plasmid transfection

For the purpose of silencing SPNS2 and LINC01503, specific shRNAs and negative control (NC)-shRNAs were devised and composed by Genepharma (Shanghai, China) for transfection into HCC70 and MDA-MB-231 cells. Besides, miR-335–5p mimics/inhibitor and NC mimics/inhibitor, as well as pcDNA3.1/SPNS2 and pcDNA3.1-NC, were also synthesized by Genepharma. Lipofectamine 3000 (Invitrogen) was applied for transfection for 48 h. The experiment was repeated at least three times.

2.4. Colony formation

The transfected cells were seeded into the 6-well-plate at the density of 600 cells per well for cultivation. After fortnight, cells were fixed by 4 % paraformaldehyde and stained by 0.5 % crystal violet (Solarbio, Beijing, China). In the end, the number of colonies was counted manually. The experiment was repeated at least three times.

2.5. EdU staining assay

In accordance with the user guide, 5-ethynyl-2'-deoxyuridine (EdU) staining assay kit (Ribobio, Guangzhou, China) was applied for conducted this assay. The transfected cells were seeded into 96-well plate and EdU medium diluent were added. Then cells were fixed by 4 % paraformaldehyde, permeated by 0.5 % Troxin X-100 (Solarbio). Next cells were cultured with 1 × Apollo®488 fluorescent dye reaction solution. DAPI was utilized to stain nucleus and we finally observed through utilizing fluorescence microscope (Olympus, Tokyo, Japan). The experiment was repeated at least three times.

2.6. Flow cytometry analysis

The transfected cells were rinsed in PBS which was precooled, then cell apoptosis was evaluated through Annexin V-FITC/PI Apoptosis kit (Life Technologies, Carlsbad, CA). After cultivation for half an hour in the dark, the apoptotic situation of cells was assayed by flow cytometry (FACScan; BD Biosicences). The experiment was repeated at least three times.

2.7. Transwell assay

Transwell assay was conducted to measure cell migration and invasion by cell culture chambers. The serum-free medium including cells was seeded into the upper chamber. For invasion assay, we put a layer of Matrigel in advance. Then, culture medium containing 10 % FBS were supplemented to the bottom chamber. After that, cells which successfully migrated or invaded to the bottom chamber were fixed and dyed by 4 % paraformaldehyde and 0.5 % crystal violet separately (Solarbio). In the end, we observed by a microscope (Olympus, Tokyo, Japan). The experiment was repeated at least three times.

2.8. Subcellular fractionation

PARIS Kit (Life Technologies, Carlsbad, CA) was utilized to conduct the fraction of cytoplasm and nucleus in line with protocols of suppliers. Then RT-qPCR analysis was conducted to detect the expression of LINC01503. GAPDH and U6 served as control, respectively. The experiment was repeated at least three times.

2.9. RNA fluorescence in situ hybridization (FISH)

RNA FISH KIT for LINC01503 was devised and composed via RiboBio (Guangzhou, China) and conducted in line with user guides. DAPI (RiboBio) was applied for counterstaining the nucleus. Finally, cells were observed via fluorescence microscope. The experiment was repeated at least three times.

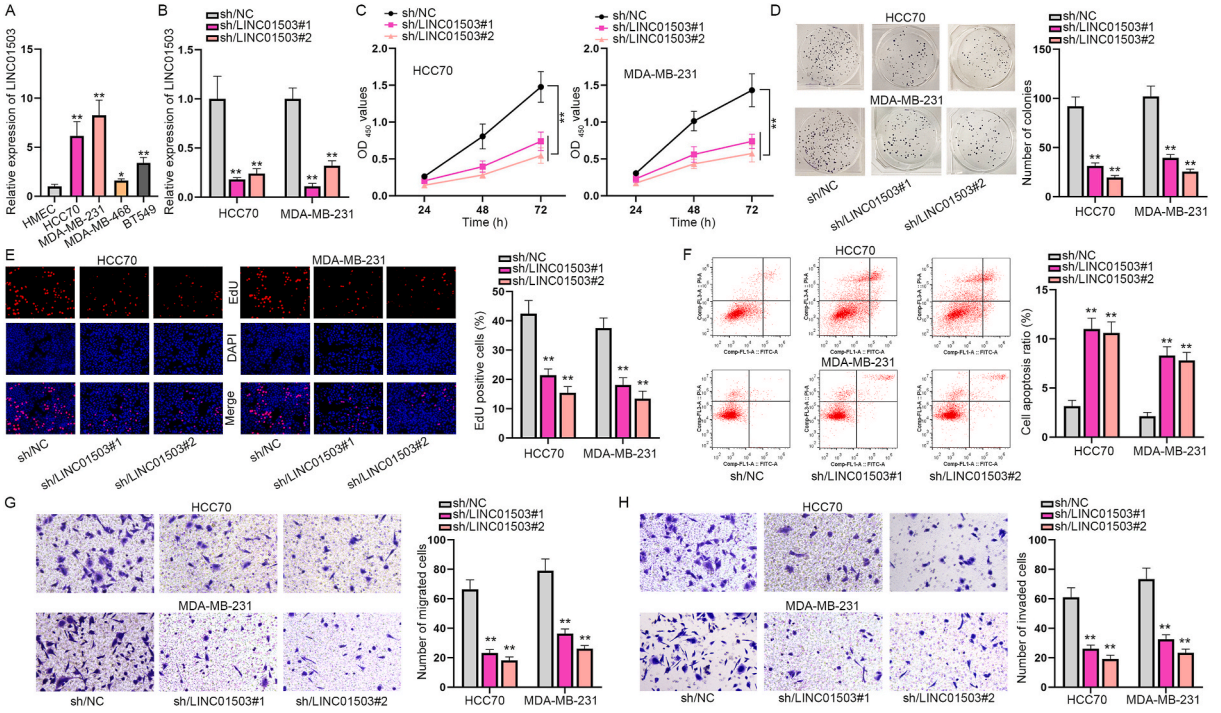

Fig. 1. LINC01503 is highly expressed in TNBC cells and acts as an oncogene in TNBC
A. RT-qPCR detected expression level of LINC01503 in the normal mammary epithelial cell line (HMEC) and TNBC cell lines (HCC70, MDA-MB-231, MDA-MB-468 and BT549). B. RT-qPCR tested the knockdown efficiency of LINC01503 in HCC70 and MDA-MB-231 cells. C. CCK-8 assay was utilized to detect cell viability in HCC70 and MDA-MB-231 cells when LINC01503 was knocked down. D-E. Colony formation and EdU staining assay were carried out to estimate proliferation of HCC70 and MDA-MB-231 cells after inhibiting LINC01503. F. Flow cytometry was applied for detecting cell apoptosis when LINC01503 was silenced in HCC70 and MDA-MB-231 cells. G-H. Transwell assays examined HCC70 and MDA-MB-231 cell migration and invasion ability after LINC01503 knocked down. $^{**}P < 0.01$.

2.10. RNA pull down assay

The utilization of Pierce magnetic RNA protein pull-down kit (Shanghai Canspec Scientific Instruments Co., Ltd.) was to conduct this assay. Protein was extracted from cells and then mixed with biotinylated probes. Following, they were incubated with magnetic beads at 4 °C for 1 h. After elution, we carried out RT-qPCR to detect. The experiment was repeated at least three times.

2.11. RNA immunoprecipitation (RIP) assay

RIP analysis was by utilizing the Magna RIP™RNA binding protein immunoprecipitation kit (Millipore, Bedford, MA). Cells were lysed via using RIP lysis buffer. Following, they were co-immunoprecipitated with Ago2 antibody. And the IgG antibody served as the negative control. Next, we added magnetic beads and conducted RT-qPCR to analysis. The experiment was repeated more than three times.

2.12. Luciferase reporter assay

The wild-type and mutated SPNS2 or LINC01503 fragments covering the miR-335–5p binding sites were inserted to the pmirGLO luciferase vector (Promega, Madison, WI). They were named SPNS2-WT/Mut and LINC01503-WT/Mut. Then they were subjected to co-transfection with miR-335–5p mimics or NC mimics into HCC70 and MDA-MB-231 cells for 48 h. In the end, the luciferase activity was detected by Luciferase Reporter Assay System (Promega). The experiment was repeated at least three times.

2.13. Statistical analysis

In our research, the whole experiments were carried out more than three times. And the data was displayed as mean ± standard deviation (SD). GraphPad Prism 5.0 software was utilized for statistical analysis with Student's t-test and one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. LINC01503 is highly expressed in TNBC cells and acts as an oncogene in TNBC

For the sake of investigating the potential role of LINC01503 in TNBC, the expression level of LINC01503 was detected at first through RT-qPCR. In contrast to the normal mammary epithelial cell line (HMEC), LINC01503 expression was singularly high in TNBC

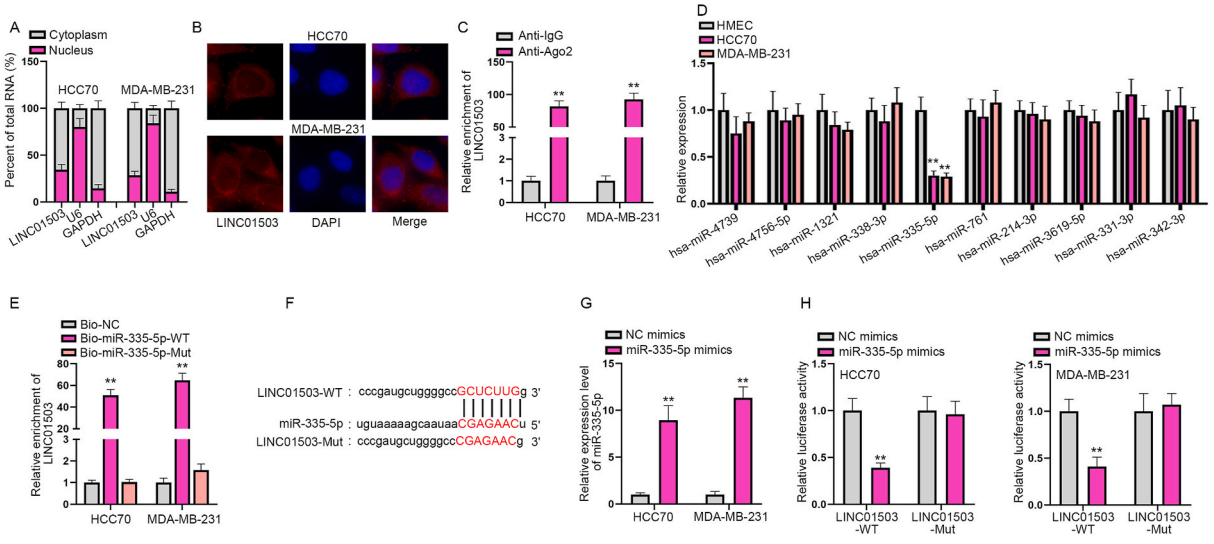

Fig. 2. LINC01503 sponges miR-335–5p in TNBC
A-B. Subcellular fractionation assay and FISH assay were utilized to detect the distribution of LINC01503 in HCC70 and MDA-MB-231 cells. C. RIP assay was applied for detecting the relation of LINC01503 and Ago2 protein. D. RT-qPCR detected expression of miR-4739, miR-4756–5p, miR-1321, miR-338–3p, miR-335–5p, miR-761, miR-214–3p, miR-3619–5p, miR-331–3p and miR-342–3p in HCC70, MDA-MB-231 and HMEC cells. E. RNA pull down assay detected the relative enrichment of LINC01503 pulled down by biotinylated wild and mutant miR-335–5p. F. Binding site of LINC01503 and miR-335–5p was predicted by ENCORI databases. G. The overexpression efficiency of miR-335–5p was detected through RT-qPCR in HCC70 and MDA-MB-231 cells. H. Luciferase reporter assay detected the luciferase activity of LINC01503-WT/Mut by miR-335–5p mimics for evaluating the interaction of LINC01503 and miR-335–5p. **$P < 0.01$.

cell lines (HCC70, MDA-MB-231, MDA-MB-468 and BT549) (Fig. 1A). Obviously, LINC01503 expression was higher in HCC70 and MDA-MB-231 cells than that in other cell lines. Hence, we chose HCC70 and MDA-MB-231 cells for subsequent experiments. Then we knocked down LINC01503 in HCC70 and MDA-MB-231 cells by transfecting with sh-LINC01503#1/2 and utilized RT-qPCR analysis to detect the transfection efficiency. As a result, we discovered that LINC01503 expression was effectively inhibited after transfection (Fig. 1B). Following, a chain of loss-of-function assays was conducted to evaluate the influence of LINC01503 depletion on TNBC cells. First of all, we observed from the CCK-8 assay that optical density at 450 nm generally displayed an upward trend. Interestingly, after transfection with sh-LINC01503#1/2, the values of optical density were decreased in comparison of negative control, suggesting that LINC01503 depletion could restrain cell viability (Fig. 1C). Then, colony formation assay was performed to measure cell proliferation and the results indicated that cell proliferation capability was hampered since the number of colonies was reduced when LINC01503 was silenced in cells (Fig. 1D). Also, cell proliferation was further estimated through EdU assay and the results illustrated that, in comparison of negative control group, EdU positive cells were declined after down-regulating LINC01503, which demonstrated that cell proliferation was suppressed by the lack of LINC01503 (Fig. 1E). As for cell apoptosis, we conducted the flow cytometry analysis. As we expected, the apoptosis rate of cells was elevated compared with negative control, suggesting cell apoptosis could be accelerated by knockdown of LINC01503 (Fig. 1F). Finally, the capabilities of cell migration and invasion were detected via transwell assays. The results illustrated that the number of migrated and invaded cells was reduced in sh-LINC01503-mediated cells, indicating cell migration and invasion could be repressed when we transfected sh-LINC01503#1/2 into HCC70 and MDA-MB-231 cells (Fig. 1G–H).

3.2. *LINC01503 sponges miR-335–5p in TNBC*

After having a basic understanding of LINC01503 role, we began to explore the mechanism of LINC01503 in TNBC. Researches have confirmed that lncRNA can serve as a sponge to bind with miRNAs, so as to release mRNA expression at post-transcriptional level [23]. This regulatory mechanism is called ceRNA [24]. To exert this regulatory mechanism, the location of lncRNA needs to be in the cytoplasmic part of cells. Thus, we first to detect the distribution of LINC01503 in cells by subcellular fractionation assay and FISH assay. We could observe that LINC01503 was mostly located in the part of cytoplasm, indicating that LINC01503 might act as a ceRNA at post-transcriptional level (Fig. 2A–B). Then RIP assay detected the relationship of LINC01503 and Ago2, and the results indicated that LINC01503 was enriched in anti-Ago2 group, which implied that LINC01503 could bind with Ago2 and exert the sponge function (Fig. 2C). Accordingly, we made predictions on the target genes of LINC01503 in TNBC cells through utilizing ENCORI (http://

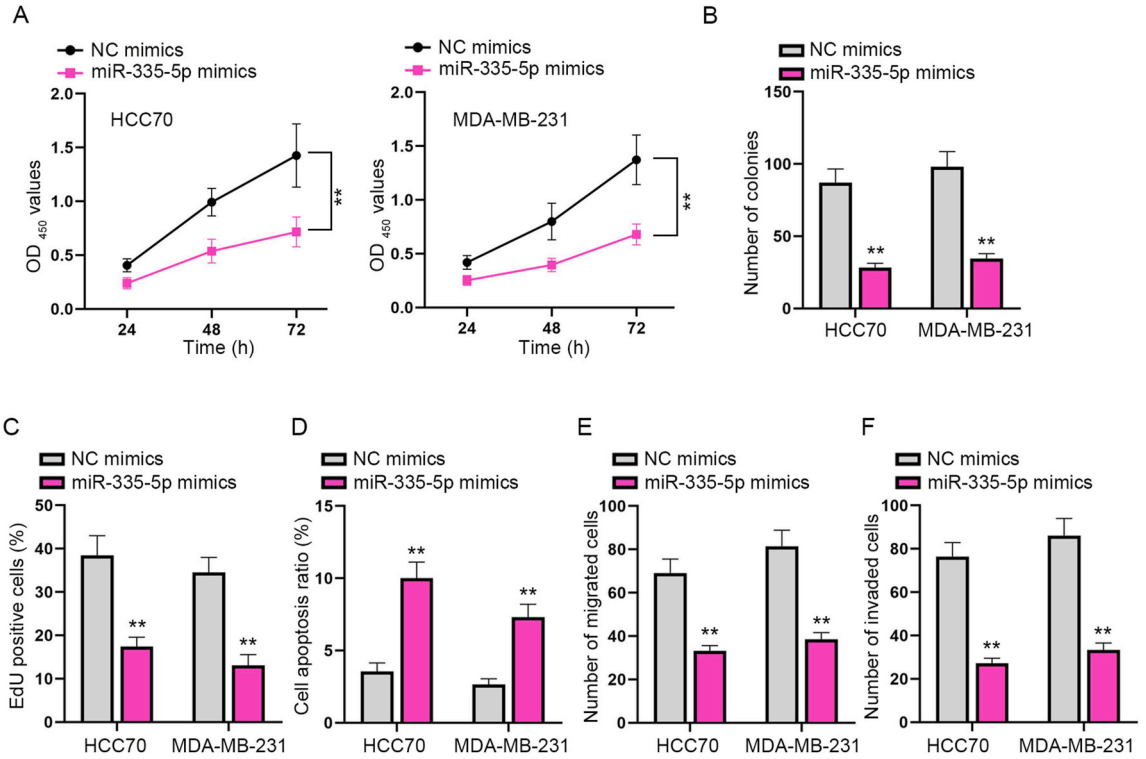

Fig. 3. MiR-335–5p inhibits cell proliferation, migration and invasion of TNBC
A. CCK-8 assay was utilized to detect cell viability after miR-335–5p was overexpressed. **B–C.** Colony formation and EdU staining assay estimated the influence of overexpressing miR-335–5p on cell proliferation. **D.** Flow cytometry was applied for detecting cell apoptosis when miR-335–5p was upregulated in cells. **E-F.** Cell migration and invasion ability was estimated by transwell assay in cells transfected with miR-335–5p mimics. **P < 0.01.

starbase.sysu.edu.cn/) databases. Ten miRNAs (miR-4739, miR-4756–5p, miR-1321, miR-338–3p, miR-335–5p, miR-761, miR-214–3p, miR-3619–5p, miR-331–3p and miR-342–3p) were predicted and we detected their expression levels in HCC70, MDA-MB-231 and HMEC cells through RT-qPCR. We discovered that only miR-335–5p expression was down-regulated in HCC70 and MDA-MB-231 cells compared with HMEC cells (Fig. 2D). Thus, we selected miR-335–5p for the later experiments. RNA pull down assay was conducted for detect the bind situation between miR-335–5p and LINC01503, and the result displayed that LINC01503 was heavily enriched in the pull down of biotinylated miR-335-5p-WT, indicating that LINC01503 could bind with miR-335–5p (Fig. 2E).

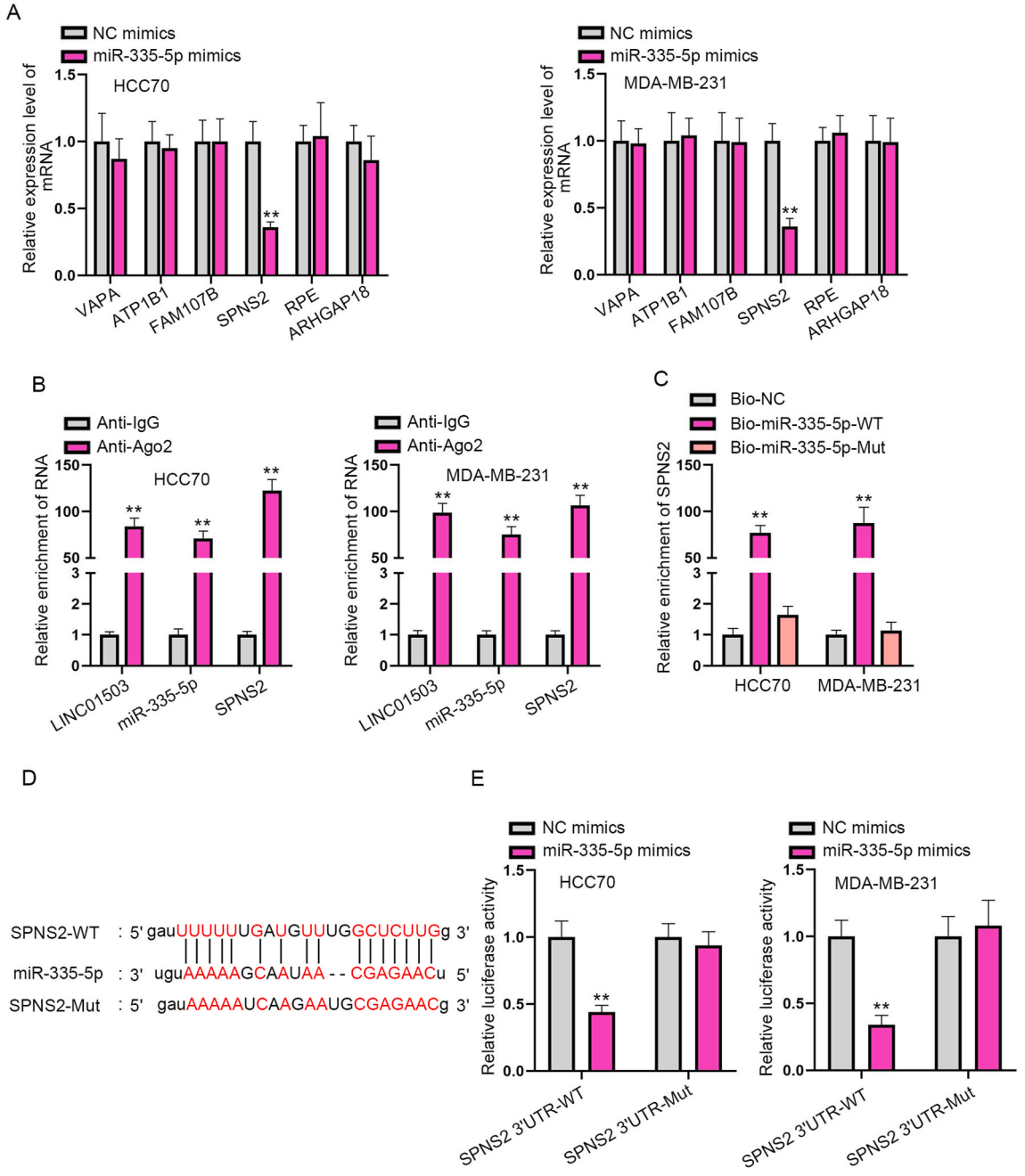

Fig. 4. SPNS2 is the target gene of miR-335–5p in TNBC

A. RT-qPCR examined the expression of VAPA, ATP1B1, FAM107B, SPNS2, RPE and ARHGAP18 in cells when miR-335–5p was overexpressed. **B.** RIP assay detected enrichment of SPNS2, miR-335–5p and LINC01503 in Ago2 and IgG group for evaluating their connection. **C.** RNA pull down assay further verified the interaction of SPNS2 and miR-335–5p. **D.** Binding site of SPNS2 and miR-335–5p was predicted through ENCORI. **E.** Luciferase reporter assay was conducted to detect the luciferase activity of SPNS2 3'UTR-WT/Mut when miR-335–5p was subjected to up-regulation. $^{**}P < 0.01$.

Following from that, we found the binding site of miR-335–5p and LINC01503 through ENCORI databases (Fig. 2F). Then we overexpressed miR-335–5p via transfecting miR-335–5p mimics into HCC70 and MDA-MB-231 cells (Fig. 2G). Next, we carried out the luciferase reporter assay to verify their correlation. From the results of luciferase reporter assay, we discovered that the luciferase activity of LINC01503-WT was reduced by overexpressed miR-335–5p. However, the luciferase activity of LINC01503-Mut almost unchanged under the overexpression of miR-335–5p (Fig. 2H).

3.3. MiR-335–5p inhibits cell proliferation, migration and invasion of TNBC

Next, we continued to detect the specific functions of miR-335–5p in TNBC. Like the process of detecting the function of LINC01503, we carried out a series of functional assays. In CCK-8 assay, we observed that the optical density value was inhibited in cells after miR-335–5p was overexpressed, indicating cell viability was restrained by overexpressed miR-335–5p (Fig. 3A). Then colony formation and EdU assay further conducted to detect cell proliferation and we discovered that upregulated miR-335–5p exerted the inhibitory function on cell proliferation (Fig. 3B–C). What's more, flow cytometry analysis also indicated that cell apoptotic ability could be expedited through miR-335–5p upregulation (Fig. 3D). And it was illustrated through transwell assay that cell migration and invasion could also be suppressed when miR-335–5p was upregulated in cells (Fig. 3E–F).

3.4. SPNS2 is the target gene of miR-335–5p in TNBC

For the sake of investigating the downstream target of miR-335–5p in TNBC, we utilized ENCORI and predicted six mRNAs (VAPA, ATP1B1, FAM107B, SPNS2, RPE and ARHGAP18) that possessed the possible binding ability with miR-335–5p under the specific condition (Degradome-Data $\geq$2) in TargetScan database. Then we conducted RT-qPCR to detect their expressions when miR-335–5p was overexpressed in cells. The RT-qPCR results indicated that only sphingolipid transporter 2 (SPNS2) expression could be inhibited by upregulated miR-335–5p, while other mRNAs expressions didn't be affected by overexpressed miR-335–5p (Fig. 4A). Thus, SPNS2 was selected to continue this research. RIP assay was applied for ascertaining that LINC01503, miR-335–5p and SPNS2 were enriched in anti-Ago2 group, suggesting they were coexisted in RISC complex (Fig. 4B). Moreover, in Fig. 4C, RNA pull down assay further proved that SPNS2 could be pulled down by biotinylated miR-335-5p-WT, indicating that miR-335–5p combined with SPNS2. In order to further probe whether miR-335–5p targeted SPNS2 directly, we found the binding site of miR-335–5p and SPNS2 according to ENCORI (Fig. 4D). Then the luciferase reporter assay demonstrated that the luciferase activity of SPNS2 3'UTR-WT was decreased via

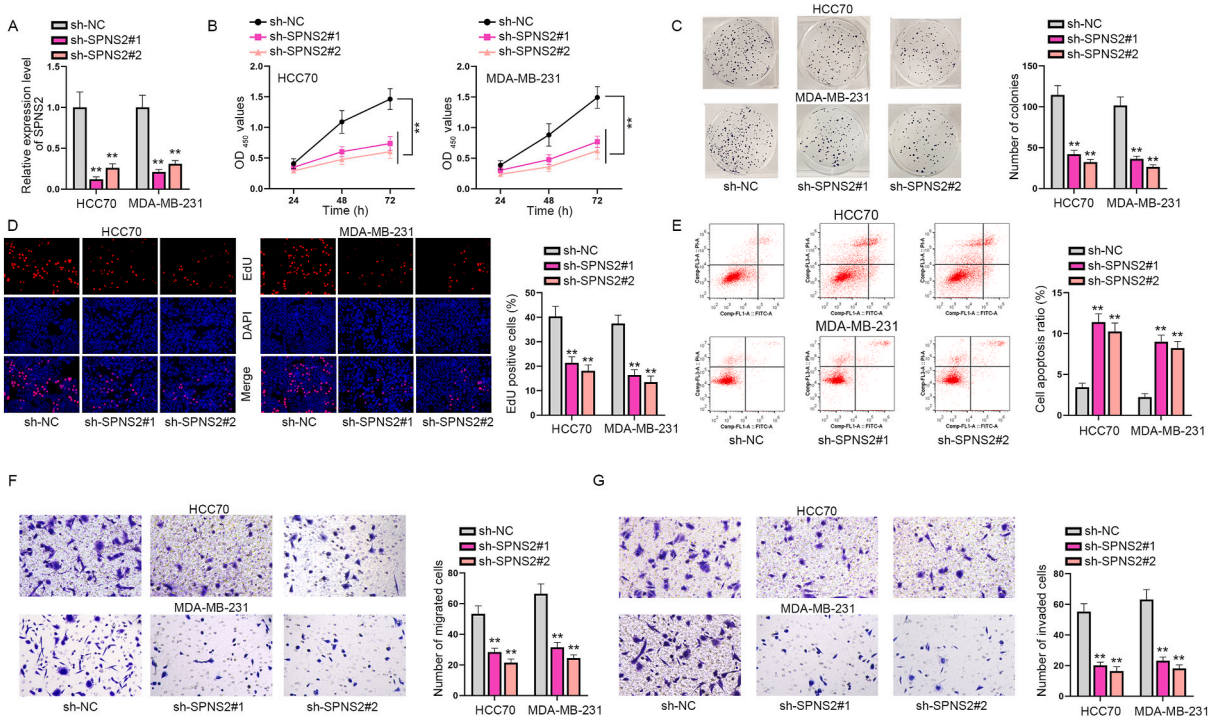

Fig. 5. Knockdown of SPNS2 inhibits the progression of TNBC
A. The interference efficiency of SPNS2 was detected through RT-qPCR. **B.** CCK-8 assay was conducted to access cell viability of HCC70 and MDA-MB-231 after SPNS2 was silenced. **C-D.** Colony formation and EdU assay was utilized to estimate the influence of inhibiting SPNS2 on cell proliferation. **E.** Flow cytometry was performed to test cell apoptosis when SPNS2 was knocked down in cells. **F-G.** Cell migration and invasion ability was measured through transwell assay in cells transfected with sh-SPNS2. **P < 0.01.

overexpressed miR-335–5p (Fig. 4E).

3.5. Knockdown of SPNS2 inhibits the progression of TNBC

After we verified SPNS2 as the target of miR-335–5p, we began to search its function in TNBC cells. We transfected the sh-SPNS2#1/2 into HCC70 and MDA-MB-231 cells for inhibiting SPNS2. Then RT-qPCR analysis was conducted and the results displayed the successful transfection efficiency (Fig. 5A). Following, CCK-8 assay revealed a reduction of OD_{450} value in sh-SPNS2-transfected cells, suggesting cell viability could be weakened by SPNS2 depletion (Fig. 5B). Next, the decreased number of colonies in transfection group also confirmed that cell proliferation could be inhibited by knockdown of SPNS2 (Fig. 5C). Similarly, EdU assay also displayed the same results, which verified the inhibitory function of silenced SPNS2 on cell proliferation again (Fig. 5D). On the contrary, cell apoptosis was measured through flow cytometry analysis and the results indicated that cell apoptosis rate could be accelerated by inhibited SPNS2 (Fig. 5E). Moreover, in transwell assays, we observed that the number of migrated and invaded cells was declined after cells were subjected to transfection with sh-SPNS2 (Fig. 5F–G).

3.6. LINC01503 accelerates the progression of TNBC by sponging miR-335–5p to upregulate SPNS2

For verifying whether LINC01503 could regulate cell behavior of TNBC via sponging miR-335–5p and regulate SPNS2, we conducted the rescue assays. Firstly, in CCK-8 assay, we observed that the repressed cell viability by silenced LINC01503 could be recovered by miR-335–5p inhibitor or SPNS2 overexpression (Fig. 6A). Besides, colony formation and EdU assay indicated that cell proliferation ability could be hampered by silenced LINC01503, but then reversed by miR-335–5p down-regulation or SPNS2 upregulation (Fig. 6B–C). Moreover, transwell assays demonstrated that cell migration and invasion was weakened by silenced LINC01503, but inhibited miR-335–5p or upregulated SPNS2 offset the inhibitory function (Fig. 6D–E). Finally, we discovered from flow cytometry analysis that miR-335–5p inhibition or SPNS2 upregulation counteracted the promoting role of silencing LINC01503 on cell apoptosis (Fig. 6F).

4. Discussion

Triple-negative breast cancer (TNBC) is a common cancer with high aggressiveness and high mortality in women [1]. Since there is

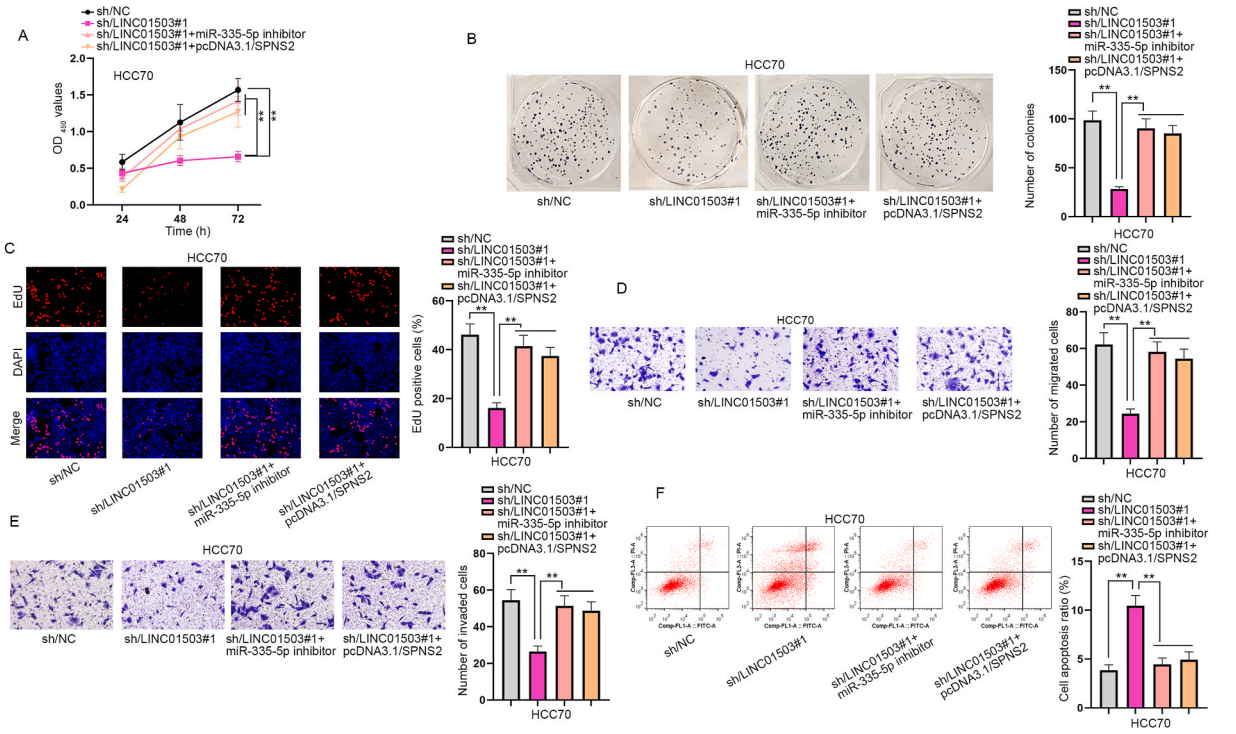

Fig. 6. LINC01503 accelerates the progression of TNBC by sponging miR-335-5p to upregulate SPNS2
A. CCK-8 assay detected cell viability when LINC01503 was inhibited and miR-335–5p was inhibited or SPNS2 was overexpressed in HCC70 cells. B–C. Colony formation and EdU assay detected cell proliferation ability in different groups in HCC70 cells. **D-E.** Transwell assays were conducted to detect cell migration and invasion capabilities in different groups of HCC70 cells. **F.** Cell apoptosis was measured by flow cytometry in different groups. **P < 0.01.

no effective target therapy, the prognosis of patients is very poor, and the overall survival rate is extremely low. Besides, the highly aggressive and invasive behavior contributes to high recurrence rate and extremely unfavorable prognosis of TNBC [4]. Thus, it is critical to explore effective treatment for HCC. Recently, the roles of lncRNA in cancer have attracted more and more attention, including TNBC [25]. For example, CCAT1 promoted TNBC cell growth by repressing miR-218/ZFX signaling [26]. And HCP5 facilitated cell proliferation and migration of TNBC by upregulating BIRC3 via sponging miR-219a-5p [21]. Moreover, MIR100HG expedited cell proliferation of TNBC by triplex formation with p27 Loci [27]. The commonality of these lncRNA was the notable high expression in TNBC and carcinogenic properties. In our study, we selected LINC01503 as our research object. According to previous reports, we discovered that LINC01503 was highly expressed in cancer cells and exerted the carcinogenic effects in esophageal squamous cell carcinoma [16], colorectal cancer [17] and cholangiocarcinoma [18]. Nevertheless, its functions in TNBC are still unclear. Thus, we firstly detected its expression pattern in TNBC cells through RT-qPCR, as we expected, the expression of LINC01503 was extremely high. Accordingly, we suspected LINC01503 to be an oncogene in TNBC. Then, loss-of-function assays were carried out and the results demonstrated that silenced LINC01503 repressed cell proliferation, migration and invasion and accelerated cell apoptosis of TNBC. Thence, we proved that LINC01503 exerted the carcinogenic functions in TNBC.

MiRNAs are the short and small RNAs with 22–24 nucleotides in length [28]. Like lncRNAs, miRNAs can also take part in the regulation of biological processes [28,29]. Meanwhile, a flow of evidence has illuminated the ceRNA network that lncRNAs can post-transcriptionally regulate the expression of miRNA targets through combining with miRNA [19,23,24]. For example, HOXD-AS1 was reported to act as a ceRNA to facilitate liver cancer metastasis through sponging miR-130a-3p to upregulating SOX4 [30]. It was also verified that CDC6 expedited breast cancer development and functioned as ceRNA to target downstream gene by sponging miR-215 [31]. In our study, we wondered whether LINC01503 could exert ceRNA function in TNBC. First of all, we discovered that LINC01503 was mostly located in cytoplasm of TNBC cells, suggesting LINC01503 could exert the ceRNA function. Following, mechanism experiments verified that LINC01503 could bind with miR-335–5p. Previously, reports indicated that miR-335–5p repressed metastasis of thyroid cancer cells via targeting ICAM-1 [32]. And miR-335–5p inhibited the development of colorectal cancer through down-regulating LDHB [33]. Similarly, we discovered that miR-335–5p was lowly expressed in TNBC cells and a chain of gain-of-function assays demonstrated that overexpressed miR-335–5p could repress cell proliferation, migration and invasion of TNBC, inhibiting the progression of TNBC.

SPNS2 has been proven as an oncogene in colorectal cancer [34]. Our research discovered that SPNS2 was confirmed to be the downstream gene of miR-335–5p and silenced SPNS2 repressed cell growth of TNBC. Ultimately, we discovered that that miR-335–5p inhibition or SPNS2 overexpression counteract the repressive function of inhibiting LINC01503 on TNBC progression, indicating the LINC01503/miR-335–5p/SPNS2 axis in TNBC.

Taken together, our research offered the vital evidence that LINC01503 was significantly upregulated in TNBC. Meanwhile, we discovered that LINC01503 accelerated cell proliferation, migration and invasion of TNBC as a ceRNA by sponging miR-335–5p to elevate SPNS2 expression. We innovatively revealed the specific molecular mechanism of LINC01503 in TNBC. However, we have to mention that our study stored in some limitations, such as the lack of clinical samples and in vivo validation. We have recognized these limitations and have made them the direction and focus of our future research to contribute to the in-depth study of TNBC.

Data availability statement

Research Data are not shared.

CRediT authorship contribution statement

Wei Weng: Writing – original draft, Resources, Investigation, Formal analysis, Data curation. **Hongyu Huang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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